10 Burton Drive Londonderry, NH 03053 (603) 437-4040 Fax (603) 437-7594 **Stonyfield.com**

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*Awesome Yogurt*

*Our Children*

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*Healthy Food*

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*Yogurt Culture*

*Uncompromising Quality*

*Employee Happiness*

*Scrumptious Flavors*

*Sustainable Farming*

*Fewer Lawyers*

*Company Pride*

*Participation*

*Believing*

January 12, 2007

Mr. Mark Bradley
Program Manager
USDA/AMS/TM/NOP
Room 4008-So.
Ag Stop 0268
1400 Independence Ave., SW
Washington, DC 20250

Dear Mr. Bradley:

Enclosed is a petition requesting the inclusion of the non-organically produced agricultural substance “Oligofructose enriched with Inulin Documented for Calcium Absorption” onto the National List section 205.606. I am the Stonyfield Farm Inc. contact and can be reached at:

Nancy Hirshberg
VP of Natural Resources
Stonyfield Farm Inc.
10 Burton Drive
Londonderry, NH 03053
603 437 4040 x 2270

Please contact me if you have any questions or if I can provide anymore information.

We appreciate your consideration of our request.

Sincerely,

Nancy B. Hirshberg
VP of Natural Resources

**Petition to add to the National List the substance**
**"Oligofructose enriched with Inulin Documented for Calcium Absorption"**

## Item A

### 1. Category

Non-organically produced agricultural products allowed in or on processed products labeled as "organic". §205.606.

### 2. Justification for this category

The petitioned substance is produced by extracting inulin from the roots of the chicory plant (*Cichorium intybus*) with hot water, treating a portion of the solution of extracted inulin with enzyme to effect a mild hydrolysis to obtain Oligofructose, and then spray-drying a solution of a mixture of the long-chain length inulin and the Oligofructose (OFS) to yield a dry powder. The process is analogous to extracting sugar from the roots of sugar beets and creating maltodextrin from corn starch.

## Item B

### 1. The common name of the substance.

The oligofructose enriched inulin with the calcium absorption claim that Stonyfield Farm uses has the trade name "BENEO®™ Synergy 1" and is described by its manufacturer as "Enriched Chicory Inulin Powder". The labels of our products containing this substance declare it as "inulin (natural dietary fiber)". In this petition we will refer to the specific Oligofructose enriched inulin powder documented for calcium absorption as BENEO®™ Synergy 1 Inulin/OFS powder. [Note: "inulin" is a polymer ("chain") of fructose. The analytical composition of the substance is fructose chains, 90% to 94%; sucrose, 2% to 4%; glucose, 0% to 6%; and fructose, 0% to 6%.]

### 2. The manufacturer.

ORAFTI Active Food Ingredients. Their U.S. address is as follows: 101 Lindenwood Drive, Malvern, PA 19355; telephone number (610) 889-9828.

### 3. The intended or current use of the substance.

BENEO®™ Synergy 1 Inulin/OFS Powder is an agricultural ingredient added to yogurt products to improve the absorption of calcium from the yogurt, as documented in published double-blind, placebo-controlled randomized clinical trials (see Appendix 1); to substantiate a label claim of improved calcium absorption as required by regulatory agencies in order to approve labeling with this claim; to add soluble dietary fiber; and to yield a satisfactory texture and consistency of the yogurt during its shelf-life. The substance is Kosher to satisfy the market requirements.

**4. The handling activities for which the substance will be used and its mode of action.**

Production of yogurt with improved calcium bioavailability.

BENEO®™ Synergy 1 Inulin/OFS Powder is used in the fermentation of milk to produce various dairy products (full-fat, reduced-fat, and fat-free) that contain a standardized level of "soluble dietary fiber". Inulin is frequently referred to as a "prebiotic" (a substance that is not digested in our intestines but reaches the bowel to nourish beneficial bacteria). Inulin improves the absorption of nutritionally essential minerals, particularly calcium. BENEO®™ Synergy 1 Inulin/OFS Powder used in our dairy formulations has documented beneficial effects on calcium absorption; see Appendix 1. The mechanism whereby calcium absorption is improved by inulin has been studied extensively in experimental animals and in human clinical trials, but the mechanism has not yet been completely elucidated.

**5. The source of the substance and a detailed description of its manufacturing or processing procedures.**

The substance is produced from the root of the chicory plant (*Cichorium intybus*). A general process for isolation of inulin and production of partially enzymatically hydrolyzed inulin materials (described as "oligofructose" or "fructose oligosaccharide") is described in a published article by Gibson, Willis, and Loo (1994) and in the "Production Process" description, both of which can be found in Appendix 2. Briefly, chicory roots, which contain up to 70% of their dry matter as inulin, are treated with hot water to extract the inulin, much as sugar beets are treated with hot water to extract the sugar. The inulin is purified and modified much as food starch and food starch hydrolysates are purified and then enzymatically treated to form more soluble and functional materials called "Oligofructose (OFS)" or "oligosaccharides".

BENEO®™ Synergy 1 Inulin/OFS Powder is produced by co-processing a long-chain quality of inulin with a pure oligofructose, in an about 50:50 ratio. The long chain length inulin is produced from native inulin by a physical separation process where the smaller molecules have been removed. The result is a distinctive distribution of the chain length of fructose polymers with unique physiological effects unlike any commercially available inulin. The manufacturer has filed for patent protection of this material and methods to produce it and to use it in Europe, the United States, and other parts of the world. A copy of United States Patent Application Publication No. 2003068429, "Inulin products with improved nutritional properties", is included in Appendix 2 and describes the process in detail.

**6. A summary of any available previous reviews of the petitioned substance by State or private certification programs or other organizations.**

No such summaries are known to be available.

**7. Information regarding EPA, FDA, and State regulatory authority registrations.**

BENEO®™ Synergy 1 Inulin/OFS Powder is a food, and not a food additive. The GRAS determination by independent expert commissioned by the manufacturer ORAFTI is included in Appendix 3. A scientific article published in 1999 by Coussement (also in Appendix 3) further describes the safety information on inulin and the process used by the manufacturer to affirm the GRAS status of its inulin and partial hydrolysates of inulin. Significantly, FDA had no questions regarding the conclusion by another manufacturer that inulin is GRAS under the intended conditions of use (GRAS Notice No. 00118; see Appendix 3).

Appendix 3 also includes copies of correspondence between the manufacturer, ORAFTI, and the State of California, wherein the California Department of Health Services indicated that it had no objection to the "improves calcium absorption" claim on the label of our yogurt containing the clinically tested BENEO® Synergy 1 Inulin/OFS Powder.

Status in the European Union: Although inulin does not appear on Annex VI of EC 2092-91 as an approved nonorganic ingredient, a request has been filed with Belgian authorities (see Appendix 7). It is currently permitted for use in EU organic products with temporary permission by local authorities.

**8. The Chemical Abstract Service (CAS) numbers of the substance and labels of products that contains the petitioned substance.**

| ***Component*** | ***Percentage*** | ***CAS Registration Number*** |
|---|---|---|
| Fructose chains (inulin) | 90 - 94 | 9005-80-5 |
| Sucrose | 2 - 4 | 57-50-1 |
| Glucose | 0 – 6 | 50-99-7 |
| Fructose | 0 – 6 | 57-48-7 |

A sample label from yogurt containing BENEO®™ Synergy 1 Inulin/OFS Powder as an ingredient is provided in Appendix 4. Note the label claim for increased calcium absorption.

**9. The substance's physical properties and chemical mode of action including:**

**(a) chemical interactions with other substances, especially substances used in organic production;**

BENEO® Synergy 1 Inulin/OFS Powder is used in organic handling, not organic production.

**(b) toxicity and environmental persistence;**

BENEO®™ Synergy 1 Inulin/OFS Powder is GRAS (Generally Recognized As Safe) when used in human food in accordance with good manufacturing practice. Inulin and its derivatives are rapidly fermented by beneficial colonic bacteria and do not persist in the environment.

**(c) environmental impacts from its use or manufacture;**

The production process involves water extraction of raw chicory roots to extract the inulin, which can represent up to 70% of the dry matter in the roots. The extracted root pulp waste and other process byproducts are used in animal feed or for other agricultural purposes; see “Production Process” in Appendix 2.

**(d) effects on human health;**

The positive effect of inulin and its partial hydrolysates has been the subject of many review articles over the past fifteen years. A sampling of these review articles can be found in Appendix 5.

**(e) effects on soil organisms, crops, or livestock.**

BENEO® Synergy 1 Inulin/OFS Powder is used in handling, not production. Many microorganisms can utilize the carbohydrate of inulin and its hydrolysates. Monogastric animals cannot digest inulin or oligofructose (OFS), which accounts for its favorable effect on beneficial colonic microorganisms (the “prebiotic” effect).

**10. Safety information about the substance.**

An MSDS for this material can be found in Appendix 6.

Appendix 3 contains a scientific article published in 1999 by Coussement who described the safety information on inulin and partial hydrolysates of inulin. Appendix 3 also includes GRAS determination of the manufacturer of BENEO®™ Synergy 1 Inulin/OFS Powder and the 2002 response of FDA indicating that the agency had no questions about the self-affirmation of inulin and its partial hydrolysates as GRAS by another manufacturer.

**11. Comprehensive research reviews and research bibliographies, including reviews and bibliographies which present contrasting positions.**

See Appendix 5 for reviews.

Organic inulin is currently available from several botanical sources: Jerusalem artichoke, agave, and chicory. However, none of these sources of organic inulin has been documented to improve calcium absorption in a double blind placebo controlled randomized human clinical trial, a requirement of the California Department of Health Services for approval of the label claim of “improved calcium absorption”.

We are urging potential suppliers of organic inulin to conduct clinical trials of calcium absorption with a form of inulin that is of an appropriate quality for incorporation into yogurt (i.e., a form that does not have an adverse effect on yogurt texture and consistency).

Note that BENEO® Synergy 1 Inulin/OFS Powder, the specific form of “inulin enriched with OFS” documented to be effective in improving the calcium absorption from yogurt, is a proprietary material and the manufacturer is seeking

patent protection in the United States and other countries. The abstract of the European patent application reads as follows:

> *The invention relates to novel inulin products and compositions thereof, to their manufacture and to their use for modulating the bacterial flora and the fermentation pattern of inulin in the large intestine of humans and mammals, to their use for providing improved inulin-associated nutritional effects/benefits, and to their use for the manufacture of a pharmaceutical composition for providing said effects/benefits in humans and mammals. The novel inulin products consist of a mixture of an easily fermentable inulin (EFI) component (preferably an oligofructose, an agave inulin, or a mixture thereof) and a hardly fermentable inulin (HFI) component (preferably a long-chain inulin with a (DP) >= 20, typically chicory inulin with a (DP) >= 23), in a weight ratio ranging from 10/90 to 70/30. The nutritional effects include improved mineral absorption, particularly calcium and magnesium, bone mineral density increase, reduction of bone mineral density loss, improvement of bone structure, modulation of lipid metabolism, stimulation of the immune system, and anti-cancer effects. The novel inulin products are particularly suitable for the manufacture of a composition or a medicament for preventing, for postponing and for treating osteoporosis in humans, particularly postmenopausal women."*

Since a patent would prevent another manufacturer from making or selling this material, merely the existence of patent applications in most of the countries where the plant sources of inulin are grown and processed is a strong disincentive for other manufacturers to develop a similar material for similar purposes.

We are urging the manufacturer of this material to produce it from organically grown chicory. There are numerous challenges to overcome for them to be willing to make it organically such as:

- Scale: Manufacture is a continuous process throughout the harvest season. They are not willing to stop production to do an organic run.
- They cannot substitute another readily available source of organic inulin, such as Jerusalem artichoke or agave as the composition of each is significantly different. The manufacturing process would have to be recreated, as well as the extensive research to gain the documented calcium absorption results.

See Appendix 7 for Orafti's letter on why they will not make organic inulin at this time.

**12. A ``Petition Justification Statement'' which provides justification for inclusion of a non-organically produced agricultural substance onto the National List.**

**Enhancing Nutritional Value**

A large gap exists between calcium intakes and calcium requirements for most of the U.S. population over the age of 11 (Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride, 1997. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Academic Press). Improving calcium absorption efficiency is another strategy to improve calcium nutrition among the population in addition to increasing calcium intakes. If the inulin powder with calcium

absorption claims is prohibited from use in organic, it will by default mean that organic yogurt is less nutritious than non-organic. We do not want to see organic become synonymous with less nutritious.

**Meeting the Consumer Demand for Calcium**
Calcium is one of the primary reasons that consumers choose yogurt, and our market research has shown that, the nutritional benefit of helping to*" boost calcium absorption"* is a very important attribute to our consumers. No organic inulin available today has been proven (to the satisfaction of our labeling regulators) to increase calcium bioavailability.

**Sampling of SF Consumer Research Indicating the Importance of the Inulin Powder with Calcium Absorption**

- Insight Express Web Survey (2004) showed that serving as a calcium source is the 2nd most important reason why consumers buy yogurt after taste. When asked," "Please indicate the importance of the following possible reasons why you choose to eat yogurt":

    - Taste
    - Low Calorie
    - Source of Calcium
    - Source of Protein
    - Convenience and Portability
    - Live Active Cultures
    - Low Cost

    76% ranked calcium as extremely or very important. This was 2nd only to taste which scored 82%.

- 52% of respondents picked "It helps boost calcium absorption" as the one characteristic they liked most about the Fountain of Yo yogurt (Oct. 2000). Followed by certified organic (15%) and low in fat (14%)

- 90% of respondents named "It boosts calcium absorption" as something they liked about the YoSelf yogurt concept. (April 2002). This attribute got the most votes, followed by "low in fat" (78%) and "Promotes good digestive health (69%).

- Consumer research conducted through Ipsos Insight (6/06), Inc confirmed the importance of extra calcium to yogurt consumers. Among 5 different new yogurt cup concepts tested, a product called 2-a-Day - differentiated by it's 50% RDI of calcium per cup - received the top scores.

**Documented Unique Properties Allow for Structure Function Claim Unavailable to Other Inulins**
Several sources of organic inulin are commercially available. Comparing the non-organic BENEO®™ Synergy 1 Inulin/OFS Powder that we use with the currently available sources of organic inulin, the non-organic form is the only material that is compatible with the product's physical characteristics **and** is backed by

conclusive, peer reviewed published clinical results. Peer reviewed human clinical results are required by the State of CA for retention of the current label claim of “Helps boost calcium absorption”.

Organic inulin is currently produced from the agave plant. Industrializadara Integral del Agave S. A. de C. V. (IIDEA) produces organic and kosher certified agave syrup in Mexico. Recently IIDEA added organic inulin to their line of products. Their agave syrup and inulin are extracted from the juice of organically grown agave plants by using a simple thermal process without any chemicals or enzymes. Unfortunately, none of the published clinical trials of inulin (or the inulin derivatives Oligofructose or fructose oligosaccharides) have involved inulin extracted from agave. We have been in conversations with them about conducting the necessary research to document calcium absorption and they have indicated a willingness to do so. They have contacted several institutions regarding conducting the research in the future.

Organic inulin, oligofructans, and fructose are currently produced by the German company TOPINA Diät-Rohstoff from Topinambur tubers, a plant better known in North America as Jerusalem artichoke (*Helianthus tuberosus*). Inulin from Jerusalem artichoke has been studied more extensively than inulin from agave, but not in published randomized clinical studies of calcium absorption.

Organic inulin made from chicory root is available in several qualities from TIC Gums (http://www.ticgums.com/store/search_list.asp ). However, none of these qualities from this manufacturer have been specifically tested in clinical studies of calcium absorption.

If the patent applications filed by the manufacturer of BENEO®™ Synergy 1 Inulin/OFS Powder mature into one or more patents, its manufacturer will be granted the right to exclude other manufacturers from making the same quality material.

As soon as a source of organic inulin has been shown in randomized clinical trials to promote calcium absorption to the satisfaction of the State of CA to allow us to make the calcium claim, and is commercially available to us, be assured that we will use it and we will follow up with a petition to the NOSB to remove this item from §205.606.

Contents of the Appendices

# Appendix 1

## Petition to add to the National List the substance "Oligofructose enriched with Inulin Documented for Calcium Absorption"

**January 12, 2007**



Nutritional File

# Improvement of Calcium Absorption

ORAFTI

BENEO

# CONTENT






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afi@orafti.com • www.orafti.com

# Inulin and oligofructose as prebiotic fibres to improve calcium absorption and to increase calcium bone density.

## 1. Introduction

### 1.1. Background

The World Health Organisation has defined osteoporosis as the second leading health care problem after cardiovascular disease. Approximately 200 million women worldwide are estimated to have osteoporosis (Leatherhead, 2002). For women, the incidence of fractures increases substantially after the age of 45. Osteoporosis affects one-third of women aged between 60 – 70 years, and two-thirds of women aged 80+. It is a growing global problem, and osteoporotic fractures are expected to increase at least 2-fold over the next 50 years, with the incidence of hip fractures at least 4-fold (Food Industry Updates, March 2002). A clear difference in bone structure can be seen in Figure 1.

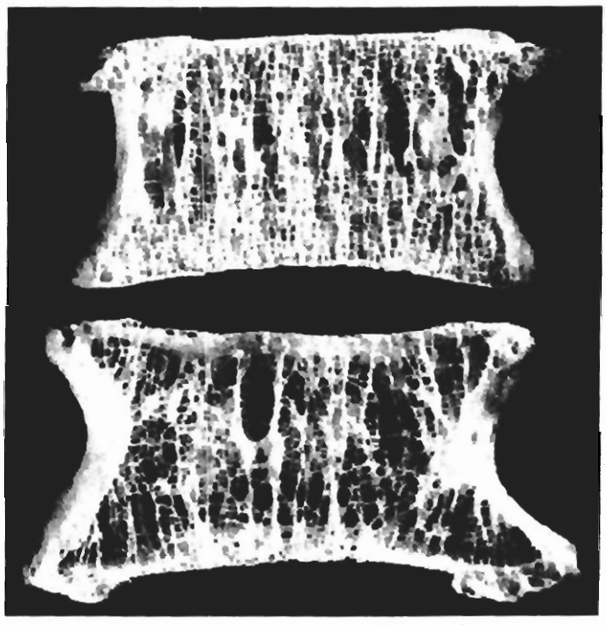

*Figure 1: Difference in bone structure (vertebrae). Top: woman at age of 30 years. Bottom: woman at age of 60 years, clearly suffering from reduced bone structure.*

Historically, to combat concerns over bone health, consumers chose products traditionally rich in calcium (Ca). Therefore, until recently Ca-fortified products have seen a lot of activity, but today attention is being focussed on how the body's absorption and usage of Ca can be optimised. In this concept, inulin and oligofructose are functional ingredients that have been demonstrated to increase the absorption of Ca from the diet, and to increase the Ca density in the bones.

## 1.2. Inulin & oligofructose

### 1.2.1. Definitions.

Inulin is a dispersed polysaccharide that can be found in many plants, a.o. chicory root, onion, leak, garlic, Jerusalem artichoke, banana... (Van Loo *et al.*, 1995).

It has a linear structure of fructosyl-units, linked by ß(2-1) glucosydic bondage, and can be represented as GFn, in which :

G = glucosyl unit;

F = fructosyl unit;

n = number of fructosyl units, and is between 2 and 60.

Native inulin also contains a fraction of oligofructose. Inulin can be further enzymatically hydrolysed to oligofructose. This hydrolysed oligofructose is a mixture of GFn and Fn compounds, in which:

n = between 2 and 9.

Because inulin and oligofructose consist of ß(2-1) linkages, they are resistant to the human digestive hydrolysis, and are world-wide accepted and used as dietary fibres. Their structure is visualised in Figure 2.

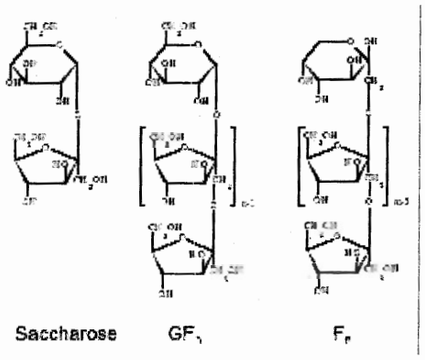


*Figure 2: The structure of the $GF_n$ and $F_n$ compounds of inulin and oligofructose, compared to saccharose.*

### 1.2.2. Overall nutritional properties

As dietary fibres, inulin and oligofructose are not metabolised by the human digestive system, but they are completely fermented in the colon. This gives them a number of beneficial properties:

- Improvement of stool weight and frequency;
- Prebiotic or bifidogenic effect;
- Decrease of serum triglycerides;
- Increase in calcium absorption.

These nutritional properties of inulin and oligofructose have been demonstrated in human volunteer studies with daily doses ranging between 5 and 10 g. This document will focus on the increased effect of Ca absorption.

# 2. Calcium absorption

## 2.1. Animal Models

Unlike some other types of dietary fibres (containing phytic or uronic acids), inulin and oligofructose do not impair but improve the bioavailability of minerals such as calcium (Ca), magnesium (Mg) and iron (Fe).

Shimura *et al.* (1991), Levrat *et al.* (1991), Rémésy *et al.* (1993), Brommage *et al.* (1993), Ohta *et al.* (1994, 1995a), Delzenne *et al.* (1995), Taguchi *et al.* (1995) and Scholz-Ahrens *et al.* (1998) all reported studies with rats in which an increased intestinal absorption of calcium (and in certain cases also of other minerals such as magnesium) was demonstrated by the consumption of inulin-type fructans (inulin or oligofructose). Ohta *et al.* (1995b, 1997) and Baba *et al.* (1996) formulated the hypothesis that the effects of non-digestible oligosaccharides on Ca and Mg absorption occur at the level of the large intestine. This was a new concept, as it is generally accepted that mineral absorption occurs mainly via the small intestine.

The study of Delzenne *et al.* (1995) indicated that a diet supplemented with 10% of either inulin (Beneo™ST) or oligofructose (Beneo™P95) leads to a significant increase (of about 60%) in the apparent retention of calcium, magnesium and iron in rats (Figure 3). A similar increase (about 65%) in Ca absorption was also observed in the study of Brommage *et al.* (1993) with rats fed a diet supplemented with 5% of oligofructose (Beneo™P95) or other non-digestible carbohydrates.

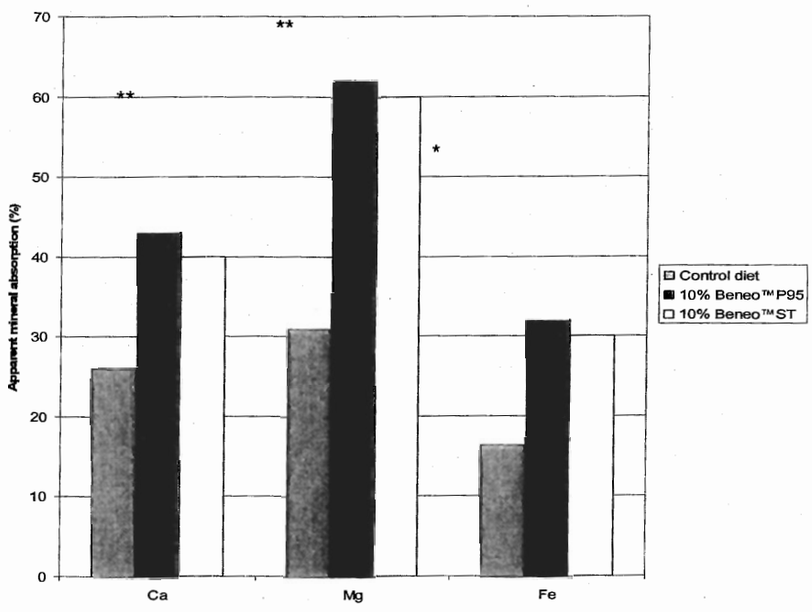


*Figure 3: Effect of oligofructose and inulin on the apparent absorption of Ca, Mg and Fe in rats (after Delzenne et al., 1995). *: p<0.05; **: p<0.01.*

Taguchi *et al.* (1995) demonstrated that oligofructose (2.5 and 5% in the diet) increases Ca and Mg absorption in ovariectomised rats (an experimental model for post-menopausal women) and that it prevents bone loss caused by oestrogen deficiency. In a similar model, Scholz-Ahrens *et al.* (2002) observed a dose-effect of oligofructose (Beneo™P95, 2.5 - 5 and 10% in the diet) on the increase of both Ca absorption and bone mineralisation (calcium content) in femur and lumbar vertebrae. The increased uptake of calcium into the bone tissue upon oligofructose ingestion was confirmed (Figure 4).

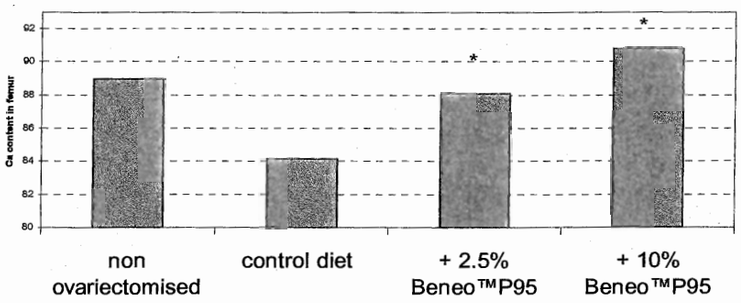


*Figure 4: Effect of oligofructose (Beneo™P95) on the femur mineralisation in ovariectomised rats. (after Scholz-Ahrens et al., 2002) *: p< 0.05.*

Using microradiography followed by computerised image analysis, the same authors showed that oligofructose (2.5-10% in the diet) significantly prevented ovariectomy-induced loss of trabecular bone (tibia) structure (Figure 5), thus strengthening the bones. These effects were more pronounced in case of high dietary calcium intake (1% Instead of 0.5%).

1% Ca; 5% oligofructose

1% Ca; 0% oligofructose

*Figure 5: Microradiography of the tibia trabecular in ovariectomised rats ; effect of oligofructose on the calcium density, visualised as the white structure in the bone. Scholz-Ahrens et al., 2002.*

An increase in whole-body bone mineral density was further confirmed by Roberfroid *et al.* (2002) in conventional rats fed a diet supplemented with 5 or 10% of inulin (Beneo™HP), together with different levels of Ca enrichment (Figure 6).

Ohta *et al.* (1998) reported an increased ratio of colon / small intestinal concentrations of calbindin-D9k (a calcium-binding protein thought to play an important role in intestinal active calcium transport) in rats upon intake of a diet supplemented with 5 or 10% of oligofructose. These results suggest that the stimulatory effect of oligofructose may also relate to the transcellular route of calcium absorption in the large intestine.

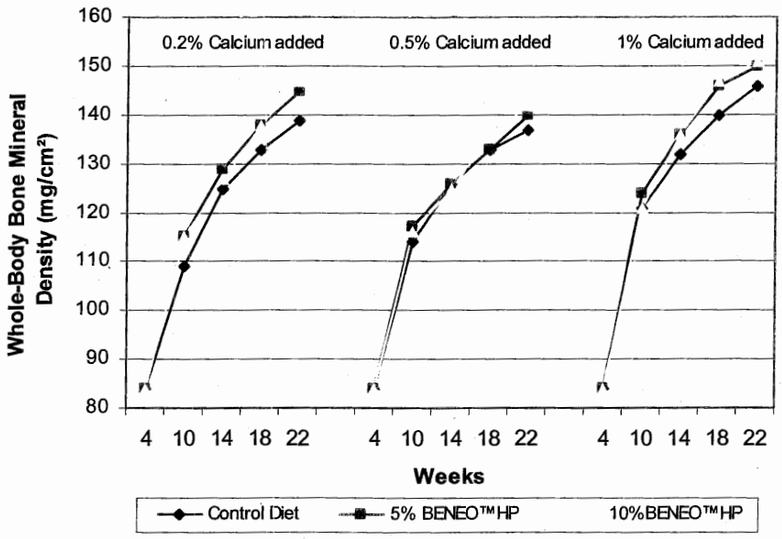


*Figure 6: Effect of inulin/oligofructose on bone mineral density in rats. $p<0.05$. After Roberfroid et al., 2002.*

## 2.2. Human intervention studies.

The homeostasis of calcium, and most importantly calcium ions ($Ca^{++}$) concentration in the blood is regulated very strictly by a complex mechanism (Figure 7). When this concentration drops too low, the parathyroid hormone (PTH) and calcitriol bring Ca blood concentration up to the required level by mobilising Ca from bone, increasing the absorption or encouraging its reabsorption from the kidneys. When blood Ca concentration is too high, calcitonin ensures that Ca is shifted back into bone or excreted in urine.

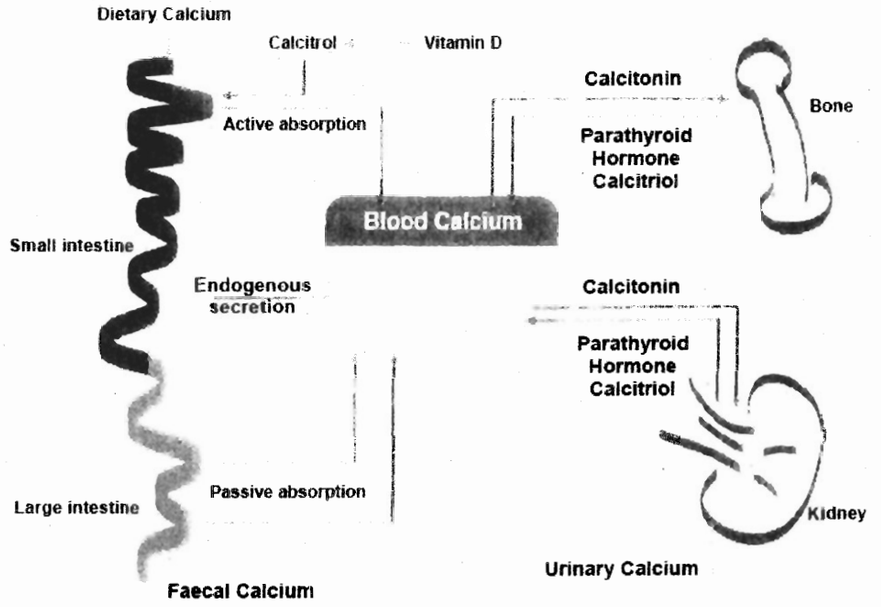


*Figure 7: A schematic presentation of the mechanisms that control the homeostasis of Ca in the blood. Calcitriol and PTH increase the Ca concentration in the blood, while calcitonin decreases the Ca concentration. Picture taken from: Calcium in nutrition – ILSI Europe Concise.*

All the Ca that is necessary to build up our bones and maintain them in good health needs to be ingested through our diet. It is generally accepted that there are two mechanisms at work for absorption.

- The active absorption: a paracellular process that takes essentially place in the small intestine and is calcitriol (vitamin D metabolite) mediated.
- The passive absorption: a trans-cellular process which takes place in the colon, which is gradient-driven.

However a third mechanism, which takes already absorbed Ca from the extracellular fluids back into the gastro-intestinal tract, also takes place, and is called the endogenous secretion.

Our adolescent part of life is a crucial period during which our bone mass is built up dramatically. We need to make maximum use of all calcium that is available in our diet. If we are able to maximise our peak bone mass during this period, and maintain high absorption levels during our adolescence, we may be able to postpone the occurrence of osteoporosis later in life (Figure 8).

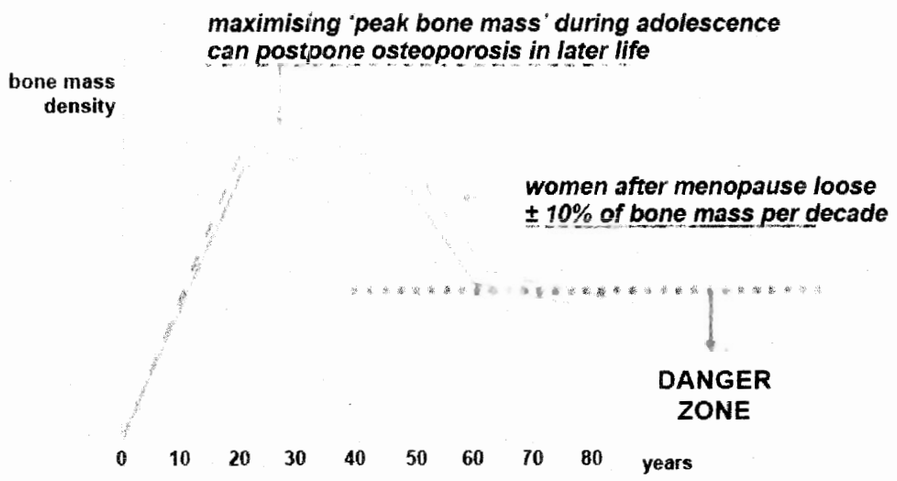


*Figure 8: A schematic presentation how maximisation of peak bone mass during adolescence can postpone osteoporosis.*

To measure calcium absorption in humans, two methods can be used:

- **Balance technique:** this technique is based on measuring the intake of Ca in the diet, and the excretion of Ca in the faeces. This method is not very accurate, and cannot account for the endogeneous excretion. Therefore the result is always indicated as the "apparent" absorption.
- **Double stable isotope technique:** this technique was developed a few years ago. It is based upon the oral administration of a specific Ca isotope with the diet, and the simultaneous intravenous injection of a second different Ca isotope. Both are recovered in the urine, and allow for the very accurate calculation of the "true" (total or fractional) absorption.

Ellegård *et al.* (1997) determined the mineral balance in ileostomy volunteers who were administered about 15g of either inulin (Beneo™ST) or oligofructose (Beneo™P95). The intake of the fructans did not alter the mineral (Ca, Mg, Fe, Zn) excretion from the small intestine and thus, it hardly affected their absorption in this part of the gut, suggesting that any effect of the fructans on mineral absorption must originate mainly in the colon.

In a first study to confirm the same concept in humans, Coudray *et al.* (1997) performed a study with nine healthy adult volunteers, who were given up to 40 g/day of chicory inulin for a period of 26 days (2 days of control diet followed by 14 days of progressive increase in inulin amount and then 12 days at max. inulin consumption). The administration of either a control diet, a diet supplemented with inulin or a diet supplemented with sugar beet fibre occurred according to a 3x3 latin square experimental design.

They measured the apparent Ca absorption by determining the mineral balance during 8 days (days 20-28) of urine and faecal samples. Upon inulin ingestion, the apparent Ca absorption increased significantly ($p<0.01$) from 21.3% (±12.5) to 33.7% (±12.1), which represents a relative increase of 58% (Figure 9). The increase in Ca absorption did not negatively influence the absorption of other minerals such as magnesium, iron or zinc. There was no increase in the apparent Ca absorption upon ingestion of the sugar beet fibre diet.

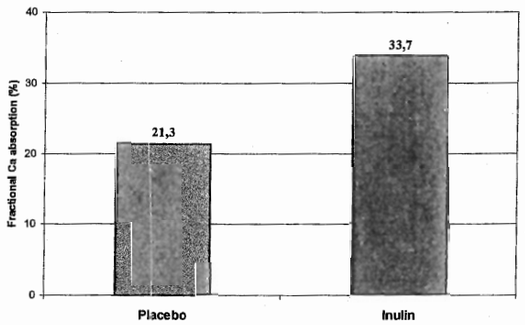


*Figure 9: Effect of chicory inulin (up to 40g/day, for 4 weeks) on apparent calcium absorption in adult men. p<0.01. After Coudray et al., 1997.*

In an experimental protocol with humans, van den Heuvel *et al.* (1998) validated the double stable isotope technique to determine true intestinal calcium (and iron) absorption. This technique allows one to make a distinction between exo- and endogeneous Ca, and therefore to measure "true" calcium absorption. The measurements were based on the contents of calcium isotopes in urine after a 24-hour period of collection. In this experiment with healthy young adults, no significant differences were observed in mineral absorption with 15 g/day of either a placebo, inulin or oligofructose. If the large intestine is the major place where fructans enhance calcium absorption, a 24-hour period of urine collection may be too short to make up a complete balance and to detect the effect of fructans. This experiment at least confirmed that fructans have no adverse effects on Ca absorption.

Based on these results, van den Heuvel *et al.* (1999) then performed a similar study using stable isotopes, but this time with a group of male adolescents, and by collecting the urine samples for 36 instead of 24 hours. Adolescents were chosen since it is thought that the mineral absorption rate is highest during adolescence. Twelve volunteers consumed 15 g/day of either oligofructose (Beneo™P95) or a placebo (sucrose), during a period of nine days. A significant increase (+26%; $p<0.05$) in the fractional Ca absorption from 47.8% (placebo) to 60.1% (oligofructose) was observed upon ingestion of oligofructose (Figure 10). This indicates that oligofructose may help to maximise the peak bone mass in adolescents.

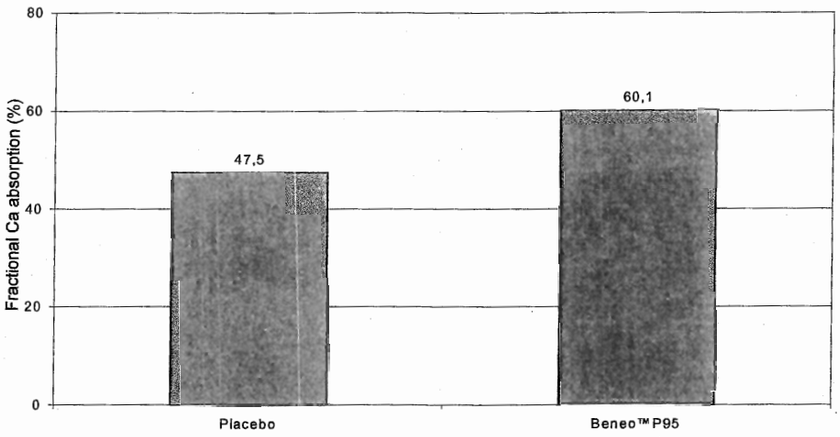


*Figure 10: Effect of oligofructose (15g/day Beneo™P95, for 9 days) on true calcium absorption in adolescent boys. p<0.05. After Van den Heuvel et al., 1999.*

## 2.3. Beneo™Synergy1.

### 2.3.1. Definition

The continuous strive for innovation resulted in the development of a new product, called Beneo™Synergy1. It is an enriched form of inulin, produced through a careful selection of the chain length distribution. This distribution is achieved by co-processing a long-chain quality of inulin with a pure oligofructose, in an about 50/50 ratio. As a result, the distribution of the chain length has dramatically changed, which turns this enriched inulin in a distinct quality on its own. By manipulating this profile of chain length distribution, we can also manipulate the fermentation process and location throughout the passage through the colon. The difference in chain length distribution with standard inulin (Beneo™ST) and oligofructose (Beneo™P95) can be clearly seen in Figure 11.

This new product has been evaluated both in animal and in human studies.

Figure 11a.

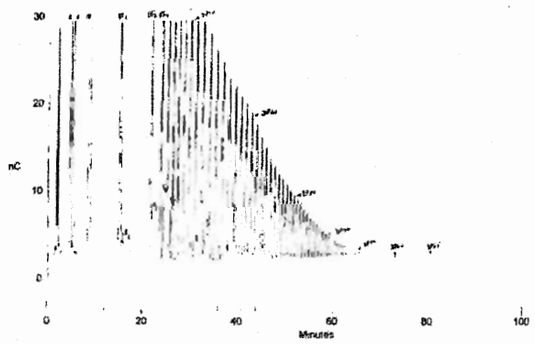


Figure 11b.

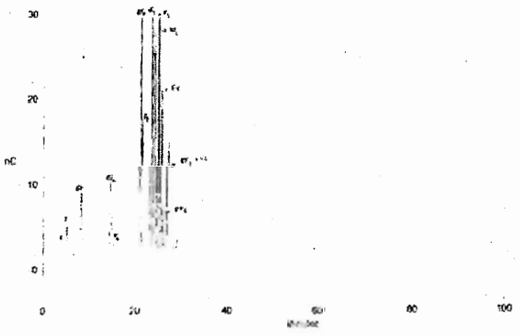

Figure 11c.

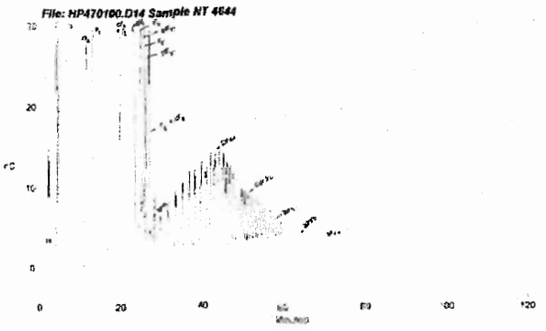


*Figure 11: The different structures of inulin and oligofructose, as in Beneo™ST (Figure 11a), Beneo™P95 (Figure 11b) and Beneo™Synergy1 (Figure 11c).*

### 2.3.2. Animal models

In a study with 50 rats, Coudray *et al.* (2003) investigated the absorption of calcium and magnesium after the intake of 10% of oligofructose and several inulin types, different in chain length. The rats were fed during 4 weeks, with increasing concentrations of the test compounds up to 10%, and with a 0,5% enrichment of calcium in the diet (Figure 12).

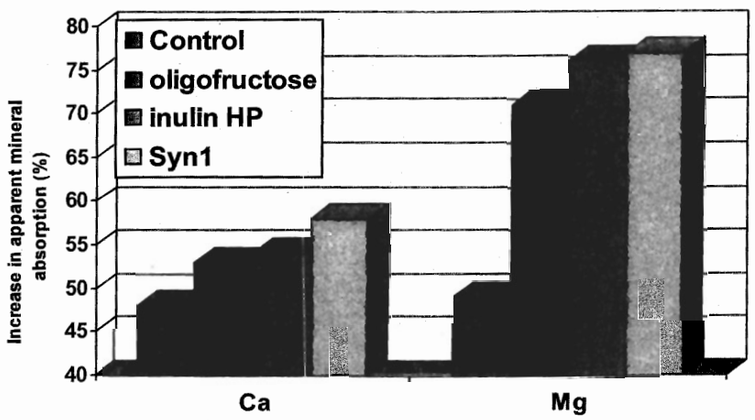


*Figure 12: Increase in calcium and magnesium absorption after intake of different types of oligofructose/inulin (10%) in rats. After Coudray et al., 2003.*

The intake by rats of the new enriched inulin, Beneo™Synergy1, substantially increases the calcium absorption ($p<0,05$), and this effect is superior compared to the other tested compounds, the normal inulin and oligofructose. Also the absorption of Mg was significantly enhanced (+ 57% compared to control).

Also the amount of solubilised Ca in the cecum of the rat was measured, and big differences were observed between the control and the fibre enriched groups (Figure 13). This is a clear support that most probably the mechanism of increased Ca absorption can be explained by an acidification of the lumen of the colon by the fermentation process. More short chain fatty acids are produced, the overall pH of the lumen decreases, and more complexed Ca that is present in the cecum and colon becomes soluble. Only Ca in its solubilised status becomes candidate for absorption.

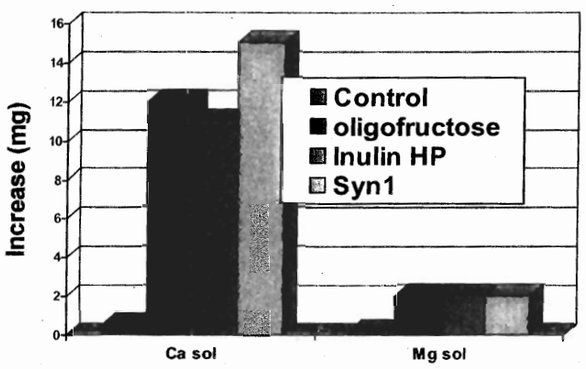


*Figure 13: Increase in solubilised calcium and magnesium after intake of different types of oligofructose/inulin (10%) in rats. After Coudray et al., 2003.*

In a recent study in ovariectomised rats (Zafar *et al.*, 2004), the influence of Beneo™Synergy1 on bone formation was investigated. The inclusion of 5% of Beneo™Synergy1 resulted in a decrease in bone formation (probably still caused by estrogen deficiency through the menopause, simulated by the ovariectomy), but the bone resorption rate was further suppressed, resulting in a net positive Ca retention and balance (Figure 14).

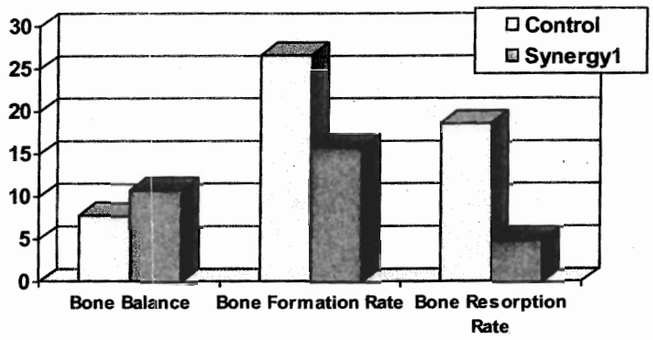


*Figure 14: Net positive Ca balance and retention in ovariectomised rats after the ingestion of 5% Beneo™Synergy1. Expressed as mg Ca/day. After Zafar et al., 2004.*

### 2.3.3. Human intervention studies.

Based upon the encouraging results in rats, a research programme was started to obtain a clinical confirmation of the findings of Coudray *et al.* (1997)

In a study by Griffin *et al.* (2002), true calcium absorption was measured in 29 young adolescent girls. In a randomised, double-blind, cross-over design the girls received two 4 g servings of the new enriched inulin, Beneo™Synergy1, daily for 3 weeks, and two 4 g servings of a placebo (sucrose), separated with a wash-out period of 2 weeks. The calcium absorption was measured using a double stable isotope technique (46Ca/42Ca) on urine samples, collected over 48 hours. The double stable isotope technique is the most accurate technique available to measure true calcium absorption. Eight (8) grams per day of Beneo™Synergy1, resulted in a relative increase of Ca fractional absorption of 18% (Figure 15) and in an absolute increase of Ca absorption of about 90 mg per day (Figure 16). This effect was established while taking daily 1500 mg of Ca, where previous work has suggested that at an intake above 1200 – 1400 mg of Ca, further increase of Ca uptake is very difficult to demonstrate. As no increase in urinary Ca excretion was observed, it can be concluded that also the net retention of Ca was increased by Beneo™Synergy1. The data suggest that the maximum effect occurs in people with the lowest Ca absorption rates, i.e. people who benefit the most of it.

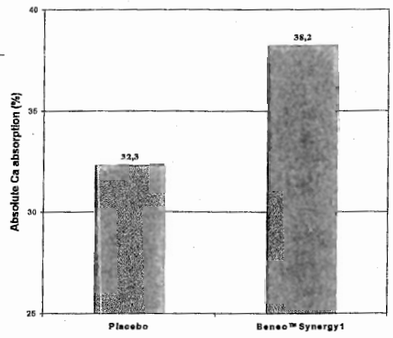


*Figure 15: True fractional calcium absorption in adolescent girls after the daily intake of 8 g of Beneo™ Synergy1 during 3 weeks. p<0.007. After Griffin et al. (2002).*

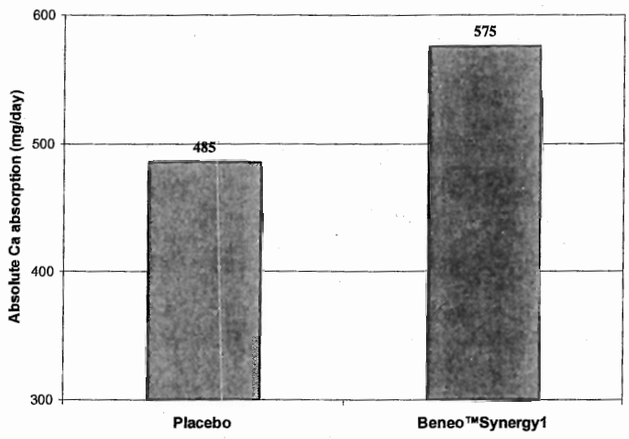


*Figure 16: True absolute calcium absorption in adolescent girls after the daily intake of 8 g of Beneo™ Synergy1 during 3 weeks. p<0.004. After Griffin et al. (2002).*

The researchers state that "if even part of this additional calcium was utilised for bone mineral production, it could lead to a significant increase in peak bone mineral density during this critical period."

In a multi-centre extension of this study (Griffin *et al*, 2003), 8g Beneo™Synergy1 per day again confirmed the increased Ca absorption by almost 10%. The authors conclude that "regular intake of 8g nondigestible oligosaccharide as Beneo™Synergy1 led to a significant increase in calcium absorption in these girls. Those subjects most likely to benefit were those with low fractional calcium absorption during the placebo period."

In a recently conducted randomised double-blind cross-over study, the effect of Beneo™Synergy1 was monitored in fifteen post-menopausal women (Holloway, 2004). The ingestion of 10 g per day during 6 weeks resulted in a significant increase in both Ca and Mg absorption as compared to the placebo group, who experienced a decrease in Ca and Mg absorption (Figure 17). Also the parathyroid hormone and blood osteocalcin were monitored, both indicating an increased Ca absorption and increased bone formation, respectively.

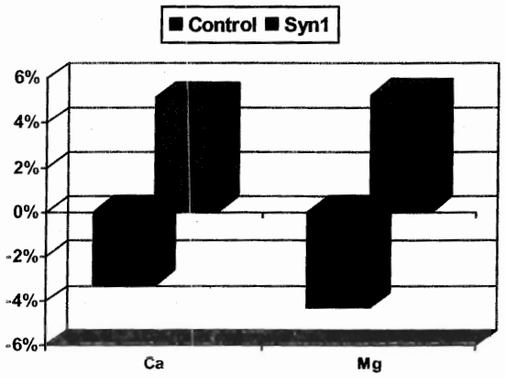


*Figure 17: Absolute change in Ca and Mg absorption after the intake of 10 g/day of Beneo™Synergy1 during 6 weeks. After Holloway et al., 2004.*

A very recent, large-scale study was finalised. A full-year randomised double-blinded intervention study with 100 children (50 boys and 50 girls) aged between 9 and 13 years monitored the effect of ingestion of 8 g Beneo™Synergy1 per day (Abrams *et al.*, 2005). Bone mineral content and bone mineral density were determined prior to randomisation, and after 1 year, through DEXA (dual X-ray Absorptiometry). Calcium absorption was measured using the double stable isotope technique at baseline, after 8 weeks and after 1 year. An enhanced calcium absorption (Figure 18) and bone mineralization has been seen compared with adolescents receiving maltodextrin, a placebo control.

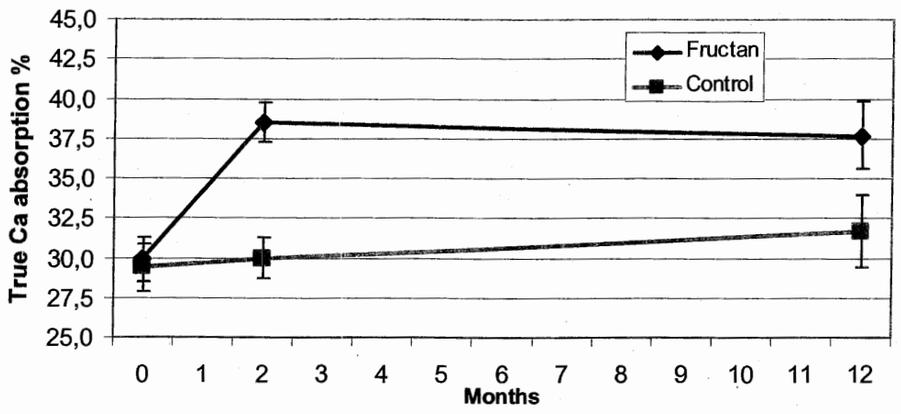


*Figure 18: Ca absorption at baseline, 8 weeks and after 1 year. * p<0.05 Beneo™Synergy1 vs Control. After Abrams et al, 2005.*

In addition, the supplemented group had a greater increment in both whole body bone mineral content (+17%; p=0.03) and whole body mineral density (+47%; p=0.01; Figures 19 & 20). Furthermore, it seems that - at least initially - the magnitude of the benefit was affected by genetic modifiers of calcium absorption, including polymorphisms of the *Fok1* vitamin D receptor gene. The net benefit in daily calcium accretion to the skeleton of supplementation found in this study was an average of approximately 30 mg.

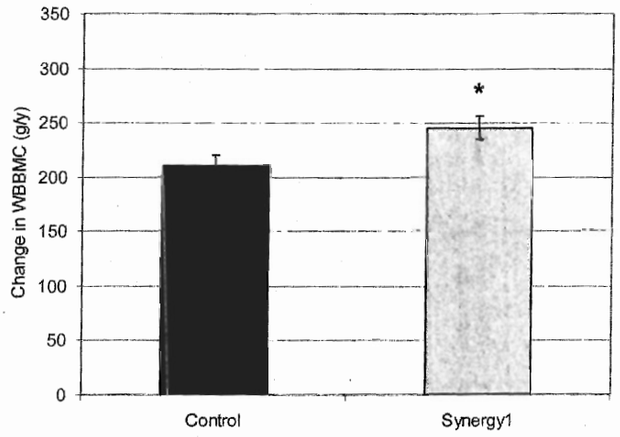


*Figure 19: Change in whole body bone mineral content (WBBMC) (g/y) of the subjects (mean + stdev) after supplementation of the diets with Beneo™ Synergy1 or maltodextrin for 1 year: p=0.03. After Abrams et al., 2005.*

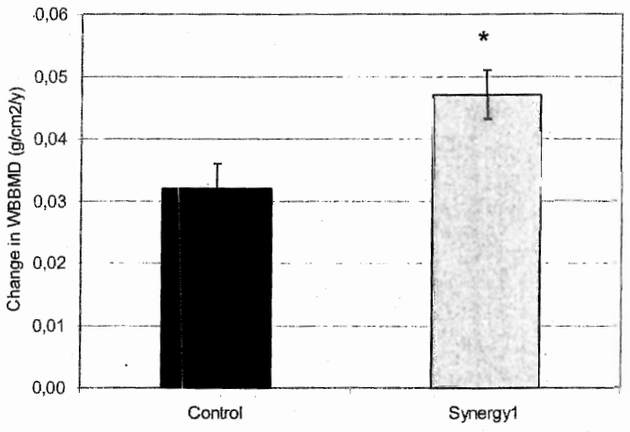


*Figure 20: Change in whole body bone mineral density (WBBMD) (g/cm²/y) of the subjects (mean + stdev) after supplementation of the diets with Beneo™ Synergy1 or maltodextrin for 1 year: p=0.01. After Abrams et al., 2005).*

### 2.3.4. Mechanism hypothesis.

This significant increase in calcium absorption at only 8 g/day could be explained, by the special composition (chain length distribution) of Beneo™Synergy1. When the non-digestible dietary fibres (inulin/oligofructose) arrive in the proximal part of the colon, fermentation starts rapidly. Thanks to the very specific distribution of the chain length of Beneo™Synergy1, this fermentation is continued also in more distal parts of the colon. As a result, the total fermentation process is better spread out over time and colon length. A broader pH decrease is achieved throughout the colon, and the solubilised calcium can more readily be absorbed. This whole hypothesis is visualised in Figure 21.

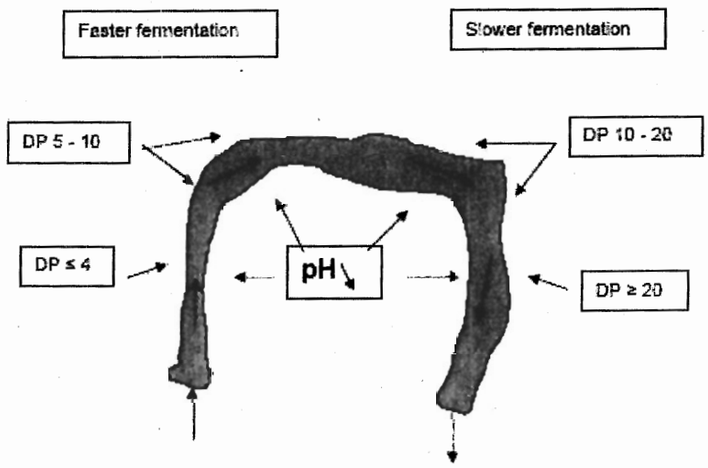


*Figure 21: Hypothesis behind the increased functionality of Beneo™Synergy1.*

## 3. Conclusions.

The stimulation of mineral (Ca, Mg) absorption by inulin-type fructans (inulin, oligofructose) is an observation which has been repeatedly confirmed in rat studies. The use of different models showed an increase in the absorption of calcium (and magnesium) at the level of the large intestine, as well as an increased calcium uptake into the bone tissue resulting in improved bone mineral density. Inulin-type fructans also prevented ovariectomy-induced loss in the bone (trabecular) structure and therefore strengthened the bones.

Human experiments showed that, as in rat models, the consumption of inulin or oligofructose by humans resulted in increased Ca absorption. Two human intervention studies showed a significant positive effect, with an increase in Ca absorption of 26% (with 15 g/day of oligofructose, in adolescents) and 58% (with 40 g/day of inulin, in young adults).

However our research has shown that not all fructans are equally efficient in stimulating calcium absorption.

A human study further demonstrated that the intake of 8 g per day of a new enriched inulin, Beneo™Synergy1, during three weeks increases the fractional and absolute Ca absorption with approximately 18 % and 90 mg/day, respectively. The authors call this observed effect "clinically significant" and state further that "if even part of this additional calcium was utilised for bone mineral production, it could lead to a significant increase in peak bone mineral density during this critical period." This significant effect was confirmed in a new study with similar protocol, recruiting an extra 25 adolescent girls.

In post-menopausal women, the ingestion of 10 g of enriched inulin, Beneo™Synergy1, significantly increased the absorption of Ca and Mg. Indirect biomarkers related to Ca absorption and bone formation were also positively altered.

In a recent study by Abrams and his co-workers, the increased Ca absorption was confirmed during a 1-year intervention study. For the first time, also Ca bone mineral density and bone mineral content were measured/calculated, and significantly increased. These data suggest that enriched inulin, Beneo™Synergy1, increases Ca absorption during pubertal growth, which enhances bone mineralisation leading to a greater bone mass during adolescence.

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# Breakthrough in Bone Health with Beneo™Synergy1

- NIH-funded (about 2 Mio US$) long-term study
- Performed by Prof.S.Abrams (Children's Nutrition Research Center, Baylor College of Medicine, Houston, Texas, USA)
- Randomised, double-blind, case-control, intervention trial
- Looking at the effects of Beneo™Synergy1 on Calcium Absorption and Bone Mineralisation
- Involving 100 girls and boys in early puberty (9-12 y. old, girls pre-menarcheal) with habitual Ca-intake of 900-1000 mg/day
- Supplementation with 8 g/day Beneo™Synergy1 (or maltodextrin as placebo), given at breakfast in calcium-fortified orange juice or milk, for 12 months

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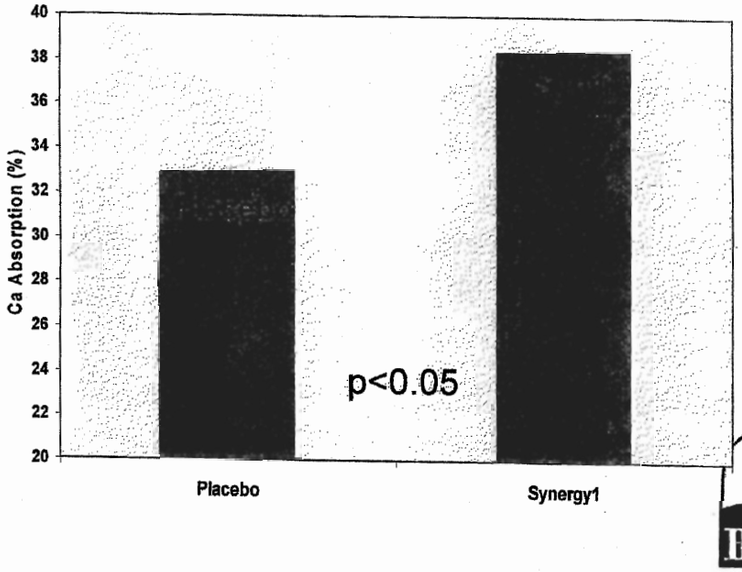
BENEO

# Breakthrough in Bone Health with Beneo™ Synergy1

Beneo™ Synergy1 significantly increased true Calcium Absorption both at 6-week and at 1-year intervention, by about 20% ($p<0.05$):
38.7% vs. 30.4% at 6-week and 38.4% vs. 32.7% at 1-year

True Ca absorption (%) at 1-year intervention

Ca Absorption measured by the dual stable isotope technique $^{46}Ca/^{42}Ca$, with 48h urine collection

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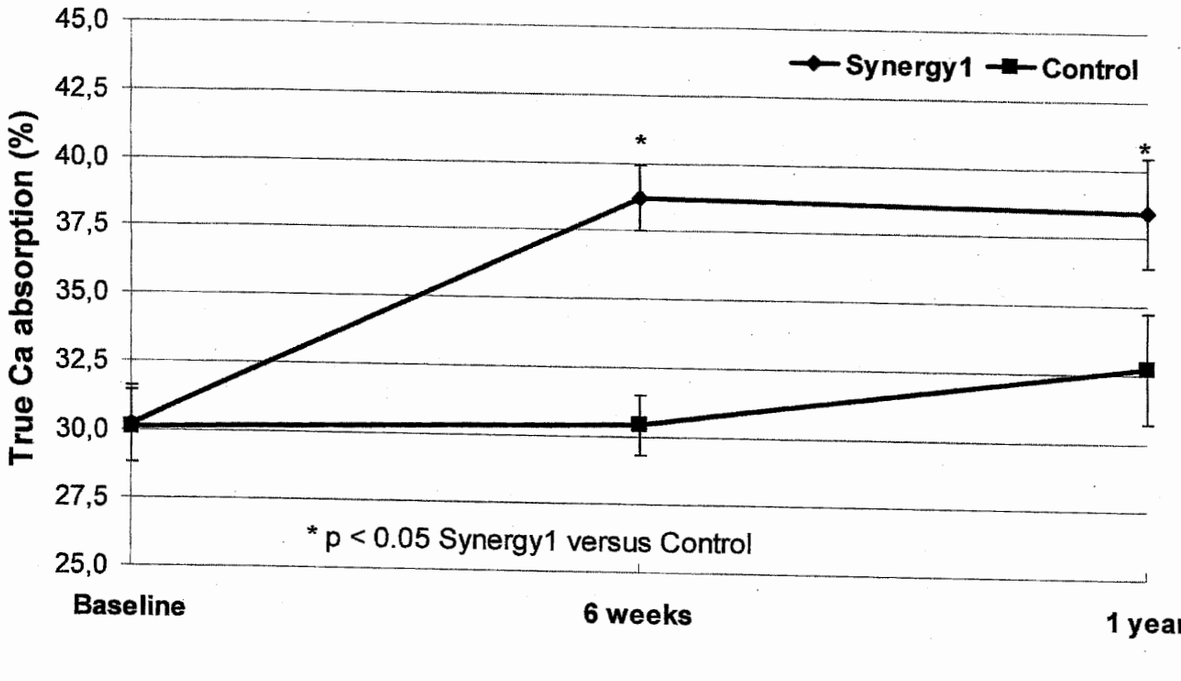
BENEO

# Breakthrough in Bone Health with Beneo™ Synergy1

## Change in true Calcium Absorption at 6-week and at 1-year intervention

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BENEO

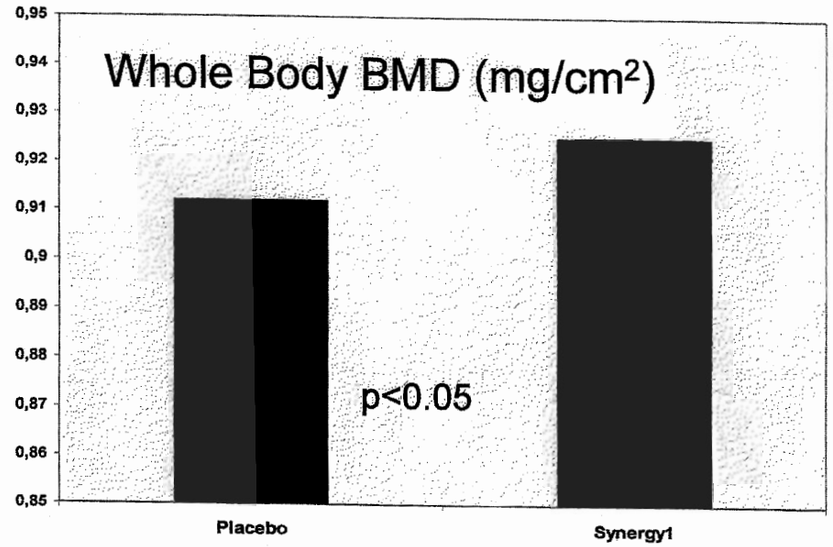
# Breakthrough in Bone Health with Beneo™Synergy1

Beneo™Synergy1 significantly increased Whole Body Bone Mineral Density (BMD) at 1-year intervention:

926 vs. 913 mg/cm$^2$ (p=0.03)

demonstrating a positive effect on bone mineralisation

BMD
measured by DXA

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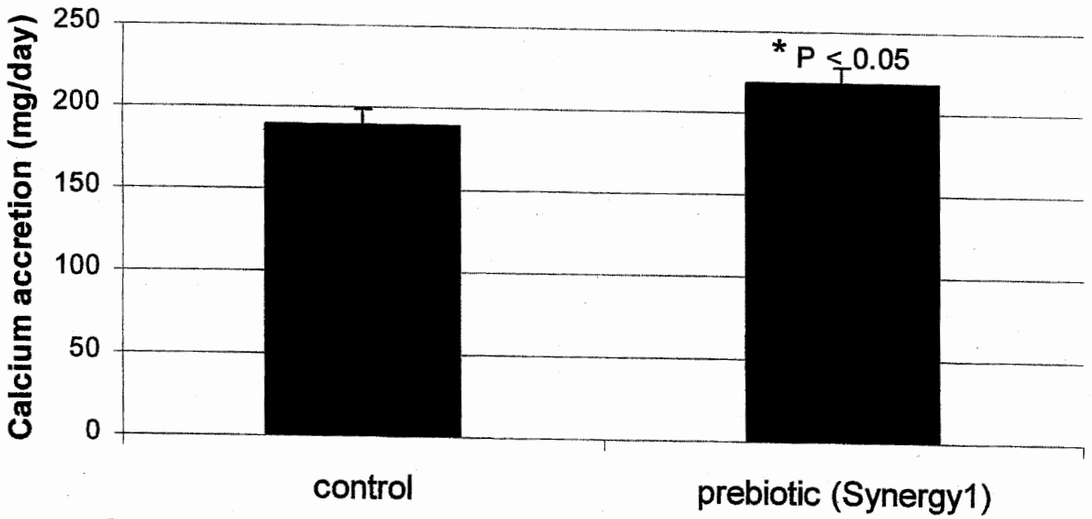
# Breakthrough in Bone Health with Beneo™ Synergy1

Beneo™ Synergy1 also significantly increased average Bone Calcium Accretion during 1 year, by about 15%: 218 vs. 189 mg/day (p=0.04)




Ca Accretion measured through the change in Whole Body Bone Mineral Content (BMC), assessed by DXA

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# Breakthrough in Bone Health with Beneo™ Synergy1

The 1-year changes in Whole Body BMC and BMD differed significantly between the Beneo™ Synergy1 group and the Placebo group, not only statistically but also physiologically:

Relative difference in Δ BMC: 17%

Relative difference in Δ BMD: 47%

| | Beneo™ Synergy1 | Placebo | p-value |
|---|---|---|---|
| Change in Whole Body BMC (g/year) | 245 | 210 | 0.03 |
| Change in Whole Body BMD ($g/cm^2$/year) | 0.047 | 0.032 | 0.01 |

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# Breakthrough in Bone Health with Beneo™ Synergy1

- Beneo™ Synergy1 had a significant beneficial effect on true Calcium Absorption at 6-week intervention
- The beneficial effect of Beneo™ Synergy1 on Calcium Absorption persisted at 1-year intervention
- Beneo™ Synergy1 increased Calcium Accretion after 1-year supplementation
- Beneo™ Synergy1 improved Whole Body Bone Mineral Content (BMC) and Bone Mineral Density (BMD) at 1-year intervention, in a physiologically relevant way

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# Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men

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**Objectives:** This study is aimed at investigating the effect of feeding a soluble or partly soluble fibre rich-diet on the apparent absorption and balance of calcium, magnesium, iron and zinc in healthy young men, by using a chemical balance technique.

**Study design:** Nine healthy young men were given a control diet or the same diet complemented with either inulin (soluble) or sugar beet fibre (partly soluble) during 28 d periods according to a 3 × 3 latin square design with three repetitions. During the 20 d adaptation period to fibre ingestion, experimental fibres were incorporated into bread (60%) and liquid foods (40%) up to a maximum of 40 g/d. Ca, Mg, Fe and Zn were measured in diets and in a 8 d urine and faecal composites to assess mineral absorption and balance.

**Results:** The dietary mineral intake provided (mg/d) 859 ± 196 of Ca; 311 ± 43 of Mg; 11.6 ± 1.7 of Fe; and 11.1 ± 1.6 of Zn from the control diet. The apparent absorption of minerals from the control diet was (%) Ca: 21.3 ± 12.5; Mg: 46.3 ± 10.9; Fe: 21.8 ± 12.3 and Zn: 14.0 ± 14.5 (mean ± s.d.). Ingestion of inulin significantly increased the apparent absorption and the balance of Ca. Sugar beet fibre ingestion resulted in a significant increase in Ca intake and balance, without modification its apparent absorption. Apparent absorption and balance of Mg, Fe and Zn were not significantly altered by the ingestion of either experimental fibre.

**Conclusions:** Addition of the two experimental fibres (inulin or sugar beet fibre) to normal mixed diets can improve Ca balance without adverse effects on other mineral retention.

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**Discriptors:** dietary fibres; inulin; sugar beet fibre; intake; absorption; balance; calcium; magnesium; iron; zinc; humans



## Introduction

Many degenerative diseases of Western society, such as atherosclerosis, diabetes, hypertension obesity and some cancers, are directly related to food intake (Jenkins *et al*, 1980; Kritchevsky & Tepper, 1995). There is now overwhelming evidence that dietary fibres are a necessary component of human and animal diets and plays a significant role in human health (Briel *et al*, 1995). According to the statistical data of the French National Institute of Economic studies, the average daily consumption of dietary fibres by French adults is about 16 g. Bagheri & Debry (1990) suggest that the recommended daily intake should be in the range of 30–40 g for French adults. However, dietary fibres and some associated substances, such as phytate, have strong *in vitro* mineral binding or complexing capacities (Persson *et al*, 1991). For this reason, dietary fibres have been suspected to impair mineral absorption. Within the last 20 y, several animal and human studies have shown that foods or diets rich in dietary fibres may alter mineral metabolism, especially when phytate is present (Sandstead *et al*, 1995). However, many studies have indicated that dietary fibres *per se* do not appear to affect trace element absorption (Sunvold *et al*, 1995). Recently, attention has been increasingly focused on fibre isolates, and more especially on fermentable poly- or oligosaccharides. Inulin, a fructo-oligosaccharide belonging to the fructan family, is currently used in various agro-food industries, and in dairy and cheese industries (Dysseler & Hoffman, 1995). In our laboratory, we showed that inulin enhanced Ca and Mg absorption in animal studies (Demigné *et al*, 1989; Levrat *et al*, 1991). This observation was recently confirmed by other workers also in animal studies (Delzenne *et al*, 1995; Ohta *et al*, 1995). Unfortunately, limited information is available for humans, and only indirect evidence that this effect could occur in humans has been reported (Trinidad *et al*, 1993, 1996). As part of a larger project concerning the effects of dietary fibres in human nutrition, we have studied the consequences for human physiology and nutrition of an increased intake of soluble fibre (inulin) in comparison to partly soluble (sugar beet) fibre. The criteria studied included stool characteristics, nutrient digestibility, energy metabolism (Castiglia-Delavaud *et al*, 1997) and mineral balance. The results of the apparent absorption and the balance of calcium, magnesium, iron and zinc are reported in this

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paper. To our knowledge, this is the first time that the effect of inulin on mineral absorption has been investigated in humans.

## Subjects and methods

*Experimental fibres*

Two types of dietary fibre were studied. Inulin, a polymer of fructose (15 units) with one unit of glucose, belonging to the fructan family, was extracted from Chicory roots. It is soluble, fermentable (Nilsson & Bjorck, 1998) and capable of retaining large quantities of water. The second was extracted from sugar beet and was composed of hemicellulose (27%), cellulose (23%), pectin (18%) and lignin (3%). Only the pectin component of the of the last fibre is soluble and the fermenticibility of this fibre is less and slower than that of inulin. Analysis of studied fibres showed that inulin contained negligible amounts of phytic acid (< 0.3 mg/g), whereas the level of phytic acid in beet fibre was as high as $15.9 \pm 0.3$ mg/g ($n = 6$). Both fibres were prepared by Agro Industries, Recherche et Developpment, (Compiegne, France). The work of Flourié *et al* (1985) showed that a supplementary intake of 40 g of fibre per day was the maximum tolerable quantity to the Western human organism. In the control diet, fibre intake was 18 g/d, whereas in the two experimental diets, the total fibre intake amounted to 58 g/d. Soluble or partly soluble fibres were added by incorporation into bread (24 g/d) and liquid foods (16 g/d). The experimental design was described previously in detail in Castiglia-Delavaud *et al* (1997). The main aspects dealing with mineral and trace element balance are presented below.

*Subjects and experimental design*

Nine male students ($21.5 \pm 2.5$ y; $174.9 \pm 8.6$ cm; $69.3 \pm 5.0$ kg) volunteered for this study. All subjects were considered to be in good health, completed a medical questionnaire, and underwent a medical examination by a physician. Exclusion criteria were digestive, hepatic, renal or cardiovascular diseases. The volunteers were fully informed of the aims and purposes of the study, and signed an informed consent. The protocol was approved by the local ethical committee (CCPPRB No. AV 38) of the Human Nutrition Research Centre at Clermont-Ferrand (France).

The volunteers were offered the three following diets according to a 3 × 3 Latin square design with three repetitions: a control diet (18 g dietary fibre per day); an inulin diet (18 g dietary fibres + 40 g inulin/d); and a sugar beet fibre diet (18 dietary fibre + 40 g sugar beet fibre/d). The study had a cross-over design, each subject acting as his own control for the two tested fibre-rich diets. The control diet was composed of four daily diets given alternatively to participating subjects. Meals were selected so that the mineral intakes provided by the control diet were comparable to intakes from self-selected diets consumed by different groups in industrialised countries. The meal composition and the conditions of fibre supplementation were previously described by Castiglia-Delavaud *et al* (1997). Briefly, each of the four daily diets were balanced and composed of some legumes, meat or fish, starchy foods or vegetable, cheese, fruit or compote plus biscuits. Each experimental period lasted for 28 d, and was composed of 2 d of control diet, 14 d of progressive increase in fibre intake, and 12 d of constant fibre consumption. Faeces and urine were collected between d20 and d28 and blood was sampled at d25 for the metabolic mineral balance study.

*Analytical methods*

Food intake was monitored by collection of duplicate meals. Composite samples of food were prepared using metal-free materials. Tubes and receptacles used for the mineral measurements were immersed in a 2% solution of EDTA for 24 h and then washed thoroughly with demineralized water. Acids of Suprapur® quality were used (Merck). Ca, Mg, Fe and Zn were determined in dietary fibres, tap water, diets, freeze-dried faeces and urine by atomic absorption spectrometry (Perkin Elmer 560 and 3030). About 0.5 g of fibres, diets or faeces were dry-ashed at 500°C for 10 h and the dry residue was taken up in HCl (6 M) and diluted to 50 ml and analysed for Ca and Mg. For Zn and Fe analysis, approximately 0.5 g of bread or diet, or 0.25 g of fibres or faeces were wet-ashed with a mixture of suprapur® acids (HNO3/HCl04) prior to analysis. Urine was analysed directly with or without dilution, in 0.1% lanthanum chloride solution. Plasma was diluted in 0.1% lanthanum chloride (1/50, v/v). Analytical quality was checked using total diet control standards (NIST 1548) for dietary, home built-human faeces for faecal, and Seronorm (Nycomed) for urinary and serum mineral measurements.

Phytic acid content in experimental fibres was determined by colorometric method at 500 nm, after an acid extraction of HCl 0.67 M as previously described (Latta & Eskin, 1980). Phytic acid from Sigma was used as external standard.

*Statistical analysis*

For each experimental treatment, the data are presented as means ± s.d. ($n = 9$). Data were analysed statistically according to a 3 × 3 Latin-square design with three repetitions. Comparisons between experimental treatments were done by analysis of variance using the General Linear Models Procedure (SAS, 1988) including diet, subject, period and repetition. The 'LS means' statement was used to calculate the adjusted means, and the 'contrast' statement to compare the three treatments. The results were considered significantly different when $P < 0.05$.

## Results

*Daily mineral intake*

Table 1 shows the content of Ca, Mg, Fe and Zn in both inulin and sugar beet fibres. As expected, sugar beet fibre was especially rich in Ca and Mg compared to inulin. Consequently, sugar beet fibre contributed 30%, 18%, 12% and 2.6% to daily intakes of Ca, Mg, Fe and Zn, respectively. In contrast, inulin contributed for only about 1% to daily intake of Fe and less than 0.25% to daily intake of any other tested minerals.

Subjects received four daily diets in rotation. Actual daily intake of minerals was determined from the analysis of these meals and the recording of non-consumed portions which were close to nil (Vernet & Vermoral, 1993). Tap water was also analysed for mineral content. The Table 1 shows the mineral intakes determined over an eight days period of consumption of control diet and tap water. With the control and inulin diets, the total daily mineral intake was 20% lower than the recommended allowances for Ca, Mg and Zn (RDAs, 1989). Addition of sugar beet fibre significantly increased Ca and Mg intakes ($P < 0.001$) to the recommended allowances.

**Table 1** Mineral daily intake provided by fibres and alimentation (mg/d)

| | *Ca* | *Mg* | *Fe* | *Zn* |
|---|---|---|---|---|
| *Experimental fibres* [a,b] | | | | |
| Inulin | 1.53 ± 0.06 | 0.46 ± 0.04 | 0.16; 0.12 | 0.03 ± 0.001 |
| Beet fibre | 324 ± 1.20 | 68.4 ± 0.38 | 1.33; 1.35 | 0.274 ± 0.02 |
| *Daily mineral intake from tap water*[c] | | | | |
| Control diet | 31.2 ± 13.2 | 12.1 ± 5.11 | < 0.005 | < 0.03 |
| Inulin diet | 30.1 ± 12.9 | 11.7 ± 4.99 | < 0.005 | < 0.03 |
| Beet fibre diet | 31.8 ± 11.5 | 12.3 ± 4.45 | < 0.005 | < 0.03 |
| *Daily total mineral intake*[c] | | | | |
| Control diet | 859 ± 196 | 311 ± 4.26 | 11.6 ± 1.70 | 11.1 ± 1.59 |
| Inulin diet | 852 ± 190 | 303 ± 41.7 | 11.5 ± 1.77 | 10.9 ± 1.61 |
| Beet fibre diet | 1229 ± 218* | 373 ± 49.6* | 12.3 ± 1.55 | 11.2 ± 1.56 |

[a]Each value is the mean and s.d. of 2–4 replicates.
[b]Calculated on the basis of 40 g of fibres per day.
[c]Each value is the mean and s.d. of 9 data.
*Significantly different from control or inulin diets ($P < 0.001$).

*Mineral apparent absorption*

As indicated in the Table 2, the mineral amounts apparently absorbed from the control diet (intake-faeces) were as follows 179 ± 115; 144 ± 38; 2.6 ± 1.6 and 1.6 ± 1.6 mg per day for Ca, Mg, Fe and Zn respectively. Consequently, the apparent mineral absorption averaged ((intake-faeces)/intake) × 100 averaged (%) 21.3 ± 12.5; 46.3 ± 10.9; 21.8 ± 12.3; and 14.0 ± 14.5 for Ca, Mg, Fe and Zn respectively with the control diet. With regard to the effects of inulin compared to the control diet, this soluble fibre significantly increased the absorption of Ca ($P < 0.01$). The apparent quantity of Ca absorbed from the inulin diet was thus increased significantly ($P < 0.05$). However, the apparent absorption of the other minerals was not significantly affected by inulin ingestion. In other respects, the absorption of all minerals studied was not significantly altered by sugar beet fibre ingestion. However, because sugar beet fibre is rich in Ca and Mg, the quantity of Ca apparently absorbed from the sugar beet fibre diet was significantly higher ($P < 0.05$) than that from control diet (Table 2). The same trend was observed for Mg without reaching a statistically significant level.

*Apparent mineral balance*

As shown in Table 2, the mean Ca balance was slightly negative (10 mg/d) in subjects consuming the control diet. However, there was a large inter-individual variability (from −274 to 141 mg/d), because urinary Ca excretion was highly variable between subjects (98–289 mg/d). In contrast, the Mg balance was largely positive (31.5 mgd) and the inter-individual variability (from −10 to 82 mg/d) was lower than for Ca. Iron balance was positive in all participating subjects (2.52 mg/d) and ranged from 0.55–5.91 mg/d. Urinary iron excretion was very low, with low inter-individual variability (45–73 µg/d). Zinc balance was also positive in most participating subjects and ranged from −1.97 to 2.78 mg/d. Urinary zinc excretion was from 649 to 1385 µg/d with no large inter-individual variability.

Ingestion of the soluble fibre (inulin) did not significantly alter urinary Ca excretion (Table 2). However, inulin ingestion resulted in a significant positive Ca balance (+91.8 mg/d) (compared to control diet, $P < 0.05$). This positive balance reflected the enhancement of Ca absorption due to inulin intake. However, inulin ingestion did not significantly alter urinary excretion of Mg, Fe and Zn, and

**Table 2** Effect of inulin and sugar beet fibres on absorbed quantity, apparent absorption, urinary excretion and balance of Ca, Mg, Fe and Zn in humans[a]

| | *Ca* | *Mg* | *Fe* | *Zn* |
|---|---|---|---|---|
| *Apparently absorbed quantities (mg/d)* | | | | |
| Control diet | 179 ± 115 | 144 ± 37.9 | 2.58 ± 1.64 | 1.58 ± 1.61 |
| Inulin diet | 278 ± 101* | 156 ± 46.5 | 1.83 ± 2.52 | 2.21 ± 2.00 |
| Beet fibre diet | 294 ± 92.4* | 169 ± 42.6 | 2.25 ± 0.99 | 1.59 ± 0.89 |
| *Apparent absorption (%)* | | | | |
| Control diet | 21.3 ± 12.5 | 46.3 ± 10.9 | 21.8 ± 12.3 | 14.0 ± 14.5 |
| Inulin diet | 33.7 ± 12.1** | 51.1 ± 12.3 | 14.0 ± 24.8 | 20.8 ± 20.0 |
| Beet fibre diet | 24.7 ± 9.40 | 45.0 ± 7.60 | 18.1 ± 7.20 | 14.1 ± 7.80 |
| *Urinary excretion (mg/d)* | | | | |
| Control diet | 189 ± 63.6 | 112 ± 28.4 | 0.057 ± 0.012 | 0.888 ± 0.267 |
| Inulin diet | 186 ± 85.0 | 121 ± 40.6 | 0.057 ± 0.023 | 0.883 ± 0.259 |
| Beet fibre diet | 198 ± 57.1 | 130 ± 23.6 | 0.045 ± 0.020 | 0.955 ± 0.231 |
| *Apparent balance (mg/d)* | | | | |
| Control diet | −10.1 ± 136 | 31.5 ± 27.5 | 2.52 ± 1.64 | 0.689 ± 1.59 |
| Inulin diet | 91.8 ± 115* | 35.2 ± 44.4 | 1.77 ± 2.51 | 1.32 ± 2.10 |
| Beet fibre diet | 96.1 ± 64.8* | 38.9 ± 27.5 | 2.21 ± 1.00 | 0.633 ± 0.95 |

[a]Each value is the mean and s.d. of 9 data.
*Significantly different from control diet ($P < 0.05$).
**Significantly different from control and sugar beet diets ($P < 0.01$).

only a trend to increase the Mg and Zn balance and to decrease in that of Fe were observed. Similarly, ingestion of the partly soluble fibre (sugar beet) did not significantly alter urinary excretion of Ca. However, sugar beet fibre ingestion resulted in a significantly positive Ca balance (96.1 mg/d) ($P < 0.05$ compared to control diet). This positive balance was the consequence of the high mineral content of sugar beet fibre which increased Ca intake by about 30% without modifying absorption efficiency. Finally, sugar beet fibre ingestion did not significantly alter urinary excretion of Mg, Fe and Zn or their balances.

Plasma Ca and Mg concentrations were determined in the subjects participating in the study towards the end of each test diet period. Plasma Ca was $2.36 \pm 0.134$, $2.36 \pm 0.121$, $2.34 \pm 0.113$ mmol/l; and plasma Mg was $8.07 \pm 0.395$, $8.07 \pm 0.712$, $7.94 \pm 0.580$ mmol/l prior to the control, inulin and sugar beet fibre periods, respectively. These results indicated that ingestion of either dietary fibres was without effect on the Ca and Mg status in our individuals. Plasma Fe and Zn determinations were not performed because both ingested fibres showed no effect on absorption or balance of these minerals.

## Discussion

The objective of this investigation was to measure, by the chemical balance technique, the effect of feeding soluble and rapid fermentable fibre (inulin), or partly soluble and slow fermentable fibre (sugar beet fibre) -enriched diets on apparent absorption and balance of Ca, Mg, Fe and Zn in healthy young men. Inulin is a polysaccharide degradable in the first half of large intestine (fermentability 100%) whereas the sugar beet fibre is degraded throughout the large intestine (less and slow fermentability 70%).

The intestinal absorption of Ca occurs via two processes: active Ca transport mainly in the duodenum and jejunum and passive transport mainly in the ileum. The active transport of Ca is mediated by calcitriol, the active component of vitamin D, but most of the Ca absorbed from food is passively transported into the intestinal mucosal cells. Availability for absorption requires Ca to be solubilised either in a free ionic or complexed form. Carbohydrates which are able to reach the ileum may stimulate Ca intestinal absorption in its part of digestive tract. In fact, the results of the present investigation indicate clearly that ingestion of an inulin-containing diet increased significantly the apparent absorption and balance of Ca in humans. This is the first time, to our knowledge, that such an effect has been observed in humans. These results confirm previously reported data from animal investigations in our own laboratory, (Demigné *et al*, 1989; Levrat *et al*, 1991; Younes *et al*, 1996), and by other workers (Delzenne *et al*, 1995; Ohta *et al*, 1995). Several hypothesis about the mechanisms of the effect of inulin on Ca absorption could be proposed. Carbohydrates that escape digestion in the small intestine are substrates for the formation of short-chain fatty acids (SCFA) in the large intestine by the intestinal microflora. This fermentation results in a lowering of the luminal pH which raises the concentration of ionised Ca and accelerates the passive diffusion of Ca (Rémésy *et al*, 1992). The accumulation of calcium phosphate in the large intestine and solubilisation of this Ca by organic acids (SCFA) probably plays an essential role in the enhancement of Ca absorption (Rémésy *et al*, 1993; Kashimura *et al*, 1996). It is also possible that SCFA directly influence Ca absorption by modifying various electrolyte exchanges (Ca-H). It has been proposed (Trinidad *et al*, 1993) that Ca could pass through the cell membrane more readily in the form of a complex with lower charge. However, a high rate of Ca absorption in the large intestine could trigger a feedback mechanism involving inhibition of duodenal absorption since there is a control of the digestive balance of Ca by endocrine factors (Allen, 1982).

The intestinal absorption of Mg has been studied less extensively than that of Ca. There are several mechanisms by which Mg can cross the intestinal epithelium: passive diffusion, solvent drug and active transport. However, several investigations in adults have documented that net absorption of Mg is linearly related to dietary Mg over a normal range of dietary Mg intake. This indicates that absorption of Mg in adults primarily occurs by a passive process. Mg is absorbed primarily in the distal segments of the gastrointestinal tract in humans, and carbohydrates that can stimulate bacterial fermentation in the intestine are known to increase Mg absorption. As animal studies showed similar effects of inulin ingestion on Ca and Mg absorption, the same effect of inulin on Mg absorption as that on Ca could be expected in human. The results from the present investigation showed, however, that inulin ingestion did not significantly affect the absorption and balance of Mg in humans under our experimental conditions. Inulin ingestion resulted only in a small increase in Mg absorption, accompanied with a slight increase in Mg urinary excretion without reaching a significant level. Mg balance was also slightly more positive than in the case of the control diet. Several explanations could be advanced for the difference observed in the effect of inulin on Mg absorption between rat and human. It is recognised that the digestive tract physiology of rat is different from that of human. The caecum segment, absent from human, plays indeed an important role in Ca and Mg absorption (Rayssiguier & Rémésy 1977). Although each experimental period in the present study lasted for 26 d, it is also possible that the effect of inulin on Mg absorption could be slower than that on Ca, and a longer adaptation period could be needed.

Fe and Zn are essential trace elements and are absorbed mainly in the small intestine by active and passive ways. Active transport takes place in the proximal part of intestine and is predominant when the element content is low. Passive diffusion can also take place when the element level is high in the digestive tract. The results of the present investigation did not show any significant effect of inulin ingestion on the apparent absorption and balance of these elements. Few studies in the literature have documented the effect of fermentable soluble fibre on the absorption and balance of Zn and Fe. Recently, Delazenne *et al* (1995) reported a significant increase in Fe and Zn absorption in rats fed with inulin (Raftiline) or its product of hydrolysis (known as Raflitose). Other dietary fermentable compounds, such as polymers of glucose and lactose, are reported to stimulate the intestinal absorption of Zn, but the mechanisms have not been clearly elucidated (Greger *et al*, 1989).

As far as the effect of sugar beet fibre is concerned, the first salient observation is its high content of minerals, in particular Ca and Mg. The Ca, provided by this fibre, increased the daily Ca intake by more than 30%, and that of Mg by about 20%. This confirms previous data concerning the high participation of some crude fibres in mineral intake (Van Dockkum *et al*, 1982; Behall *et al*, 1987; Fairweather-Tait *et al*, 1990). Furthermore, sugar

beet fibre addition provided until 450 mg of phytic acid per day, 250 mg from beet fibre added to soup and 200 mg from beet fibre added to breads, given that phytic acid is degraded at the level of 50% during baking (Tangkongchitr *et al*, 1981). The daily phytic acid intake averages up to 750 mg/d, but varies largely between countries from 200 mg/d in Sweden to 1000 mg/d in Italy (Reddy *et al*, 1989). In the present study, the sugar beet fibre diet exerted no significant effect on Ca absorption when compared to the control diet, but significantly increased the Ca balance. This positive balance was the result of the appropriate intake of Ca provided by the sugar beet fibre. One could not exclude that the non effect of sugar beet fibre on Ca absorption reported in our study could indeed be the result of two opposite phenomena. The first one is a decrease in Ca absorption due to the high intake of Ca in the sugar beet fibre diet. It is recognised that Ca absorption decreases when Ca dietary intake is increased (RDAs, 1989). The second is that the sugar beet fibre could increase Ca absorption in our subjects. The measured apparent absorption of Ca could be the result of these two opposite tendencies and thus not be significantly modified. No similar studies have been reported in relation to the effect of sugar beet fibre on Ca absorption either in animals or humans. However, there are numerous studies investigating the effects of cereal, vegetable and fruit fibres on the absorption of Ca in animals and humans. Most of them have reported no effect on Ca absorption or balance (Wisker *et al*, 1991; Davidsson *et al*, 1996). However, some studies described a decrease in Ca absorption with ingestion of dietary fibres under certain conditions (Knox *et al*, 1991; O'brien *et al*, 1993). It seems that differences in the chemical composition, and probably in the physical properties, of fibre sources and in other dietary constituents such as phytate and oxalic acid, may be responsible for these contradictory results.

Our data show that Mg absorption was not affected by sugar beet fibre ingestion when compared with control diet. Although sugar beet fibre ingestion significantly increased Mg intake, the absolute quantity of absorbed Mg (mg/d) was not significantly affected by sugar beet fibre intake in comparison to the control diet. Consequently, Mg balance was not altered by the sugar beet fibre diet compared to the control diet. We are not aware of any similar studies concerning the effect of sugar beet fibre on Mg absorption either in animals or humans. As for Ca, some studies have dealt with the effect of vegetable and fruit fibres on Mg absorption in animal and human. These studies reported that there were no differences in Mg balance with these fibres (Andersson *et al*, 1983). Moreover, Bagheri & Guégen (1985), demonstrated clearly that Mg from wheat bran was well utilized and bran addition improved considerably the balance of Mg. It is worth noting that fruits and vegetables remain an important source of both dietary fibres and minerals.

With regard to Fe and Zn absorption and balance, the present study did not show any significant effect of partly soluble dietary fibres on any of these minerals when compared with the control diet. In agreement with our results, Sandstrom *et al* (1987) reported that sugar beet pulp-fibre did not affect the extent of Zn absorption when used as a meat extender in humans. However, it was observed that Zn absorption was higher when sugar beet fibre was included in bread than when it was used as muesli. Cossack *et al* (1992) studied the effects of a sugar beet fibre supplement on Fe and Zn status in humans over five weeks. It was concluded that consumption of sugar beet fibre did not constitute any risk with respect to trace element nutriture. Interestingly, in an animal study, Fairweather-Tait *et al* (1990) reported that Fe and Zn absorption was significantly enhanced by sugar beet fibre intake, when Fe and Zn levels in the control diet were adjusted to those of the test diet. The effect of pectin on Zn retention had been tested in humans and found to be negligible (Lei *et al*, 1980). Finally in a similar study to ours, Behall *et al* (1987) found that refined fibres had no effect on mineral balance in subjects consuming recommended dietary allowance levels of Fe and Zn when fed as part of their control diet.

## Conclusions

Our investigations indicate that ingestion of inulin, a soluble fermentable fibre, significantly increases the apparent absorption of Ca and its balance in humans, probably by solubilizing this element in the colon. Moreover, sugar beet fibre, a partly soluble fibre, did not have any negative effect on any of the minerals studied in the present work. These results clearly demonstrate that sugar beet fibre ingestion in humans can improve the utilization of Ca and Mg and their balances. These two fibres can thus be added to normal mixed diets without adverse effects on mineral retention. It is clear that an intake of both soluble and partly soluble fibre, having different degrees of fermentability, ensure the fermentation in the whole large intestine. Dietary fibres or similar compounds could exert positives effects on mineral nutrition via the fermentation and the solubilisation of several minerals, or because they are very rich in minerals. In the case of inulin, which is not rich in minerals, its utilisation in the products containing high level of Ca, such as dairy products, could improve the digestibility of Ca by solubilising this element in the colon. This will enable appropriate nutritional recommendations to be made which will enhance mineral absorption. Additional studies are needed to evaluate the effect of combining inulin and sugar beet fibre and to study the effect of other naturally fructo-oligosaccharides in various foods on mineral balance.

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# Oligofructose stimulates calcium absorption in adolescents[1–3]

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## ABSTRACT

**Background:** In rats, nondigestible oligosaccharides stimulate calcium absorption. Recently, this effect was also found in human subjects.

**Objective:** The objective of the study was to investigate whether consumption of 15 g oligofructose/d stimulates calcium absorption in male adolescents.

**Design:** Twelve healthy, male adolescents aged 14–16 y received, for 9 d, 15 g oligofructose or sucrose (control treatment) daily over 3 main meals. The treatments were given according to a randomized, double-blind, crossover design, separated by a 19-d washout period. On the 8th day of each treatment period, $^{44}Ca$ was given orally with a standard breakfast containing ≈200 mg Ca. Within half an hour after administration of $^{44}Ca$, $^{48}Ca$ was administered intravenously. Fractional calcium absorption was computed from the enrichment of $^{44}Ca$:$^{43}Ca$ and $^{48}Ca$:$^{43}Ca$ in 36-h urine samples, which was measured by inductively coupled plasma mass spectrometry.

**Results:** An increase in true fractional calcium absorption (%) was found after consumption of oligofructose (mean difference ± SE of difference: 10.8 ± 5.6; $P < 0.05$, one sided). The results are discussed in relation to the methods used.

**Conclusion:** Fifteen grams of oligofructose per day stimulates fractional calcium absorption in male adolescents. *Am J Clin Nutr* 1999;69:544–8.

**KEY WORDS** Oligofructose, true calcium absorption, male adolescents, double stable-isotope technique, nondigestible oligosaccharides

## INTRODUCTION

Oligofructose is a mixture of oligosaccharides composed of fructose units linked together by β(2→1) linkages. Part of these molecules are terminated by a glucose. The total number of fructose or glucose units in an oligofructose molecule generally ranges between 2 and 8.

Like other nondigestible oligosaccharides (NDOs), oligofructose resists hydrolysis by human alimentary enzymes (1), but is fermented by colonic microbiota and induces a decrease in the pH of the human culture medium (2). Because of this cecocolonic fermentation, large amounts of short-chain fatty acids (SCFAs) are produced, which may cause a trophic effect on intestinal epithelium as well as on the triacylglycerol- and cholesterol-lowering effects of these NDOs (1). In addition, NDOs have been shown to improve mineral absorption in rats (3–5). In healthy, adult men (mean age: 22 y) a positive effect of 40 g of the NDO inulin daily on apparent calcium absorption was found by using the chemical balance technique (6). The positive effect found in rats (3–5) and humans (6) likely originates predominantly in the colon. Younes et al (7) showed that the large intestine is a major site of calcium absorption when acidic fermentation takes place. Contrary to the results of Coudray et al (6), we did not find an effect of 15 g/d inulin, oligofructose, or galactooligosaccharides on true calcium or iron absorption in adult men (mean age: 23 y) when we used stable-isotope techniques (8). This finding might have resulted because we used a lower NDO concentration than used by Coudray et al. However, 17 g NDO/d also had no effect on apparent calcium absorption in ileostomy subjects (mean age: 54 y) (9).

Therefore, it is also possible that our previous study (8) did not include the colonic component of calcium absorption because calcium absorption was calculated from the enrichment of both isotopes in 24-h urine samples: calcium absorption takes >24 h after isotope administration to be complete (10).

In addition, the stimulating effect of a more plausible dose of oligofructose on mineral absorption could be more pronounced in younger volunteers, whose calcium requirement is larger. Therefore, the aim of the present study was to investigate whether 15 g oligofructose/d stimulates true absorption of calcium in male adolescents aged 14–16 y. A supplement of 15 g NDO/d to the diet is feasible by using NDO-enriched products and raises substantially the total amount of NDOs in the diet (from ≈4 to 19 g/d) without bringing about symptoms of intolerance because the first symptoms of intolerance (eg, excessive flatus) are expected at intakes >30 g NDO/d (11). True fractional absorption was measured by using the double stable-isotope technique. Colonic calcium absorption was included by extending the collection of urine over 36 h instead of 24 h after isotope administration.

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## SUBJECTS AND METHODS

### Subjects

Subjects were recruited through an advertisement in a local newspaper. Twelve healthy boys were selected for the study. On the basis of our experience with adults, we calculated that with 12 subjects a difference of 5% true calcium absorption could be detected with a power of 90%. At the start of the study, the subjects were aged 14–16 y ($\bar{x}$: 15.3 y) and their body mass index (in kg/m$^2$) was between 16.0 and 20.7 ($\bar{x}$: 18.4). Normal health was assessed at prestudy screening, which included a medical history, a physical examination, measurements of blood pressure and heart rate, and routine clinical laboratory tests. The study protocol was approved by the TNO external Medical Ethics Committee and all subjects and their parents signed informed consent forms.

### Preparation of stable-isotope solutions

The double stable-isotope technique involves the administration of 2 stable isotopes, one orally and one intravenously. Taking the amount administered and the natural abundances of these stable isotopes into account, true fractional calcium absorption can be calculated from the enrichment of both stable isotopes in a urine sample (12). The isotopic labels were obtained from NEDRAY (Bunschoten, Netherlands) in the form of calcium carbonate. The abundances of the different isotopic labels, as determined by inductively coupled plasma mass spectrometry (ICPMS), were as follows: enriched $^{44}$Ca (3.39% $^{40}$Ca, 0.06% $^{42}$Ca, 0.03% $^{43}$Ca, 96.5% $^{44}$Ca, <0.01% $^{46}$Ca, and 0.02% $^{48}$Ca) and enriched $^{48}$Ca (8.96% $^{40}$Ca, 0.09% $^{42}$Ca, 0.02% $^{43}$Ca, 0.24% $^{44}$Ca, <0.01% $^{46}$Ca, and 90.69% $^{48}$Ca). One subject was given calcium enriched in $^{44}$Ca that was leftover from an earlier experiment, which had the following enrichment: 2.80% $^{40}$Ca, 0.05% $^{42}$Ca, 0.02% $^{43}$Ca, 97.1% $^{44}$Ca, <0.002% $^{46}$Ca, and 0.03% $^{48}$Ca. The $^{44}$Ca carbonate was converted into chloride salt, diluted with deionized water, and then adjusted to a pH of 5. A similar procedure was followed for $^{48}$Ca carbonate, except that saline was used instead of deionized water (13). After filtration, the solution was distributed into 10-mL injection bottles and sterilized for 25 min.

### Study design

The subjects were asked to maintain their normal food intake during the study as best as possible, but to restrict consumption of fiber-rich and oligosaccharide-containing food products. During the two 9-d treatment periods, subjects drank 100 mL orange juice containing 5 g oligofructose or the control treatment (fine-powdered sucrose) 3 times daily (at breakfast, lunch, and dinner). Oligofructose is obtained by partial enzymatic hydrolysis of inulin, which is prepared by hot-water extraction of chicory roots. Because the pure oligosaccharide content of the oligofructose-containing product (Raftilose P95; ORAFTI, Tienen, Belgium) was not 100% (**Table 1**), the weight of oligofructose was adjusted for a constant intake of 5 g pure oligofructose. Aspartame was added to the oligofructose-containing product to obtain the same sweetness as the control treatment. The treatments were given to the subjects according to a randomized, crossover design. This strictly controlled study was double blind.

During the first 7 d of each treatment period, the subjects consumed the study substances at home. On the last 2 d of each treatment period, the subjects were housed in the metabolic unit of the TNO Nutrition and Food Research Institute and calcium absorption was determined. During their stay at the institute, the diet was standardized and contained 1267 mg Ca and 12 MJ energy, of which 12% was from protein, 59% from carbohydrate, and 29% from fat, as estimated from the Dutch Food Composition Table (14).

**TABLE 1**
Composition of the oligofructose-containing product

| | |
|---|---|
| Oligofructose (% of dry matter) | 95 |
| Glucose:fructose:sucrose (% of dry matter) | 5 |
| Aspartame (%) | 0.35 |
| Dry matter (%) | ≥95 |
| Ash (% of dry matter) | <0.2 |
| Degree of polymerization (%) | 2–8[1] |

[1] Range.

For the calcium absorption test, the orange juice containing the study substance was extrinsically labeled with $^{44}$Ca and given to the subjects with a standard breakfast (with ≈200 mg Ca) on the 8th day of each treatment period after a 12-h overnight fast. No food or drinks were allowed, except for water, for 4 h after $^{44}$Ca administration. $^{48}$Ca was administered intravenously within 30 min after the oral administration of $^{44}$Ca. Before and after the bolus injection, blood pressure and heart rate were recorded for safety reasons.

The exact quantity of isotopes given by each route, as calculated by weighing the bottles or syringes before and after administration, was 14.0 mg (range: 13.0–15.5 mg) true $^{44}$Ca and 1.15 mg (range: 1.09–1.16 mg) true $^{48}$Ca. $^{44}$Ca:$^{43}$Ca and $^{48}$Ca:$^{43}$Ca values in urine collected before isotope administration (basal urine sample) and over the 36 h after administration were used to compute fractional calcium absorption according to the formula reported by van Dokkum et al (12).

### Stable-isotope analysis

$^{44}$Ca:$^{43}$Ca and $^{48}$Ca:$^{43}$Ca in basal and 36-h urine samples were measured by ICPMS (Elan 500; Perkin-Elmer Sciex,, Norwalk, CT). The accuracy of this method was evaluated by analyzing enriched urine samples with our inductively coupled plasma mass spectrometer and a thermal ionization mass spectrometer. Comparable results were found with both methods, as described by Luten et al (15).

All measurements were carried out in isotope-ratio peak hopping mode. ICPMS was operated in the high-resolution mode to provide maximal accuracy. Typical conditions for operations were as follows: plasma power 1.2 kW, reflected power <5 W, coolant argon flow rate 18 L/min, dwell time 20 ms, 1 measurement per peak, 10 repeats per integration, and total measuring time 270 s.

Trichloroacetic acid (3.5%) was added to the urine samples for deproteinization. The calcium was concentrated by precipitation of calcium with saturated ammonium oxalate and dissolution of the formed calcium oxalate into 1.2 mol HCl/L (15). The calcium concentration in the HCl solution was measured by atomic absorption spectrometry and, if necessary, adjusted by dilution to ≈10 mg Ca/L.

The prepared urine samples taken during each treatment, before and after isotope administration for the same subject, were tested within 1 d. Between each 4 urine samples, one standard solution of 10 mg Ca/L and one blank solution were measured. Mean $^{44}$Ca:$^{43}$Ca values of the standard solutions, measured within 1 d, ranged between 15.560% and 15.858% (CV:

0.26–0.77%). Mean $^{48}Ca$:$^{43}Ca$ values ranged between 1.463% and 1.587% (CV: 0.46–1.75%). All values were adjusted for minor deviations from standard calcium solutions with accepted natural ratios. All samples were measured in duplicate.

### Statistics

Statistically, the null hypothesis was that there would be no positive effect of oligofructose consumption on calcium absorption. Because the animal experiments and the study in humans of Coudray et al (6) indicated a positive effect of NDOs on calcium absorption and because our first human study (8) did not indicate a negative effect of oligofructose on mineral absorption, the alternative hypothesis was that there would be a positive effect of 15 g oligofructose/d on calcium absorption. The null hypothesis was to be rejected at the 0.05 level of probability (one sided). Because one sample was lost, the differences in calcium absorption between treatments were evaluated by using the general linear models procedure for an unbalanced analysis of variance (ANOVA) (16). ANOVA was used to be able to include the advantages of a crossover design.

## RESULTS

All subjects completed the study; however, the 36-h urine sample of subject 12, collected during the treatment with oligofructose, was lost. Compliance, as checked by returned test substances and questionnaires, was good. On day 6 of the first treatment period, 2 subjects forgot to drink their orange juice containing the study substance at dinnertime. Reports of gastrointestinal complaints were no higher after consumption of 15 g oligofructose/d than after the control treatment.

All samples were measured in duplicate. Within-duplicate CVs were 0.30% for $^{44}Ca$:$^{43}Ca$ and 0.82% for $^{48}Ca$:$^{43}Ca$. Because of small amounts of calcium in some basal urine samples, no reliable counts (outside the 95% upper limit of confidence) could be measured by ICPMS. In these cases, the mean basal ratio per treatment period was used. The average basal value for $^{44}Ca$:$^{43}Ca$ ($n = 20$) was 15.46% (CV: 0.22%) and for $^{48}Ca$:$^{43}Ca$ ($n = 19$) was 1.407% (CV: 1.70%). The average enrichment value ($n = 23$) was 4.8% (range: 2.9–8.2%) for urinary $^{44}Ca$:$^{43}Ca$ and 8.8% (range: 5.0–12.5%) for urinary $^{48}Ca$:$^{43}Ca$.

Mean basal and enriched $^{44}Ca$:$^{43}Ca$ and $^{48}Ca$:$^{43}Ca$ values and the mean percentage enrichment of these ratios by treatment are shown in **Table 2.** Mean (±SD) calcium absorption during the control treatment was 47.8 ± 16.4% ($n = 12$) and during the treatment with 15 g oligofructose/d ($n = 11$) was 60.1 ± 17.2%. Mean and individual changes in calcium absorption are shown in **Figure 1.** There was an increase in percentage calcium absorption (%) after consumption of oligofructose (mean difference ± SE of the difference: 10.8 ± 5.6; $P < 0.05$, one sided).

No relation was found between calcium absorption and the total amount of urinary calcium excreted in 36 h ($y = 0.09x + 137.9$; $r = 0.02$, NS). The total amount of calcium in the 36-h urine sample was 126 ± 87 and 160 ± 101 mg for the control and oligofructose treatments, respectively ($P = 0.06$, one sided). Also, no relation was found between the difference in calcium excretion and the difference in calcium absorption ($y = 0.06x + 2.7$; $r = 0.29$, NS).

**TABLE 2**
$^{44}Ca$:$^{43}Ca$ and $^{48}Ca$:$^{43}Ca$ and the percentage enrichment by treatment[1]

| | Control treatment ($n = 12$) | Oligofructose ($n = 11$) |
|---|---|---|
| $^{44}Ca$:$^{43}Ca$ | | |
| Basal | 15.47 ± 0.03 [9] | 15.45 ± 0.04 [10] |
| Enriched | 16.13 ± 0.18 | 16.28 ± 0.18[2] |
| $^{48}Ca$:$^{43}Ca$ | | |
| Basal | 1.40 ± 0.02 [9] | 1.41 ± 0.03 [10] |
| Enriched | 1.53 ± 0.03 | 1.54 ± 0.04 |
| Enrichment (%) | | |
| $^{44}Ca$:$^{43}Ca$ | 4.3 ± 1.2 | 5.4 ± 1.1[2] |
| $^{48}Ca$:$^{43}Ca$ | 8.9 ± 1.8 | 8.8 ± 2.1 |

[1] $\bar{x}$ ± SD; $n$ in brackets.
[2] Significantly different from control treatment, $P < 0.01$ (one sided).

## DISCUSSION

An increase in true fractional calcium absorption was found in male adolescents after consumption of oligofructose. In addition, the study in humans by Coudray et al (6) and experiments in rats (3, 4) showed a positive effect of NDOs on apparent calcium absorption. Contrary to these results, we did not find an effect of NDOs on true calcium absorption in an earlier study (8). This might have been because colonic calcium absorption was not considered in that study. Coudray et al's study (6) and most experiments in rats used the chemical balance technique to measure apparent calcium absorption. The advantage of the chemical balance technique is that it measures complete calcium absorption, including that from the colon. Absorption from the colon should be considered when investigating the effect of NDOs on calcium absorption, mainly because the large intestine may represent a major site of calcium absorption when acidic fermentation take place (7), but also because it is hypothesized that SCFAs, produced during fermentation of NDOs, improve calcium absorption through an exchange of intracellular $H^+$ for $Ca^{2+}$ in the distal colon (17). Experiments in rats have shown that fermentable oligosaccharides facilitate colorectal (18) and cecal absorption of calcium (3, 19). A disadvantage of the chemical balance technique, however, is that it does not distinguish

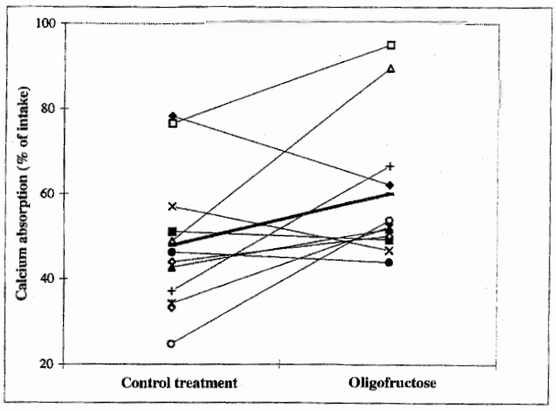


**FIGURE 1.** Individual changes in calcium absorption in male adolescents after consumption of 15 g oligofructose/d or a control substance. The thick black line represents the mean change in calcium absorption (mean difference ± SE of difference = 10.8 ± 5.6; $P < 0.05$, one sided).

between unabsorbed and endogenously secreted minerals, so true absorption cannot be determined. This was the main reason for using the double stable-isotope technique in the present study. In addition, we compared the results of the present study with those of our earlier study in humans (8). The measurement of colonic calcium absorption was included by extending the collection of urine from 24 h to 36 h after isotope administration.

One of the oldest approaches developed for the measurement of true fractional calcium absorption is determination of the ratio of isotopes excreted in complete urine collections. This method was used in our work with stable isotopes administered orally in a single meal (8, 12). Use of complete urine collections over a period of time instead of a single urine or blood sample gives better approximations of fractional calcium absorption (20, 21) because intravenously injected isotopes and absorbed orally administered isotopes do not necessarily arrive in the bloodstream at the same time or are metabolized in parallel. Measurement of isotopes in a complete 24-h urine sample largely reflects the ratio of the areas under the plasma disappearance curves for the 2 isotopes (20) and is therefore much less dependent on equal metabolic kinetics of the orally and intravenously administered isotopes. Measurement of calcium absorption, based on 24-h urinary excretion of isotopes, is a proven, valid method; therefore, there is no reason to doubt the validity of the method when urine collection is extended to 36 h to include colonic calcium absorption.

Nevertheless, we cannot exclude the possibility that the metabolic kinetics of calcium absorbed in the colon are different from those of calcium absorbed earlier in the duodenum or ileum or from that of the injected isotope because of the lag time between the entrance of these isotopes in the blood and the existence of a diurnal rhythm in the metabolism of calcium (22, 23). Such a bias would, however, not change the outcome of the present study because such a bias would exist with both treatments.

If the only difference between the earlier and the present study in humans had been the duration of urine collection, we have speculated that most of the enhancement of calcium absorption due to oligofructose takes place in the large intestine. However, there were other differences between the 2 studies: age, the duration of adaptation to oligofructose consumption, and the carrier dose of calcium given at breakfast with the stable isotopes.

Calcium absorption was not correlated with urinary calcium excretion, which is consistent with the results found in white girls and boys (24, 25). Apparently, during the period of bone development, absorbed calcium is largely taken up by the bone tissue so that no relation between absorption and urinary excretion becomes apparent. The oligofructose-induced enhancement of calcium absorption was not reflected by an increase in urinary calcium excretion. Moreover, the enrichment of $^{48}Ca$:$^{43}Ca$, and hence the excretion of injected calcium, was not affected by oligofructose. In rats, a positive effect of nondigestible carbohydrates on bone development was found (26–28). Therefore, oligofructose may help to maximize the peak bone mass in boys.

In conclusion, 15 g oligofructose/d increases calcium absorption in adolescent boys. More research is warranted to explore in which part of the intestine the oligofructose-induced enhancement takes place and whether it can improve calcium balance in humans.

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# Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes[†]



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Non-digestible oligosaccharides such as inulin and oligofructose have been shown to consistently increase calcium absorption in experimental animals, but data in humans are less clear-cut. The objective of this study was to assess the effect of 8 g/d of oligofructose or a mixture of inulin and oligofructose on calcium absorption in girls at or near menarche. A total of fifty-nine subjects were studied using a balanced, randomized, cross-over design. They received, in random order, 8 g/d placebo (sucrose), oligofructose or the mixture inulin+oligofructose for 3 weeks, separated by a 2-week washout period. Throughout the study, subjects consumed a total of approximately 1500 mg/d dietary calcium, by adding two glasses of calcium-fortified orange juice to their diet. Four grams of placebo, oligofructose or the mixture inulin+oligofructose was added to each glass of orange juice immediately before it was consumed. At the end of each 3-week adaptation period, calcium absorption was measured, using a dual stable isotope technique, from the cumulative fractional excretion of an oral and an intravenous tracer over 48 hours. Calcium absorption was significantly higher in the group receiving the inulin+oligofructose mixture than in the placebo group (38·2 ± 9·8 % *v.* 32·3 ± 9·8 %; $P=0·01$), but no significant difference was seen between the oligofructose group and the placebo group (31·8 ± 9·3 % *v.* 31·8 ± 10·0 %, $P$=NS). We conclude that modest intakes of an inulin+oligofructose mixture increases calcium absorption in girls at or near menarche.



**Calcium absorption: Non-digestible oligosaccharides: Oligofructose: Inulin: Prebiotics: Stable isotope**

## Introduction

Maintenance of an adequate calcium intake at or near puberty is essential for the development of optimal peak bone mass (Matkovic, 1992) An adequate calcium intake during this pivotal time period is vital for optimum bone mineral accretion (Chan, 1991; Nieves *et al.* 1995). Interventions aimed at preventing the morbidity and mortality associated with osteoporosis may, therefore, best be aimed at this vulnerable age group (Matkovic, 1992). Despite the importance of an adequate calcium intake during this period, self-selected diets of children during this stage typically provide insufficient calcium (Eck & Hackett-Renner, 1992), leading to inadequate calcium retention (Abrams & Stuff, 1994).

There has been increasing interest in the effect of prebiotic non-digestible oligosaccharides as modifiers of mineral absorption in animals (Brommage *et al.* 1993; Delzenne *et al.* 1995; Rémésy *et al.* 1993) and humans (Roberfroid, 1999; Van Loo *et al.* 1999). These compounds resist digestion by human alimentary enzymes, and undergo fermentation in the large intestine (Van Loo *et al.* 1999). It is speculated that the volatile short-chain fatty acids produced by this fermentation lower cecal pH, increase calcium concentration in the liquid phase of the cecal contents, and increase colonic absorption of calcium (Greger, 1999; Schulz *et al.* 1993). They may also have trophic effects in the gastrointestinal tract, which may increase calcium absorption either in the colon or throughout the entire gut (Chonan & Watanuki, 1995; Greger, 1999; Rémésy *et al.* 1993).

The most widely studied non-digestible oligosaccharides

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**Note:** For the definition of the terms inulin and oligofructose please refer to the introductory paper (p. S139) and its footnote.

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in humans are inulin and oligofructose, which are naturally occurring components of the Western diet (Van Loo *et al.* 1995; Moshfegh *et al.* 1999). Human studies examining their effect on calcium absorption have been contradictory. Two studies have shown a beneficial effect of 40 g/d inulin (Coudray *et al.* 1997) or 15 g/d oligofructose (van den Heuvel *et al.* 1999) on calcium absorption in young men and adolescent boys on modest calcium intakes. A third study, however, found no effect of 15 g/d of inulin, oligofructose or galacto-oligosaccharides on calcium absorption in a similar population (van den Heuvel *et al.* 1998). These discrepant findings may be due to methodological differences (Coudray & Fairweather-Tait, 1998) or due to a type II error, as all the studies were relatively small.

All three previous studies involved male subjects on moderate calcium intakes (approximately 800 mg/d), where calcium retention can be increased significantly by increasing calcium intake (Matkovic & Heaney, 1992). In children at or near puberty calcium retention appears to reach a maximum at an intake of about 1200 mg/d; calcium intakes above this threshold do not seem to lead to increased calcium retention (Matkovic & Heaney, 1992). It is not known whether non-digestible oligosaccharides will increase calcium absorption at higher levels of calcium intake. Further, because previous studies have examined the effect of relatively large doses of these ingredients on calcium absorption, it is unclear whether significant increases in calcium absorption can be achieved at lower intakes.

The object of this study was, therefore, to examine the effect of relatively modest intakes of oligofructose and a mixture of oligofructose and inulin, on calcium absorption in a large sample of girls at or near menarche, with calcium intakes approximating the currently recommended dietary intake.

## Materials and methods

Sixty healthy girls, 11·0–13·9 years of age, were recruited from the greater Houston area by public advertisement. Subjects were interviewed by a pediatric dietician prior to enrolment, and calcium intake was estimated using a food frequency questionnaire. Only subjects with a habitual calcium intake between 500 and 1400 mg/d were considered eligible for the study. Subjects were excluded from the study if they had chronic gastrointestinal disease, renal failure, or disorders of calcium homeostasis; were taking prescription medications (including oral contraceptives); smoked; or weighed more than the ninetieth percentile for age.

Subjects were studied using a randomized, double-blind, cross-over design. Subjects were randomized to receive two 4-g servings of non-digestible oligosaccharides daily for 3 weeks and two 4-g servings of placebo daily for 3 weeks, separated by a 2-week washout period. The oligosaccharides and placebo were given in random order, and investigators were blinded to the treatment assignment. Two similar protocols were carried out simultaneously. In Protocol I, the non-digestible oligosaccharide used was chicory oligofructose (Raftilose® P95, Orafti, Tienen, Belgium); Protocol II used an inulin+oligofructose mixture (Raftilose® Synergy1, Orafti, Tienen, Belgium). In both protocols, the placebo (sucrose) was packaged and presented in a manner that was identical to that of the oligosaccharides.

Subjects were instructed to maintain a calcium intake aimed at providing a total of approximately 1200–1300 mg/d during the study period by consuming an 8-ounce glass of calcium-fortified orange juice with breakfast and with the evening meal, and an 8-ounce glass of milk, calcium-fortified orange juice or a serving of yogurt with the midday meal. The placebo (sucrose), oligofructose or the inulin+oligofructose mixture were provided as packets containing 4 g of carbohydrates. One packet was added to both the morning and evening glasses of orange juice and gently mixed until it dissolved completely. The same dietary regime was continued during the 2-week washout period, but without oligosaccharides or placebo added to the glasses of orange juice. During the third week of each study period, subjects kept a weighed dietary record involving two weekdays and one weekend day. Calcium intake was calculated from these records by a pediatric dietician using the Nutrition Data System for Research (version 4.02, University of Minnesota, Minneapolis, MN)

At the end of each 3-week study period, the subjects were admitted to the Metabolic Research Unit of the Children's Nutrition Research Center. Calcium absorption was measured using a double stable isotope method. All used and unused packets of the placebo, oligofructose or the inulin+oligofructose mixture were returned, and compliance was measured by a packet count.

### *Measurement of calcium absorption*

Calcium absorption was measured using a modification of a previously validated dual-isotope methodology (Yergey *et al.* 1994; Yergey *et al.* 1990). On the morning of the study, subjects were admitted to the Metabolic Research Unit of the Children's Nutrition Research Center, and a baseline urine sample was collected. Subjects consumed a low-calcium breakfast and an 8-ounce glass of calcium-fortified orange juice to which was added one packet of placebo, oligofructose or the inulin+oligofructose mixture and 10 μg of $^{46}Ca$. The oligosaccharide was added immediately prior to consumption of the drink, and the $^{46}Ca$ 18–24 h earlier. Immediately after breakfast, 1·5 mg of $^{42}Ca$ was infused intravenously over 3–5 min. The midday meal contained approximately 400 mg calcium in the form of calcium-fortified orange juice, milk or yogurt. The evening meal contained another serving of calcium-fortified orange juice, 10 μg of $^{46}Ca$, and a packet of oligosaccharide or placebo. Immediately after the first dose of calcium isotopes was administered, a complete 48 h urine collection was begun. After the evening meal, subjects were discharged to their homes, where they completed the urine collection.

Stable isotopes were purchased from Oak Ridge National Laboratories (Oak Ridge, TN) as the carbonate salts. Aqueous solutions were prepared as previously described (Eastell *et al.* 1989) by the Pharmacy Department of Texas Children's Hospital, and tested for sterility

and pyrogenicity prior to use. All isotopes were dispensed in medicinal syringes which were weighed before and after use, and the exact dose of isotope given was calculated from the change in weight. A complete 48 h urine collection was started immediately following the infusion of $^{42}Ca$. Samples were purified using an oxalate precipitation method (Yergey *et al.* 1980) and isotope ratios measured by thermal ionization magnetic sector mass spectrometry (Finnigan, MAT 261 Bremen, Germany). Isotope ratios were expressed with regard to the non-administered isotope $^{43}Ca$ and corrected for fractionation using the ratio of $^{44}Ca$ to $^{43}Ca$. Repeated blocks of ten scans were performed until the desired degree of precision was obtained. Calcium absorption was measured from the ratio of the fractional excretion of the oral and intravenous isotopes in the 48 h urine collection, as described elsewhere (Eastell *et al.* 1989; Yergey *et al.* 1980).

### *Statistical analysis*

Based on previous studies, we had anticipated a mean calcium absorption of approximately 30 %, with a standard deviation of 8 %. We considered a 5 % difference in calcium absorption to be the smallest clinically significant difference, so thirty subjects were required to have a 90 % power ($\beta = 0{\cdot}10$) to detect this difference at a statistical significance of $P<0{\cdot}05$ ($\alpha = 0{\cdot}05$).

The effect of oligofructose or the inulin+oligofructose mixture on calcium absorption was assessed using paired *t*-tests, using StatView v4.5.1 for Macintosh (Abacus Concepts Inc., Berkeley, CA). Statistical significance was taken at $P<0{\cdot}05$ All data are presented as mean (standard deviation) unless otherwise stated.

### *Ethical considerations*

The study received ethical approval from the Institutional Review Board of Baylor College of Medicine. Informed written consent was obtained from the subjects (where age-appropriate) and their parents.

**Table 1.** Demographic data for the subjects in Protocol I (placebo *v.* oligofructose) and Protocol II (placebo *v.* Synergy1)

| | Protocol I | Protocol II | *P*-value‡ |
|---|---|---|---|
| Number | 30 | 29 | |
| Age (years)* | 12·1 (0·7) | 11·8 (0·8) | 0·12 |
| Weight (kg)* | 42 (9) | 46 (9) | 0·14 |
| Height (cm)* | 152 (8) | 154 (6) | 0·24 |
| Ethnicity (%)† | | | 0·08§ |
| African-American | 27 % | 7% | |
| Hispanic | 13 % | 28 % | |
| Caucasian | 57 % | 52 % | |
| Asian/others | 3 % | 14 % | |
| Pubertal status | | | 0·53§ |
| Premenarcheal (%) | 67 % | 59 % | |

* Mean (SD).
† Totals may not equal 100, due to rounding.
‡ Unpaired *t*-test, unless otherwise stated.
§ Chi-squared test.

## Results

Of the sixty subjects recruited, a total of fifty-nine completed the study. One subject in Protocol II defaulted from the second visit, and it was not possible to re-arrange her admission, so she was excluded from analysis. This was not related to intolerance of the study product. Demographics of the populations for the two protocols were similar (Table 1). The majority of subjects were Caucasian and premenarcheal.

### *Compliance with non-digestible oligosaccharide*

Compliance was good, with a mean of 95·4 % of the expected number of packets of the mixture inulin+oligofructose taken (SD 7·3 %, range 71 %–100 %, median 100 %), and 95·2 % of the expected number of packets of oligofructose taken (SD 7·9 %, range 68 %–100 %, median 100 %). All but four subjects achieved a compliance rate of at least 80 % of the expected number of packets of oligosaccharides (two in Protocol I, and two in Protocol II). There was no difference in compliance between oligofructose and of the inulin+oligofructose mixture (unpaired *t*-test, $P=0{\cdot}59$).

### *Calcium intake*

There were no significant differences in calcium intake between the placebo and the oligofructose or the inulin+oligofructose mixture phases of the study, either for Protocol I (oligofructose 1524 (265) mg/d *v.* placebo 1611 (326) mg/d; $P=0{\cdot}34$) or Protocol II (inulin+oligofructose mixture 1525 (282) mg/d *v.* placebo 1495 (280) mg/d; $P=0{\cdot}50$).

### *Calcium absorption*

In Protocol I, there was no significant difference in fractional calcium absorption on placebo (31·8 (9·3 %)) or on oligofructose (31·8 (10·0 %); $P=0{\cdot}75$). In Protocol II, calcium absorption on the inulin+oligofructose mixture was 38·2 (9·8 %), significantly greater than on placebo (32·3 (9·8 %), $P=0{\cdot}007$). Calcium absorption during the placebo period did not differ significantly between Protocol I and Protocol II ($P=0{\cdot}97$). The absolute calcium absorption (the product of fractional calcium absorption and dietary calcium intake) was significantly increased by the inulin+oligofructose mixture (575 (148) mg/d *v.* 485 (154) mg/d, $P=0{\cdot}004$), but not by oligofructose (489 (169) mg/d *v.* 490 (153) mg/d; $P=0{\cdot}99$), in comparison with placebo.

### *Urinary calcium excretion*

Calcium excretion during the 48 h study period was variable, and did not differ significantly between groups. In Protocol I, calcium excretion was 71 (48) mg/d during the placebo period and 79 (50) mg/d during the oligofructose period ($P=0{\cdot}26$). In Protocol II, the corresponding values were 65 (54) mg/d for placebo and 71 (50) mg/d for the inulin+oligofructose mixture ($P=0{\cdot}92$).

*Estimated calcium balance*

Net calcium balance was estimated by subtracting measured urinary calcium excretion and an estimate of endogenous fecal excretion, 1·4 mg/kg (Abrams *et al.* 1991) from the product of the fractional calcium absorption and the dietary calcium intake. This was similar during the sucrose and oligofructose periods of Protocol I (430·7 (153·4) mg/d *v.* 438·5 (169·0) mg/d; *P*-value=0·87). In Protocol II, estimated calcium balance was significantly greater during the inulin+oligofructose mixture period than the sucrose period (511·4 (151·4) mg/d *v.* 421·1 (154·0) mg/d; *P*-value=0·004).

## Discussion

In this study, we examined the effect of 21 days' adaptation to modest amounts of two non-digestible oligosaccharides, oligofructose and an inulin+oligofructose mixture, on calcium absorption in girls at or near puberty who were consuming a diet containing the recommended dietary allowance of calcium. Calcium absorption, measured using a dual stable isotope method, was significantly higher when subjects consumed 8 g/d of the inulin+oligofructose mixture than whilst consuming placebo. No significant benefit was seen from 8 g/d of oligofructose.

The dual stable isotope method of evaluating calcium absorption has been discussed in detail elsewhere (Yergey *et al.* 1990; Yergey *et al.* 1994); and a 24 h urine collection is adequate to allow accurate measurement of calcium absorption (Yergey *et al.* 1994). Indeed, in some populations, as brief a collection as 12 h may be adequate (Hillman *et al.* 1988). In normal circumstances, very little calcium absorption occurs in the colon (Hillman *et al.* 1988); however, oligosaccharides may significantly increase colonic absorption of calcium (Van Loo *et al.* 1999). It has been argued that a longer urine collection is required to capture this late, colonic, phase of absorption (Barger-Lux *et al.* 1989; van den Heuvel *et al.* 1998). For this reason, we extended the urine collection in our study to 48 h after administration of the intravenous tracer, or at least 36 h after administration of the second dose of oral calcium tracer.

Three studies had evaluated the effects of different oligosaccharides on calcium absorption in humans, with conflicting results. Coudray *et al.* (1997) showed in a study of nine men that 40 g/d of inulin significantly increased calcium absorption, measured by a metabolic balance, from 21·3 % (SD 12·5 %) to 33·7 % (SD 12·1). Van den Heuvel *et al.* (1998), however, found no effect of 15 g/d inulin, oligofructose or galacto-oligosaccharides on calcium absorption in young men, using a dual-isotope tracer method. One criticism of this study was that urine was only collected for 24 h, potentially missing the late colonic phase of absorption (Coudray & Fairweather-Tait, 1998). Indeed, a subsequent study by the same group (van den Heuvel *et al.* 1999), using a 36 h urine collection, showed that 15 g/d oligofructose significantly increased calcium absorption from 47·8 % (SD 16·4) to 60·1 % (SD 17·2). Our data show a significant increase in calcium absorption in response to the consumption of 8 g/d of an inulin+oligofructose mixture, but no beneficial effect of 8 g/d of oligofructose. This suggests that this mixture may be a more potent promoter of calcium absorption than oligofructose.

The present study differs from previous studies in a number of important aspects. All previous studies have been very small, involving only nine to twelve male subjects, and used rather high intakes of oligosaccharides (15–40 g/d) (Coudray *et al.* 1997; van den Heuvel *et al.* 1998; van den Heuvel *et al.* 1999). The dose of oligosaccharides used in the current study was only 8 g/d. This compares to a typical dietary intake of 2·6 g/d of inulin and 2·5 g/d of oligofructose in the Western diet (Moshfegh *et al.* 1999), and well below the amount of oligosaccharides that may cause abdominal symptoms (Briet *et al.* 1995).

Previous studies have evaluated subjects with calcium intakes in the order of 800 mg/d, well below the RDA for this age group of 1300 mg/d (Institute of Medicine Food and Nutrition Board's Standing Committee on the Scientific Evaluation of Dietary Intervals, 1997). Work by Matkovic & Heaney (1992) has suggested that during the stage of life approaching and at the achievement of puberty, net calcium balance increases with increasing calcium intake, to a maximum of about 1200 mg/d. Beyond this threshold, further increases in calcium intake do not improve calcium balance. Despite the fact that our subjects averaged total daily calcium intakes that achieved and even surpassed this threshold, a mixture of inulin+oligofructose significantly increased their absorption of calcium. It is not clear from our data whether the additional amount of absorbed calcium was utilized for bone mineral production; however, we did not find an increase in urinary calcium excretion that would have negated the increase in calcium absorption. The absolute increase in calcium absorption due to consumption of a mixture of inulin+oligofructose was approximately 90 mg/d, which is, clinically, highly significant. If even part of this additional calcium was utilized for bone mineral production, it could lead to a significant increase in peak bone mineral density during this critical period. Preliminary data from ovariectomized rats show that not only did galacto-oligosaccharides increase calcium absorption, but the additional calcium absorption resulted in an increased bone mineral mass (Chonan *et al.* 1995). If this was the case in our study population, the results could have significant public health implications.

The effects of non-digestible oligosaccharides on more sophisticated measures of calcium metabolism and on bone mineral accretion rates will require further study, and were not the purpose of this study. Our findings, however, show that regular intake of modest amounts of a mixture of inulin+oligofructose significantly increases calcium absorption in girls at or near menarche, with adequate or high calcium intakes, without any compensatory increase in urinary calcium excretion.

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# A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents[1–4]

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## ABSTRACT

**Background:** Short-term studies in adolescents have generally shown an enhancement of calcium absorption by inulin-type fructans (prebiotics). Results have been inconsistent; however, and no studies have been conducted to determine whether this effect persists with long-term use.

**Objective:** The objective was to assess the effects on calcium absorption and bone mineral accretion after 8 wk and 1 y of supplementation with an inulin-type fructan.

**Design:** Pubertal adolescents were randomly assigned to receive 8 g/d of a mixed short and long degree of polymerization inulin-type fructan product (fructan group) or maltodextrin placebo (control group). Bone mineral content and bone mineral density were measured before randomization and after 1 y. Calcium absorption was measured with the use of stable isotopes at baseline and 8 wk and 1 y after supplementation. Polymorphisms of the *FokI* vitamin D receptor gene were determined.

**Results:** Calcium absorption was significantly greater in the fructan group than in the control group at 8 wk (difference: 8.5 ± 1.6%; $P < 0.001$) and at 1 y (difference: 5.9 ± 2.8%; $P = 0.04$). An interaction with *FokI* genotype was present such that subjects with an *ff* genotype had the least initial response to fructan. After 1 y, the fructan group had a greater increment in both whole-body bone mineral content (difference: 35 ± 16 g; $P = 0.03$) and whole-body bone mineral density (difference: 0.015 ± 0.004 g/cm$^2$; $P = 0.01$) than did the control group.

**Conclusion:** Daily consumption of a combination of prebiotic short- and long-chain inulin-type fructans significantly increases calcium absorption and enhances bone mineralization during pubertal growth. Effects of dietary factors on calcium absorption may be modulated by genetic factors, including specific vitamin D receptor gene polymorphisms. *Am J Clin Nutr* 2005;82:471–6.

**KEY WORDS** Calcium absorption, vitamin D receptor, stable isotopes, inulin, prebiotics, pubertal growth, bioavailability

## INTRODUCTION

Absorption of an adequate amount of calcium is particularly important during early adolescence to help achieve peak bone mass. The current recommended daily intakes of calcium are largely based on dose-effect relations to maximize net calcium retention, which in adolescents is primarily determined by calcium absorption (1). In addition to dietary intake, intestinal absorption is a key factor that controls the retention of calcium. This is especially important, given the large disparity between recommended and typical intakes of calcium in adolescents.

Recent data have shown that prebiotic inulin-type fructans (ITFs) added to the daily diet significantly increase the absorption of both calcium and magnesium in growing animals and in adolescents. Numerous animal studies have shown that ITFs significantly increase calcium absorption (2) and bone mineralization (3). In humans, the most convincing data, up until now, have been obtained in adolescents (4–6) and in postmenopausal women (7, 8). These data suggest that a mixed short and long degree of polymerization (DP) fructan product is most effective for enhancing mineral absorption (2, 5, 6).

However, all of the reported studies in humans have been relatively short term and none have directly assessed the potential benefits of supplementation with ITFs on bone mineralization. It is important that such data be available in considering the inclusion of ITFs in the diet on a daily basis, as would occur with more widespread food fortification with ITF. We therefore evaluated the effects of a mixed short- and long-DP fructan on calcium absorption and bone mineralization in young adolescents. We further sought to evaluate the interactions of genetic factors in the response of calcium and bone mineral metabolism to ITFs.

[1] From the US Department of Agriculture/Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics (SAA, IJG, KMH, LL, and KJE); the Section of Endocrinology, Department of Pediatrics (SKG); and the Department of Pathology (GD), Baylor College of Medicine and Texas Children's Hospital, Houston, TX.

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## SUBJECTS AND METHODS

### Subjects

Through public advertising, we identified 50 girls and 50 boys for this study. All subjects were between 9.0 and 13.0 y of age and had a body mass index between the 5th and 95th percentiles for age and sex. The subjects were selected to approximately match the ethnic distribution of the greater Houston area. All subjects received a screening physical examination, which included Tanner staging. To be enrolled in the study, the subjects had to be healthy and have a Tanner stage of 2 or 3 (breast stage for girls and pubic hair stage for boys). Girls had to be premenarcheal. Subjects with any chronic illnesses requiring them to take medications regularly were ineligible for the study.

Written informed consent was obtained from a parent or legal guardian for each subject; written assent was obtained from all of the study subjects. The Institutional Review Board of Baylor College of Medicine and Affiliated Hospitals approved this protocol.

### Initial study visit

Within 8 wk of the screening visit described above, the subjects were admitted for 24 h to the General Clinical Research Center of Texas Children's Hospital in Houston, TX. During this stay, calcium absorption and bone mineralization were measured as described below. Blood was collected for DNA analysis of vitamin D receptor polymorphisms.

At the end of the baseline study, the subjects were randomly assigned in a double-blinded fashion and stratified by sex to 1 of 2 carbohydrate supplement groups: fructan group (8 g/d oligosaccharides of an inulin-type fructan, Raftilose Synergy1; Orafti NV, Tienen, Belgium) or control group (maltodextrin placebo). The ITF was a cospray dried 1:1 mixture of oligofructose (average DP:$DP_{av}$ = 4) and long-chain inulin ($DP_{av}$ = 25). Subjects were instructed to mix the carbohydrate supplement with 180–240 mL of calcium-fortified orange juice and to drink it with breakfast daily for 12 mo. Maltodextrin (Glucidex IT38; Roquette Freres, Lestrem, France) was chosen as the placebo control because, contrary to the ITF, it is completely digested in the upper intestinal tract and does not interfere with the metabolic activity of the colonic flora. Its sensory and other characteristics were virtually indistinguishable from those of the ITF; therefore, it served as a better control than did sucrose. To provide some dietary variation, the subjects were also allowed to use milk to mix the carbohydrate supplement. However, they were provided the orange juice free of charge. Dietary recalls and discussions with families showed that all subjects primarily used orange juice on >95% of the study days.

After 8 wk of receiving the carbohydrate supplement to which they had been randomly assigned, the subjects returned for a repeat calcium absorption study. Twelve months after the initial baseline study, the subjects returned for a follow-up visit during which time calcium absorption and bone mineralization were measured.

### Calcium absorption measurements

Stable-isotope studies were performed as previously described (9, 10). Most of the subjects received a breakfast that contained approximately one-third of their daily intake of calcium (including the tracer-containing juice). For subjects with low calcium intakes (<800 mg/d), the total breakfast represented a higher proportion of their total daily calcium intake (≤50%), consistent with their usual dietary practices. Subjects with higher usual intakes had other meal components (primarily dairy products) provide up to an additional 350 mg with their meal, depending on their usual intake. Toward the end of breakfast, the subjects were given 20 $\mu$g $^{46}$Ca, which had been mixed with 240 mL calcium-fortified orange juice. Different breakfast items were used to reflect the usual pattern of calcium intake of the subjects, but the calcium content of the isotope-containing meals was the same in each subject in all 3 studies.

After breakfast, $^{42}$Ca (1.2 mg) was infused over 2 min via a heparin-lock catheter. Beginning with breakfast, a complete 24-h urine collection was obtained. Subsequently, subjects collected a second 24-h urine collection at home after discharge from the General Clinical Research Center (6). Calcium absorption was calculated from the relative recovery of the oral and the intravenous tracers during the entire 48-h study period. A 48-h time period was chosen because of evidence that ITFs may increase the absorption of calcium in the large intestine. This would necessitate a longer collection period than the 24-h time period usually used in such studies to fully identify an effect (5, 11, 12). The subjects were required to note any lost urine or failure to collect a urine sample at home.

### Dietary methods

At the screening visit, the study dietitian asked the subjects what foods they usually ate on a normal day to determine food preferences. Inpatient menus for the overnight study visit were based on normal calcium intake (13). All foods and beverages used during the inpatient and outpatient visits were weighed before and after intake to accurately determine intake. The subjects were instructed to keep weighed food records for 6 d during the study: a 2-d period after the first overnight visit, a 2-d period 2 mo later, and a 2-d period after the 1-y visit. The subjects were called at home during the 1-y period to obtain a 24-h dietary recall of the previous day's intake and to ensure that the subject maintained a relatively consistent calcium intake. To reflect the marketplace changes in dietary food contents during the study, dietary intake data were collected with the use of the NUTRITION DATA SYSTEM FOR RESEARCH software (versions 4.03 and 4.05; Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

To monitor compliance, subjects were provided with a calendar and instructed to put a sticker on the calendar for every day that they remembered to drink the juice with the supplement. They were instructed to keep all supplement packets after they had mixed them into the orange juice and return them to the research center every 3 mo along with their calendars and any unused supplement packets. The study staff counted compliant days and the number of supplement packets consumed. Subjects were mailed a small gift if they returned their calendars and packets in a timely fashion. If subjects chose to consume the supplement with milk, they were required to indicate this in writing on the calendar.

### Analytic methods

Urine samples were prepared for thermal ionization mass spectrometric analysis as previously described by using an oxalate precipitation technique (9, 10). Samples were analyzed for

isotopic enrichment with a magnetic sector thermal ionization mass spectrometer (model MAT 261; Finnigan Bremen, Germany). The accuracy and precision of this technique for natural-abundance samples compared with standard data are 0.15%.

### Dual-energy X-ray absorptiometry

Bone mineralization measurements were performed by using a Hologic QDR-4500A dual-energy X-ray (DXA) absorptiometer (Hologic, Inc, Waltham, MA). The whole body was scanned in the fan-beam mode. Whole-body bone mineral content (BMC) and whole-body areal bone mineral density (BMD) were measured. Whole-body BMD precision was <1%, whereas whole-body BMC precision was <1.5% (14, 15).

### Genetic methods

Genomic DNA was isolated from 3 mL whole blood collected in EDTA-coated tubes with the use of the Wizard TM Genomic DNA Purification Kit (Promega, Madison, WI). The DNA was analyzed for *FokI* genetic polymorphisms by the Gene Expression Core of the Texas Coast Digestive Disease Center. The VDR receptor phenotypes (*FokI*) were analyzed as previously described (16). Primer sequences for *FokI* were obtained from Mark Johnson, Creighton University School of Medicine, Omaha, NE.

### Statistical methods

Comparisons of carbohydrate supplement and genotype groups for fractional absorption of calcium were made by using analysis of variance and analysis of covariance (ANCOVA) techniques, in which changes over the time course of the study were determined by repeated-measures ANCOVA with subsequent determination of differences at specific measurement time points by ordinary ANCOVA. Covariate adjustments were based on the specific analysis conducted. Sex, ethnicity, and Tanner stage at enrollment were included as covariates in models of calcium absorption. These analyses were implemented by using the general linear models (univariate and repeated-measures options) provided in SPSS 13.0 for WINDOWS (SPSS Inc, Chicago, IL). In addition, the proportion of responders with an increase in calcium absorption of ≥3% after 8 wk of the ITF treatment was determined by using the multiple logistic regression option of this program. All data are presented as means ± SEMs.

Sample size was determined on the basis of our earlier study, in which we found a 6% change (SD: 9%) in fractional calcium absorption in girls after adding an ITF to their diet for 3 wk. Therefore, enrollment of 80 subjects had a power >0.9 ($P < 0.05$) to identify this difference. We enrolled 100 subjects (50 of each sex) based on a 20% dropout rate by 1 y, whereas ultimately only 8% of the subjects failed to complete all aspects of the study.

We have separately reported the relation between vitamin D receptor polymorphisms, including the *FokI* gene, and calcium absorption and bone mineralization (17). Because we found a significant *FokI* genotype–related effect on calcium absorption and bone mineralization, *FokI* genotype was used as a covariate in evaluating the effects of the carbohydrate supplement on calcium absorption and bone mineralization.

**TABLE 1**
Anthropometric characteristics of the children at baseline[1]

| | Fructan group ($n = 48$) | Control group ($n = 50$) | $P^2$ |
|---|---|---|---|
| Age (y) | 11.8 ± 0.2[3] | 11.4 ± 0.2 | 0.10 |
| Height (cm) | 148.9 ± 1.3 | 148.3 ± 1.2 | 0.74 |
| Weight (kg) | 42.7 ± 1.3 | 41.4 ± 1.4 | 0.48 |
| Tanner stage 2 (%)[4] | 73 | 76 | 0.73 |

[1] Children were randomly assigned to receive 8 g/d of either inulin-type fructan or maltodextrin placebo (control).
[2] ANOVA.
[3] $\bar{x}$ ± SEM (all such values).
[4] All subjects were either Tanner stage 2 or Tanner stage 3.

## RESULTS

### Subject and dietary description

A total of 100 subjects met the study criteria and were randomly assigned to the fructan or the control group; 98 subjects completed the baseline and 8-wk absorption studies. Both dropouts were from the fructan group, which left 48 subjects in the fructan group and 50 in the control group. One subject dropped out because of a failure to tolerate the ITF (increased stool frequency and diarrhea), and the other subject dropped out because of noncompliance with the study procedures, which was unrelated to the carbohydrate assignment. All other subjects tolerated the study protocol well. Three additional subjects (all in the control group) dropped out between 8 wk and 1 y for personal reasons that were unrelated to the group assignment. At 1 y, 3 additional subjects were unable to complete the absorption studies, but did complete the bone mineral measurements. Thus, the total sample number at 1 y was 95 for the bone mineral measurements and 92 for the calcium absorption measurements.

Anthropometric characteristics of the study subjects are shown in **Table 1**. The mean (±SEM) age of the subjects at the start of the study was 11.6 ± 0.1 y. The fructan group consisted of 24 whites, 5 African Americans, 11 Hispanics, and 8 Asians; the control group consisted of the 28 whites, 9 African Americans, 11 Hispanics, and 2 Asians. Compliance with daily carbohydrate supplementation was not significantly different between groups (84% in the fructan group and 81% in the control group). There was no significant relation between fractional absorption and compliance at any time period.

Total urinary calcium at the 3 time points was compared. Mean (±SEM) urinary calcium was 81 ± 7 mg/d at baseline, 78 ± 5 mg/d at 8 wk, and 87 ± 6 mg/d at 1 y ($P = 0.10$, repeated-measures analysis of variance). These results suggest no differences in the completeness of the urine samples collected at home and those collected while the subjects were inpatients. There were no differences in urinary calcium between the fructan and control groups at baseline or any time point ($P > 0.2$ at each time point after correction for ethnicity, sex, and Tanner stage).

Calcium intake was maintained throughout the study at the subject's usual intake, and there were no significant differences in calcium intake between the carbohydrate supplement groups. The mean (±SEM) calcium intake at baseline was 907 ± 33 mg/d, at 8 wk was 959 ± 33 mg/d, and at 1 y was 906 ± 29 mg/d.

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**TABLE 2**
Calcium absorption in the children at baseline, 8 wk, and 1 y[1]

| Time of study | Fructan group | Control group | *P* |
|---|---|---|---|
| | % | % | |
| Baseline | 29.9 ± 1.4 [48][2] | 29.4 ± 1.5 [50] | 0.76 |
| 8 wk[3] | 38.5 ± 1.2 [48] | 30.0 ± 1.3 [50] | <0.001 |
| 1 y[3] | 37.7 ± 2.1 [47] | 31.7 ± 2.3 [45] | 0.04 |

[1] Children were randomly assigned to receive 8 g/d of either insulin-type fructan or maltodextrin placebo (control). All values were adjusted for genotype, ethnicity, Tanner stage at enrollment, sex, and calcium intake. Repeated-measures analysis of covariance showed a significant interaction of time of measurement and use of fructan ($P = 0.04$). Subsequent comparisons were made by using ordinary analysis of covariance.

[2] $\bar{x}$ ± SEM; *n* in brackets (all such values).

[3] Adjusted for calcium absorption at baseline.

**TABLE 3**
Effect of genotype on calcium absorption in children at 8 wk and 1 y[1]

| *Fok1* genotype | Fructan group | Control group | *P* |
|---|---|---|---|
| 8 wk | | | |
| *FF* | 45.6 ± 1.7 [12][2] | 33.2 ± 1.3 [22] | <0.001 |
| *Ff* | 37.3 ± 1.7 [26] | 30.2 ± 2.0 [20] | 0.02 |
| *ff* | 31.9 ± 1.6 [10] | 28.5 ± 1.8 [8] | 0.22 |
| 1 y | | | |
| *FF* | 44.9 ± 4.5 [11] | 35.8 ± 3.3 [20] | — |
| *Ff* | 38.9 ± 2.5 [26] | 30.6 ± 3.1 [18] | — |
| *ff* | 31.0 ± 3.5 [10] | 32.1 ± 4.2 [7] | — |

[1] Children were randomly assigned to receive 8 g/d of either inulin-type fructan or maltodextrin placebo (control). All values were adjusted for baseline absorption, calcium intake at baseline and 8 wk or 1 y, Tanner stage, sex, and ethnicity. Repeated-measures analysis of covariance showed a significant genotype by carbohydrate treatment by time of measurement interaction ($P = 0.04$). Subsequent pairwise comparisons of genotypes were made by using ordinary analysis of covariance. The interaction of genotype with carbohydrate supplementation was significant at 8 wk ($P = 0.03$) but not at 1 y ($P = 0.43$).

[2] $\bar{x}$ ± SEM; *n* in brackets (all such values).

### Calcium absorption at 8 wk and 1 y

The effects of the fructan and control groups on the fractional absorption of calcium during the study year were compared by repeated-measures ANCOVA at the baseline, 8- wk, and 1-y time points ($n = 92$). After adjustment for ethnicity ($P = 0.04$), sex ($P = 0.54$), *Fok1* genotype ($P = 0.007$), calcium intake at each visit ($P = 0.03$ at baseline; $P > 0.1$ at 8 w and 1 y), and Tanner stage at enrollment ($P = 0.15$), the effect of fructan on the fractional absorption of calcium was significant ($P = 0.02$). The interaction of time point of measurement and carbohydrate group was significant ($P < 0.01$).

We further evaluated the results for calcium absorption at 8 wk and 1 y relative to the baseline absorption values by ordinary ANCOVA (**Table 2**). After adjustment for baseline values and other covariates, calcium absorption was significantly greater at 8 wk (difference 8.5 ± 1.6%, $P < 0.001$) and 1 y (difference 5.9 ± 2.8%, $P = 0.04$) in the fructan group than in the control group.

Inclusion of the 25-hydroxyvitamin D concentration in the model had no effect on the relation between carbohydrate groups and calcium absorption. The 25-hydroxyvitamin D concentration was not significantly related to calcium absorption at 8 wk ($P = 0.83$) or at 1 y ($P = 0.51$).

### Effects of genotype on results at 8 wk and 1 y

We previously showed a significant effect of *Fok1* genotype on calcium absorption (16, 17). Therefore, we sought to identify whether there was a nutrient-gene interaction by evaluating whether fractional calcium absorption at 8 wk and 1 y was related to an interaction of genotype with carbohydrate supplementation. The three-factor interaction of genotype with carbohydrate supplementation and time point of measurement was significant ($P = 0.04$). Additionally, the interaction of genotype with carbohydrate supplementation was significant at 8 wk ($P = 0.03$) but not at 1 y ($P = 0.43$). We analyzed each genotype group at 8 wk and 1 y for the effects of the carbohydrate supplement, and the results indicate a preferential effect of fructan in the subjects with genotypes associated with higher calcium absorption (*FF* and *Ff*) at 8 wk (**Table 3**).

Additionally, we determined the proportion of individuals who were "responders" to the carbohydrate supplement. In this analysis, we chose an increase of 3% to represent a responder a priori. This evaluation was only done at the 8-wk time period because there was a mean 2.3% increase in calcium absorption in the control group compared with baseline at 1 y, which would have made the 3% definition of a responder difficult to interpret. The interaction of carbohydrate supplement group with *Fok1* genotype was significant in determining responders at 8 wk ($P = 0.01$) with sex, Tanner stage, and ethnicity as covariates. The percentage of responders by genotype is shown in **Table 4**. Overall, 67% (32/48) of the fructan group and 34% (17/50) of the control group were responders ($P = 0.004$).

### Bone mineralization results

Comparisons of groups for changes during the study year in whole-body BMC and BMD are shown in **Table 5**. After 1 y, fructan resulted in a greater increase in whole-body BMC (35 ± 16 g; $P = 0.03$) and BMD (0.015 ± 0.004 g/cm$^2$; $P = 0.01$) than did the control treatment. To calculate the approximate effect of this difference in whole body BMC on daily calcium accretion, we used a factor of 0.322 for the fraction of calcium per mg of BMC (14). This leads to a calculation of an average net difference of 30 mg/d in calcium accretion between groups.

**TABLE 4**
Percentage of children with an increase in calcium absorption of ≥3% after 8 wk of treatment[1]

| *Fok1* genotype | Fructan group | Control group | *P* |
|---|---|---|---|
| | % | % | |
| *FF* | 92 [12] | 18 [22] | 0.002 |
| *Ff* | 62 [26] | 40 [20] | 0.07 |
| *ff* | 50 [10] | 63 [8] | 0.53 |

[1] *n* in brackets. Children were randomly assigned to receive 8 g/d of either inulin-type fructan or maltodextrin placebo (control). All values were adjusted for sex, Tanner stage, and ethnicity. In the initial 2-factor multiple logistic regression, the interaction of genotype by carbohydrate treatment was significant ($P = 0.01$). Subsequent pairwise comparisons of genotypes were made by using a single-factor logistic regression.

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their being chosen by consumers. Furthermore, we have shown that a benefit of ITF on calcium absorption exists across a range of calcium intakes (5), which indicates that consumers have several strategies to choose from to enhance their calcium status.

We conclude that the daily inclusion of a modest amount of a commercially available nonabsorbable ITF with a mixture of short and long DP enhances calcium absorption and bone mineralization in pubertal adolescents. Genetic interactions, however, may modulate this affect.

We acknowledge the assistance of the nursing staff of the General Clinical Research Center of Texas Children's Hospital for caring for the study subjects; Cynthia Edwards for study recruitment; Holly Endris, Angela Freeman, Melissa Knox, Courtney Edwards, Lora Plumlee, Yana Kriseman, Rachel Wolfson, Michelle Lopez, and Anh Mai for subject assistance; and E O'Brian Smith for statistical advice.

SAA was responsible for the overall conduct of the study. IJG was responsible for the daily supervision of the study and Tanner staging of the boys. SKG was responsible for the medical management of the subjects during the study and the Tanner staging of the girls. KMH supervised all dietary aspects of the study. LL was responsible for the protocol design and the laboratory analysis in the stable-isotope studies. GD was responsible for the protocol design, the determination of the *FokI* genotype, and the interpretation of the genetic aspects of the study. KJE was responsible for all bone mineralization measurements. All authors were involved in the preparation of the manuscript for publication. SAA is a consultant for The Coca-Cola Company (member of the Beverage Institute for Health and Wellness). None of the other authors had a conflict of interest.

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# Appendix 2

## Petition to add to the National List the substance "Oligofructose enriched with Inulin Documented for Calcium Absorption"

**January 12, 2007**

# The production process



Chicory Roots

Washing, slicing

Hot water extraction

Filtration, purification

Raw inulin

Natural recycle of co-products for Agriculture & Cattlefeed

Processing for Oligofructose

Processing for high soluble inulin

Short Chain Removal

Purification, Filtration

Spray drying

L60 L85 L95* P95* P85 Synergy1*
Beneo™ oligofructose Family

HSI

ST, ST-Gel*, GR
Beneo™ inulin

HP, HP-Gel, HPX
Beneo™ inulin

*Patented or Patents pending

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BENEO

# Non-digestible oligosaccharides and bifidobacteria - implications for health

By Glenn R. Gibson*, Caroline L. Willis* and Jan Van Loo**

Glenn R. Gibson

Caroline L. Willis

Jan Van Loo

### *Introduction*

Inulin and oligofructose belong to the group of carbohydrates known as non-digestible oligosaccharides (NDO), found commonly in a range of foodstuffs in the standard Western diet[1] (Table I). Both are currently receiving interest as important food ingredients. It is has been shown that these sugars have a much reduced calorific value when compared to sucrose[2] and are suitable for intake by diabetics[3] (they do not influence serum glucose levels nor stimulate the secretion of insulin). The may also have dietary fibre-like properties in that they cause decreased intestinal transit, reduce blood cholesterol and stool pH but increase stool weight[4].

A further factor which is highly important for health relies on their effect on the composition of the mass of bacteria that normally reside in the human colon. These particular oligosaccharides seem to be preferentially utilised by bifidobacteria in the large intestine, causing elevated growth of this potentially salutary bacterial genus. This article will review some of the evidence for this observation and discuss the relevance in terms of human health.

### *Inulin and oligofructose*

Structures of these carbohydrates are shown in Fig. 1. Chemically, both are β-D- fructans with degrees of polymerisation (DP) varying between 2 - 60 (inulin) and 2 - 20 (oligofructose). Basically, oligofructose is a subset of of inulin. Inulin can exist as a variably branching form, dependent on its plant of origin. For example, chicory inulin is 1 - 2% β (2-6) branched with short side chains. Inulin may also contain small amounts of fructan side chains without a glucose moeity[5].

The synthesis of oligofructose and inulin in plant cells starts by the transfer of a fructosyl moiety between two sucrose molecules. The β (2-1) osidic bond, including the first glucose-fructose linkage, is not hydrolysed to a significant extent by human digestive enzymes[6]. This means that the majority of these carbohydrates present in food can enter the colon relatively intact where they are then susceptible to metabolism by bacteria, thus appropiating their classification as NDO.

**Table I. Inulin and oligofructose content of various foodstuffs**

| Foodstuff | Inulin content (%) | Oligofructose content (%) |
|---|---|---|
| Onion | 2-6 | 2-6 |
| Jerusalem artichoke | 16-20 | 16-20 |
| Chicory | 15-20 | 5-10 |
| Asparagus | 1-30 | 1-20 |
| Leek | 3-10 | 2-5 |
| Garlic | 9-16 | 3-6 |
| Artichoke | 3-10 | <1 |
| Banana | 0.3-0.7 | 0.3-0.7 |
| Wheat | 1-4 | 1-4 |
| Rye | 0.5-1 | 0.5-1 |
| Barley | 0.5-1.5 | 0.5-1.5 |
| Dandelion | 12-15 | NA |
| Burdock | 3.5-4 | NA |

NA = Data not available

Inulin is the energy reserve in over 36000 plant species, of which many are used as a staple food ingredient. Major sources of inulin or oligofructose include wheat, onion, banana and garlic. The estimated average daily per capita intake of both carbohydrates ranges between 4 and 12g/d in Europe, and between 2 and 4g/d in the USA. Peak intakes of up to 20g may occur after eating a bowl of onion soup or dish of salsify[1].

### *Production and manufacture*

#### *History*

Both inulin and oligofructose are nowadays commercialised as 'new food ingredients.' In Belgium, Raffinerie Tirlemontoise market products known as Raftiline® and Raftilose® using a manufacturing process summarised in Fig. 2. Inulin was discovered by Rose in 1804. Around the middle of the 19th Century, their biochemical production was elucidated, while their indigestibility properties were noted at about the start of the 20th Century. In the 1950's dried plants with a relatively high inulin content were first advised as food ingredients for diabetics. It has only been discovered recently however that oligofructose and inulin can impact on the composition of the human large intestinal microflora in such a manner that the health status of the host may be improved (see later). As such, there is now increasing nutritional and physicochemical interest in these NDO.

An additional reason for this interest is that these carbohydrates have only in the last few years been produced on a realistic industrial scale and become commonly available as a food ingredient for human consumption. However, between 1920 and 1930, inulin was produced on a pilot scale in Germany. Further action for commercial production in several sugar refineries would also have occurred. Past literature mentions a lack of economic feasibility of the production process with inulin finally being recovered by means of precipitation by chilling.

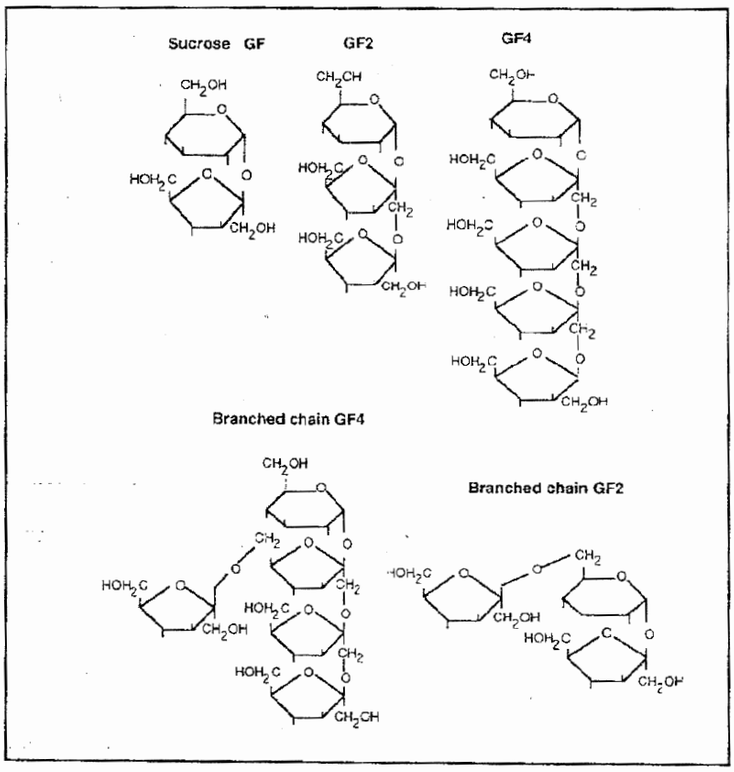


**Fig. 1. Chemical structure of some non-digestible oligosaccharides (G = glucose; F = fructose)**

*Industrial inulin crops*

Both Jerusalem artichoke *(Helianthus tuberosus)* and chicory *(Cichorium intybus)* are sufficiently rich in inulin to be used as industrial crops. The Jerusalem artichoke tuber has been a very important staple food since Medieval ages in countries such as France, Belgium and Holland. However, after the introduction of the potato in these countries, the relevance of this food plant drastically reduced. The chicory root needs a rather difficult culture that requires a mild maritime climate, a rich soil with good texture and a long period of light per day. As such, the number of places in the world where it can be successfully grown is limited. Despite this, some countries have many decades of experience in growing this crop where nowadays it still is cultured for roasting the root which may be used as a coffee substitute. The shoots of a variety of *C. intybus* from the well known Belgian food crop called 'Witloof.'

*Production of inulin and oligofructose*

From these chicory roots, which contain up to 70% (on D.S.) inulin, the carbohydrate is extracted with hot water, much the same way as sucrose is extracted from the sugar beet root. The

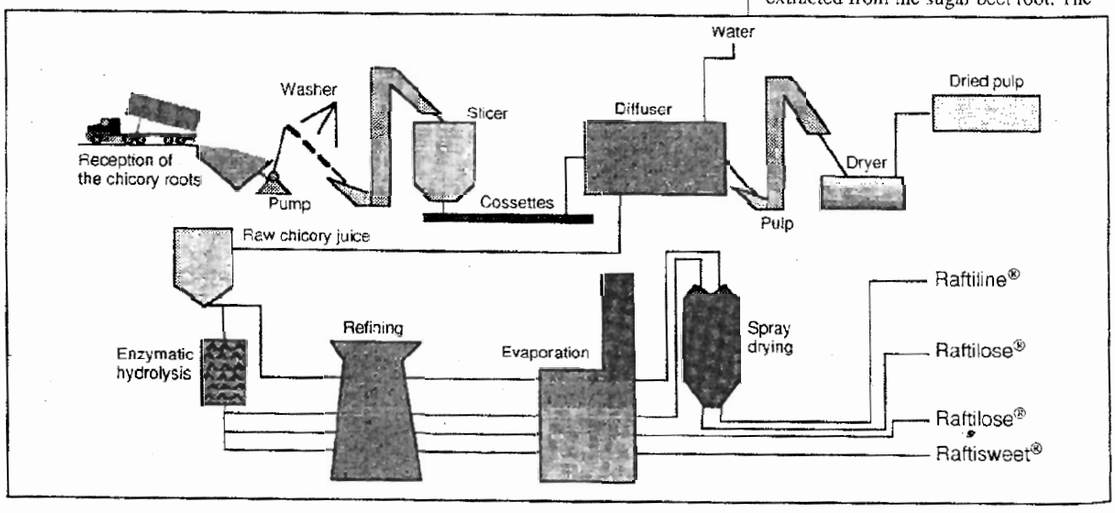


**Fig. 2. Manufacturing process of Raftiline® and Raftilose®**

further purification scheme of the watery extract very much resembles the purification of starch hydrolysates. Chicory inulin is a distinctly polydisperse molecule and cannot be crystallised. It is only slightly soluble in water (approximately 10% in cold water). It is spray dried for further commercial handling. Inulin is enzymatically converted into oligofructose and this is even more soluble in water than sugar.

The commercial preparations of inulin and oligofructose also contain fructose, sucrose and glucose. Raftiline® for example, contains either 92% inulin (ST) or over 99% inulin with an average DP of 10 (LS). Raftilose® contains several blends of lower sugars with up to 95% oligofructose (Fig. 3).

*Applications of inulin and oligofructose*

Both carbohydrates may add functional and nutritional properties to foods in which they are applied. Inulin is a white powder with a neutral, only slightly sweet, taste. Physicochemically, it behaves as a particle in watery solutions. The Rafticreming® process allows a reduction in particle size to 1 micron or less. In this form inulin has a lipid-like texture and is variably used as a fat replacer.

Oligofructose is very soluble in water and therefore can be used in foodstuffs where inulin can be discounted because of its relatively low solubility. It has roughly 30% the sweetness of sugar and can be well applied to products such as chocolates, dairy products and ice creams.

Inulin and oligofructose have similar nutritional properties. Importantly, both positively influence the composition of the colonic microflora. Evidence for this is described below, but will be preceded by a brief summary of the human gut microbiota.

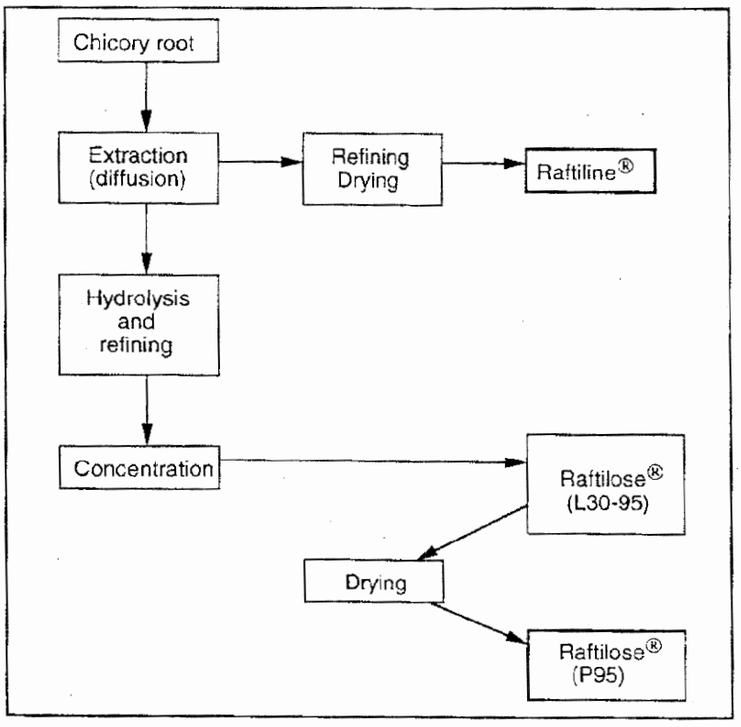


**Fig. 3. Flow diagram of the production process for Raftiline® and Raftilose®**

*Overview of colonic bacteriology*

The large intestine starts at the ileocecal junction, at the terminus of the small intestine, and continues to the anus. In the past, major functions of the colon have been considered as being the absorption and secretion of certain electrolytes and water, as well as the storage and excretion of waste materials[7]. Nowadays, however, it is generally recognised that this organ is much more physiologically and biochemically important than previously thought. This is directly attributable to the microflora that reside in the colon. There are up to $10^{12}$ micro-organisms in every gram of gut contents, with over 200 grams normally being present in the large gut at any one time. In real terms, this means that over 50% of faeces is comprised of bacteria with the large intestine being easily the most heavily colonised region of the human body.

Carbohydrates and proteins that are not absorbed in the upper gastrointestinal tract enter the colon where they may be metabolised by this resident flora. In addition, the colon itself makes a contribution, through endogenous secretions, towards bacterial growth substrates. The predominant carbohydrates available for bacterial fermentation are resistant starch and non-starch polysaccharides (dietary fibre), which together are thought to contribute between 16 and 60 g/d. Through the process of fermentation, colonic bacteria are able to produce a wide range of compounds that have both positive and negative effects on gut physiology. For instance, colonic bacteria produce short chain fatty acids (SCFA) as major fermentation end products. The host may then salvage energy from SCFA absorption. It is thought that about 6% of a healthy adult's energy intake is derived from this process. In addition the remainder of the SCFA absorbed may exert other important systemic influences in the body[8].

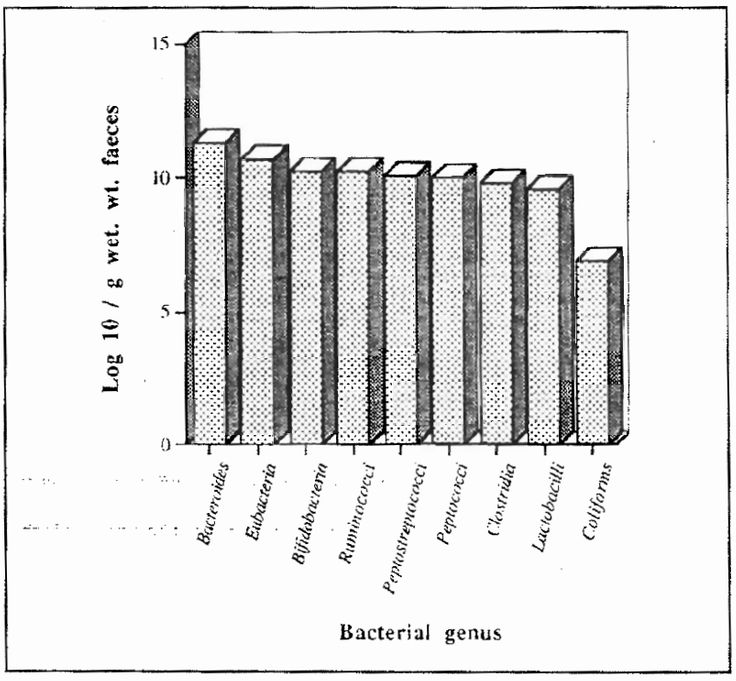


**Fig. 4. Predominant bacterial genera in human faeces**

At least 50 different genera of bacteria reside in the colon, comprising several hundred individual species[9-11]. Each of the bacteria present have fluctuating activities in response to substrate availability, redox potential, pH and distribution in the colon. The vast majority of colonic bacteria have a strictly anaerobic metabolism, whilst numbers of facultative anaerobes are many orders of magnitude lower than those of the obligate anaerobes. The numerically predominant anaerobes belong to the genus Bacteroides. Other important groups are bifidobacteria, clostridia, eubacteria, lactobacilli, ruminococci, peptococci and peptostreptococci. Approximate numbers of these groups found in faeces is shown in Fig. 4.

Each of the vast range of bacterial species which grow in the colon has a specialized ecological niche to fulfil. Some may be considered remedial micro-organisms with respect to human health. In particular, bifidobacteria are thought to exert a number of potentially beneficial properties. Stimulation of growth of these bacteria and suppression of potential pathogens, which are either resident in the colon or enter in diet and are transients ('food poisoning' bacteria), should be advantageous to the host. There is much interest in adding health promoting components to diet to cause this effect. The data summarised below indicate that certain NDO, but especially oligofructose, are suitable candidate materials.

### *Bifidobacteria and human health*

The history of bifidobacteria dates back almost 100 years, with the first isolation of these micro-organisms, by Tissier, from the faeces of infants[12]. Since then, studies on their physiology, ecology, biochemistry and taxonomy have constantly increased to reach their most important stage now. The genus Bifidobacterium consists of 29 separate species, 10 of whose main area of colonisation is the human large intestine[13]. The bacteria are Gram positive bacilli, that often have an unusual and characteristic branching morphology (Fig. 5). Differences exist in the species profile of these bacteria in the adult and infant guts, with *Bifidobacterium longum* and *B. adolescentis* predominating in the former, but *B. infantis* in the latter. In adults, bifidobacteria comprise, on average, 25% of the total flora. However, this proportion may be much higher in infants (up to 95%), particularly those that are breast fed. It is thought that this observation can be attributed to the presence of certain glycoproteins in human milk that stimulate their growth and may contribute towards increased colonisation resistance seen in these infants.

Although bifidobacteria have been implicated in some opportunistic infections[14], they are generally regarded as non-pathogenic and beneficial. That bifidobacteria are today receiving a great deal of biological attention is due to the range of purported helpful aspects that they are able to exert on their host organism, most significantly man. These are summarised briefly in Table II. One of the most important is their effects on potential pathogens. Undoubtedly, this is

**Fig. 5. Typical bifidobacterial morphology. Photograph courtesy of A.J. McBain**

in part due to their ability to produce the strong acids, acetate and lactate, from a saccharolytic fermentation. This function lowers the pH, in a microniche, to levels below those at which the pathogenic bacteria are able to effectively compete. However, a different aspect may also be considered. Using a variety of *in vitro* fermentation systems in which culture pH was irrelevant, bifidobacteria still exerted an inhibitory effect on a range of both Gram positive and negative bacteria[14]. These included *Salmonella* spp., listeria, shigellas, campylobacters, enteropathogenic *Escherichia coli* and clostridia. This more direct mechanism of inhibitory action has not yet been fully clarified, but further emphasizes the potential microbiological importance of these bacteria.

Because of the above, it is more than likely that increased bifidobacterial numbers in the colon will be of benefit to the host. One approach is the addition of live cultures to fermented milk products (e.g. "active", "bio", "live" or "bifidus" yoghurts). It is proposed that the exogenous bacteria reach the large intestine in a viable form and thus contribute towards a healthier flora. However, a number of physical and chemical barriers exist in the upper gastrointestinal tract such as stomach acid, rapid transit and bile acid secretion. It has been shown that specially adapted strains may survive these parameters and reach the colon relatively intact. However, culture viability is questionable and it seems that the added bacteria do not effectively establish therein. This is not surprising considering that the ecosystem contains a vast range of metabolically diverse, previously established, species.

**Table II. Potential health promoting properties associated with bifidobacteria**

- Inhibit the growth of pathogenic bacteria
- Act as immunomodulators
- Produce vitamins, mostly of the B group
- Reduce blood ammonia concentrations
- Lower blood cholesterol
- Help restore the normal intestinal flora after antibiotic therapy

**Table III. Selective media used for the enumeration of faecal bacteria (available from Oxoid)**

| Bacteria | Selective media | Supplements (mg/l) |
|---|---|---|
| Total aerobes | Nutrient agar | - |
| Coliforms | MacConkey no. 2 | - |
| Gram +ve cocci | Azide blood agar base | - |
| Total anaerobes | Wilkins Chalgren agar | - |
| Bifidobacteria | Reinforced clostridial agar | Iodoacetate (0.0125)<br>Nalidixic acid (0.02)<br>Kanamycin (0.05)<br>Polymycin (0.009) |
| Bacteroides | Modified bacteroides agar | Nalidixic acid (0.01)<br>Vancomycin (0.003) |
| Lactobacilli | Rogosa agar | - |
| Clostridia | Perfringens agar base | Oxoid supplements |

Our approach has examined the potential for dietary components (inulin and oligofructose) to stimulate bifidobacteria that are already present in the healthy colon.

***Carbohydrate screening tests***

In the first phase of this study, the fermentability of various dietary components was compared *in vitro* using incubations of mixed faecal bacteria. Briefly, these consisted of anaerobic, pH and temperature controlled glass fermenters to which colonic bacteria, as well as individual carbon and energy sources for their growth, were added. Patterns of carbohydrate metabolism were obtained by means of SCFA production and gas formation in batch culture systems, these being the major end products of anaerobic fermentation by gut bacteria. Results showed that both oligofructose and inulin were metabolised at least as well as other, more common, dietary carbohydrates such as starch, pectin and arabinogalactan[16].

In a separate experiment, carbohydrates (oligofructose, inulin, glucose, sucrose, polydextrose and starch) were added to slurries to give final concentrations of 7g/l. The effect of these additions on bacteria present in the fermenters was then determined during a 24 hour period. Predominant groups of bacteria found in faeces were enumerated using selective growth media given in Table III. After incubation, single colonies were removed from the plates and grown in liquid media for subsequent identification. Viable populations of bacteria increased during the initial 12 hours of carbohydrate fermentation. The enhanced abilities of bifidobacteria to grow on fructose containing NDO in comparison to other carbohydrates was demonstrated, oligofructose being preferred to inulin. In contrast, the other carbohydrates tested exerted a more general effect on overall bacterial growth[15]. Moreover, when bifidobacteria grow on oligofructose or inulin, they seemingly do so at the expense of potential pathogens such as bacteroides, clostridia or coliforms that are maintained at low levels or may even be reduced. Such a high specificity of bifidobacteria for these NDO is likely to be due to the production of appropiate enzymes involved in their metabolism.

Continuous culture studies, where carbohydrates were constantly fed to a fermenter, showed that the most favourable conditions for the bifidogenic effect of inulin and oligofructose were during low pH and high carbohydrate availability, which resembled physicochemical conditions that occur in the proximal (right) side of the colon. These results were confirmed using a 3-stage continuous culture system which

modelled the heterogenous nature of the human large intestine[17].

Pure culture studies showed that, at the species level, both inulin and oligofructose were variably utilised by bifidobacteria. The most adept species at metabolising these carbohydrates were *B. infantis, B. catenulatum, B angulatum* and *B. breve*. The majority of bifidobacteria tested prefer to utilise oligofructoses as sources of carbon and energy compared to glucose. The reverse is true for other colonic bacteria that have been tested[18].

Thus, laboratory studies indicated that certain NDO could potentially increase the bifidobacterial flora of the large gut. The next stage was to test this hypothesis *in vivo* using human volunteers.

### *In vivo volunteer trial*

A human volunteer trial was instigated to assess the bifidogenic effect of oligofructose *in vivo*[19]. In these experiments, the influence of this carbohydrate on the faecal bacterial composition in healthy persons was evaluated during a 45 day feeding period, when the volunteers were given a strictly controlled diet. Eight volunteers (7 male and 1 female) aged 21 - 48 years participated in this experiment. They had never suffered from any form of gastrointestinal disorder and had not taken antibiotics for at least three months before the start of the study. Energy requirements for each volunteer during the feeding regime were calculated on the basis of body weight. During the first 5 days, subjects were admitted into the Dunn Clinical Nutrition Centre metabolic suite and given a non-controlled diet. During this time, a stool sample was collected for bacteriological analysis. Subsequently, subjects were given the controlled diet supplemented with 15 g of sucrose for a 15 day period. This was then replaced by oligofructose for a further 15 days, followed by another period with sucrose. Stool samples were taken periodically for bacterial enumeration.

In summary, the use of oligofructose as a replacement for sucrose in diet caused a marked increase in bifidobacteria, whilst bacteroides, fusobacteria and clostridia all decreased. Other bacteria tested (total aerobes, total anaerobes, lactobacilli, coliforms and Gram positive cocci) remained more or less unchanged. Bacteroides was the numerically predominant genus on sucrose, whilst on oligofructose bifidobacteria became more predominant. This is a significant observation, when it is considered that bacteroides are almost always the major bacterial genus found in faeces. These results are shown in a simplified form in Fig. 6.

This study has shown how a subtle and simple change in diet composition, i.e. the substitution of 15 g sucrose by 15 g oligofructose, can lead to a potentially important alteration in the composition of the large intestinal microbiota. The next stage of the process is to demonstrate that a microbial flora high in bifidobacteria actually does exert health promoting functions. It is planned to carry out a trial with traumatised patients (e.g. those on enteral feeding) where a high risk of infection occurs. Will this be alleviated if the natural populations of bifidobacteria are increased?

### *Relevance of the work*

If one accepts that bifidobacteria

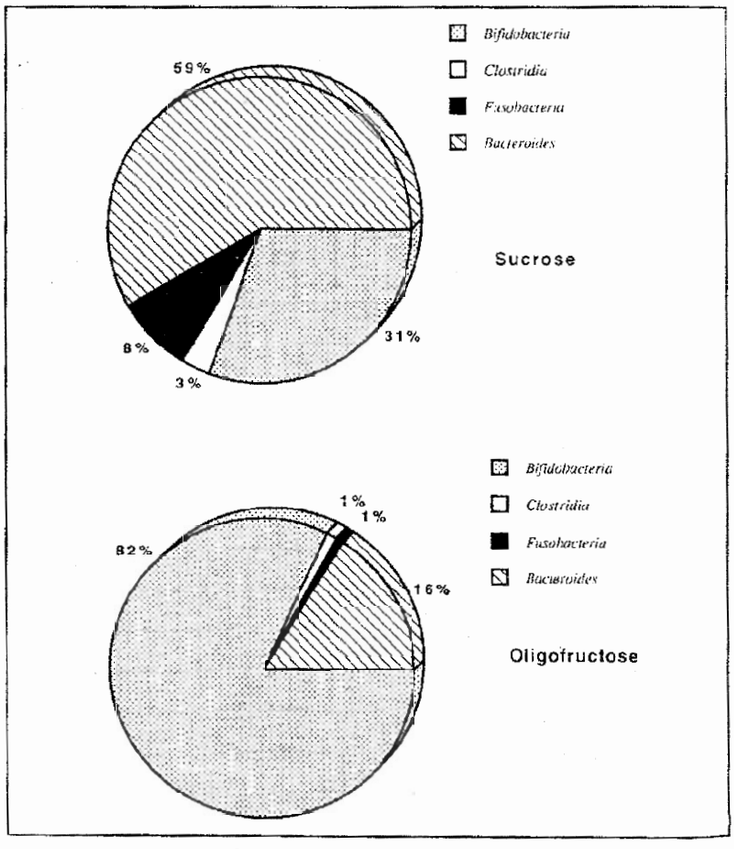


**Fig. 6. Summary diagram showing major bacterial groups in faeces of healthy volunteers given a strictly controlled diet that was supplemented with 15g/d carbohydrate (sucrose or oligofructose)**

are in fact important salutary micro-organisms in the human colon, and there is no realistic contradictory evidence thus far, then an increase in their numbers will be of some advantage. Increased oligofructose intake seems to be a possible viable mechanism whereby this may occur. However, what is the feasibility of this rationale? It is estimated that only about 2-12 g are consumed per day. Bearing in mind the type of foodstuffs that have a high oligofructose content (Table I), it is probably not realistic to advocate a significantly increased intake through these food items. This therefore implies that dietary preferences may need to be altered. However, one's diet is also a cultural entity. Therefore, other possibilities must be examined. Purified chicory inulin and oligofructose may offer a solution. They can both be incorporated into many existing sugar containing foodstuffs, and inulin may be used as a fat replacement. The biological properties of these NDO, in particular their effect on bifidobacteria resident in the gut, seem convincing. However, the final choice rests with the consumer.

*Summary*

Bifidobacteria are non-pathogenic micro-organisms in the human colon which appear to have a beneficial effect. Inulin and oligofructose, which belong to the group of carbohydrates known as non-digestible oligosaccharides (NDO), have been found to have a number of dietary advantages, but also seem to be positively involved in the growth of bifidobacteria, as confirmed by *in vitro* tests and by *in vivo* tests in which sucrose was replaced by oligofructose in diets. A trial is to be carried out to demonstrate that bifidobacteria do play a health-promoting role.

## Oligosacáridos y bifidobacterias no digeribles - las consecuencias para la salud

Las bifidobacterias son microorganismos no patogénicos en el colon humano que por lo visto tienen efectos beneficiosos. Se ha descubierto que la inulina y la oligofructosa, que forman parte del grupo de hidratos de carbono conocido por el nombre de oligisacáridos no digeribles (OND), tienen varias ventajas dietéticas , y también parece que participan activamente en el crecimiento de bifidobacterias. Esto se ha confirmado en pruebas in vitro y en pruebas in vivo en las que la sacarosa fue sustituida por la oligofructosa. Se va a realizar una prueba para demostrar que las bifidobacterias desempeñan un papel que es beneficioso para la salud.

## Les oligosaccharides et les bifidobactéries non-digestes - les conséquences pour la santé

Les bifidobactéries sont des micro-organismes non-pathogènes dans le côlon humain qui semblent avoir une action salutaire. On a découvert que l'inuline et l'oligofructose, qui font partie du groupe d'hydrates de carbone connu sous le nom d'oligosaccharides non-digestes (OND), ont plusieurs avantages diététiques, et ils participent activement aussi au développement des bifidobactéries. Cela s'est confirmé dans les analyses in vitro et dans les analyses in vivo dans lesquelles le saccharose a été remplacé par l'oligofructose. On va faire un essai pour montrer que les bifidobactéries sont très bonnes pour la santé.

## Nichtverdauliche Oligosaccharide und Bifidobakterien - ihre Bedeutung für die menschliche Gesundheit

Bifidobakterien sind nichtpathogene Mikroorganismen im menschlichen Grimmdarm, die nützlich scheinen. Es wurde gefunden, dass Inulin und Oligofructose, die zur Gruppe von als nichtverdauliche Oligosaccharide (NVO) genannten Carbohydraten, einige Diätvorteile haben; sie sollen auch positiv am Wachstum von Bifidobakterien beteiligen, wie es in *in vitro* Versuchen und in *in vivo* Untersuchungen mit Oligofructose anstatt Saccharose in den Diäten bestätigt wurde. Es soll ein Versuch durchgeführt werden, um zu zeigen, dass Bifidobakterien wirklich zur Erhaltung der Gesundheit beitragen.

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** Raffinerie Tirlemontoise, Tienen, Belgium.

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(54) **INULIN PRODUCTS WITH IMPROVED NUTRITIONAL PROPERTIES**

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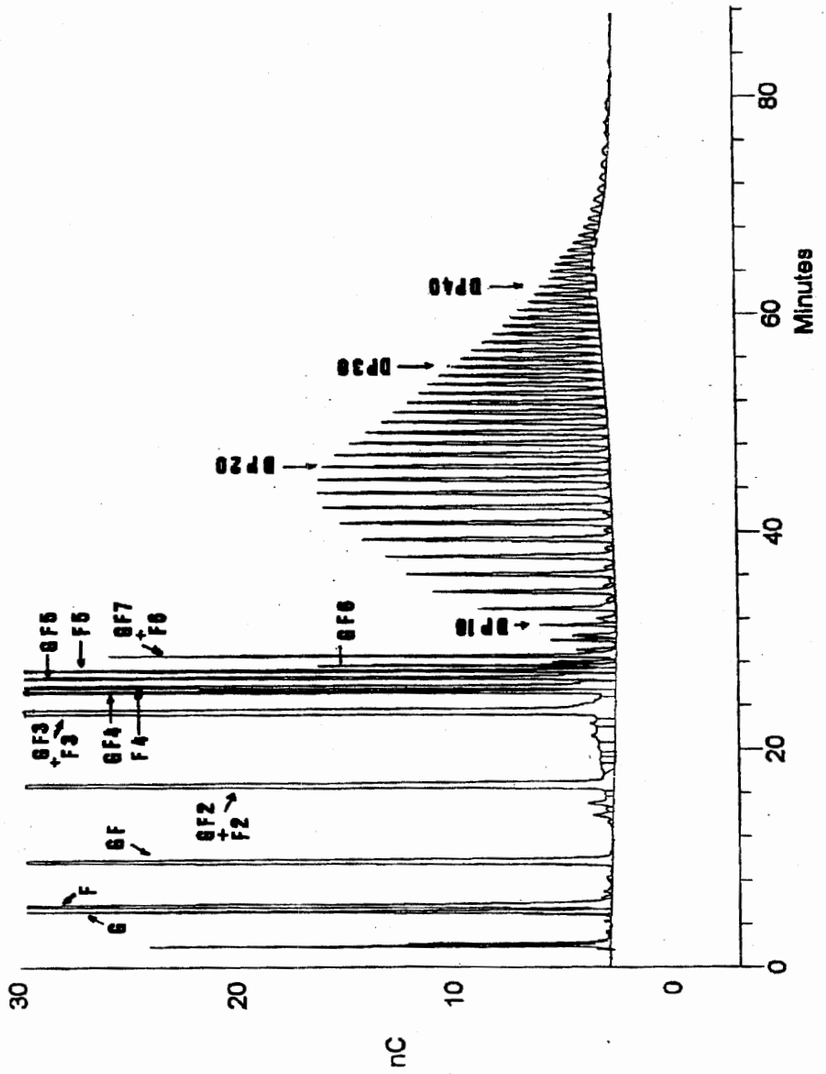
(57) **ABSTRACT**

The invention relates to novel inulin products and compositions thereof, to their manufacture, to their use for modifying and modulating the bacterial flora and the fermentation pattern of inulin in the large intestine of humans, mammals or other vertebrates, to their use for providing improved inulin-associated nutritional effects/benefits, as well as to their use for the manufacture of consumer products and compositions for providing said effects/benefits in healthy, disfunctioned and diseased humans, mammals and other vertebrates.

The novel inulin products consist of a particular mixture of an easily fermentable inulin (EFI) component and a hardly fermentable inulin (HFI) component.

The nutritional effects/benefits include dietary fibre effects, improved mineral absorption, particularly calcium and magnesium, bone mineral density increase, reduction of bone mineral density loss, modulation of lipid metabolism, stimulation of the immune system, and anti-cancer effects. The novel inulin products are particularly suitable for the manufacture of a composition or a medicament for preventing, for postponing and/or for treating osteoporosis in humans, particularly in post-menopausal women and elderly people.


BP40
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BP10
GF6
F5
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G
0
20
40
60
80
Minutes
30
20
10
0
nC

FIG. 1

# INULIN PRODUCTS WITH IMPROVED NUTRITIONAL PROPERTIES

## FIELD OF THE INVENTION

[0001] This invention relates to novel inulin products and compositions thereof, to their manufacture and to their use for modifying and modulating the bacterial flora composition and the fermentation pattern of inulin in the large intestine of humans, mammals and other vertebrates, to their use for providing improved inulin-associated nutritional effects/benefits, and to their use for the manufacture of a composition, a consumer product, a pharmaceutical or a medicament for providing said effects/benefits in humans, mammals and other vertebrates.

## PRIOR ART AND TECHNOLOGICAL BACKGROUND

[0002] Inulin is a fructan-type carbohydrate, consisting mostly of fructose units, which occurs in many plants as a reserve carbohydrate. Inulin can be produced by certain bacteria and can also be enzymatically produced in vitro from sucrose. Inulin naturally occurs as a polydisperse mixture of carbohydrate molecules which are essentially composed of fructosyl units forming chains in which the fructosyl units are mainly or exclusively linked to one another by a β(2,1) bound. The mainly linear chains are possibly bearing one or more side chains essentially composed of fructosyl units, thus forming branched inulin molecules with a fructosyl-fructosyl linkage at the branching point commonly formed by a fructosyl-fructosyl β(2,6) bound. Inulin molecules from plant origin mostly contain one terminal glucosyl unit. Accordingly, inulin molecules can be represented by the formula GFn or Fm wherein G represents a terminal glucosyl unit, F represents a fructosyl unit and n and m represent the number of fructosyl units linked to one another through a β(2,1) and/or a β(2,6) bound. The number n+1, respectively m, indicates the degree of polymerisation (DP) of the inulin molecule. Inulin is further characterised by its (number) average degree of polymerisation, represented by (DP). This is the value which corresponds to the total number of saccharide units (G and F units) in a given inulin sample divided by the total number of inulin molecules in said sample, without taking into account the monosaccharides glucose (G) and fructose (F) and the disaccharide sucrose (GF) which are possibly present in the sample. The average degree of polymerisation (DP) is commonly determined by the method described by De Leenheer et al. (1).

[0003] Native inulin from plant sources (i.e. the inulin as present in the plant) appears as a polydisperse mixture of mainly linear polysaccharide chains with a (DP) ranging from 2 to about 100, whereas inulin molecules from bacterial origin, which commonly are branched ones, usually have much higher (DP) values, even up to about 115.000. Plant inulin has a (DP) which largely depends on the plant source and on the harvest, storage and processing conditions. Natural (or standard grade) inulin indicates herein inulin which has been extracted from plant sources, purified and isolated, without applying a treatment for reducing or increasing its (DP) and it usually has a (DP) which is about 1 unit lower than the (DP) of the corresponding native inulin.

[0004] Inulin molecules with a low degree of polymerisation, usually defined as a (DP)<10, are named inulo-oligosaccharide(s), fructo-oligosaccharide(s) or oligofructose. These terms, including linear and branched inulin of (DP)<10, are commonly, also herein, used interchangeably. Oligofructose is also termed herein short-chain inulin.

[0005] Inulin is commonly manufactured from plant sources, mainly from roots of Chicory (*Cichorium intybus*), but also from tubers of Jerusalem artichoke (*Helianthus tuberosus*) and from the piña (head) of the Blue Agave plant, in which inulin can be present in concentrations up to about 20 wt % on fresh plant material (hereinafter wt % means per cent by weight). Inulin can be readily extracted from said plant parts and purified according to conventional techniques.

[0006] Natural inulin from chicory, respectively from J. artichoke, commonly appears as a polydisperse mixture of slightly branched chains (typically chains with less than 2 per cent, respectively less than 1 per cent, branching) with a (DP) ranging from 2 to about 70, respectively from 2 to about 40. Natural (standard grade) chicory inulin has a (DP) of about 10 and natural (standard grade) inulin from J. artichoke has a (DP) of about 6.

[0007] Natural inulin from agave appears as a polydisperse mixture of highly branched chains with a (DP) commonly ranging from about 14 to about 17.

[0008] At industrial scale, chicory inulin is conventionally obtained by extraction of shredded chicory roots with hot water yielding a crude inulin solution which is subsequently purified by depuration (treatment with lime followed by carbonatation and filtration) and by refining (involving treatment over ion-exchangers, treatment with active carbon and filtration). Standard grade inulin is then commonly obtained from the purified and refined solution by spray-drying. Optionally, monomeric and dimeric saccharides are removed from the purified and refined solution (e.g. by column chromatographic separation as described in EP 0670 850) to yield via spray-drying an inulin grade with a standard (DP) of about 10 which is about free of monomeric and dimeric saccharides. Optionally the purified and refined solution can be fractionated to remove monomeric and dimeric saccharides as well as oligofructose (e.g. by directed crystallisation as described in EP 0 769 026) and the fractionated inulin is then isolated in particulate form by spray-drying. Depending on the manufacturing process, chicory inulin with a (DP) ranging from about 10 (standard grade) to about 30, and even more, can be obtained.

[0009] Similarly, agave inulin can be obtained at industrial scale by squeezing, or extracting with water, shredded heads or pulp from Blue Agave, followed by conventional purification, refining and isolation of the inulin e.g. via spray-drying.

[0010] Inulin, including linear and branched inulin, with a (DP)≧20 is termed herein long-chain inulin, whereas linear and branched inulin with a (DP) from 10 to <20 is termed herein medium-chain inulin.

[0011] Inulin from chicory is for example commercially available as RAFTILINE® from ORAFTI (Tienen, Belgium) in various grades. Typical grades are RAFTILINE® ST (with a (DP) of about 10 and containing in total about 8% by weight glucose, fructose and sucrose) and RAFFILINE® HP (with a (DP) of at least 20, commonly with a (DP) of about 23 to about 25, and virtually free of glucose, fructose and sucrose).

[0012] Agave inulin is commercially available, for example industrial grade agave inulin as GAVEDIET® PR with a (DP) of 14-16 and containing in total about 5% by weight of glucose and fructose, from Industrias Colibri Azul S.A. de C.V., Mexico.

[0013] Oligofructose can be obtained according to techniques which are known in the art, including enzymatic in vitro synthesis from sucrose, as for example described in U.S. Pat. No. 5,314,810, and partial hydrolysis of inulin, as for example described in EP 0 917 588.

[0014] Oligofructose prepared by enzymatic hydrolysis of chicory inulin is commercially available in various grades, for example as RAFTILOSE® from ORAFTI (Tienen, Belgium), e.g. RAFTILOSE® L95 (liquid form) or RAFTILOSE® P95 (powder form), both with a content of about 95% oligofructose (% is wt % on total carbohydrates) with a (DP) from 2 to 9, typically with a (DP) mainly from 2 to 7, a (DP) of about 4.5, and containing about 5% in total (% is wt % on total carbohydrates) of glucose, fructose and sucrose, and RAFTILOSE® L85, liquid form with a content of about 85% oligofructose (% is wt % on total carbohydrates) with a (DP) from 2 to 9, typically a (DP) mainly from 2 to 7, a (DP) of about 3.5, and containing about 15%, maximally 20% in total (% is wt % on total carbohydrates) of glucose, fructose and sucrose.

[0015] Unless otherwise specified, the term inulin used herein refers to linear as well as branched inulin, and includes inulin molecules with a (DP)<20 as well as inulin molecules with a (DP)≧20.

[0016] In the food and feed industry, oligofructose is widely used as a low-calorie partial or complete replacement for sugar, providing sweetness, body and mouthfeel, whereas inulin of a (DP) of at least about 10, preferably of at least 20, is utilised (i) as a partial or complete low-calorie replacement for sugar in combination or not with one or more high intensity sweeteners, providing body and mouthfeel, (ii) as a texture improver, and (iii) as a low-calorie replacement for fat. The use of inulin as fat replacer results from the fact that inulin can form with water a particle gel with a stable, homogeneous, creamy structure with excellent organoleptic properties.

[0017] Inulin molecules with a (DP)≧10 as well as oligofructose molecules with a (DP)<10, are not hydrolysed by human digestive enzymes. Accordingly, these molecules pass the upper part of the digestive tract and the small intestine unaltered (Ellegård et al. (2)) and reach almost quantitatively the large intestine where they are fermented by specific intestinal bacteria (Roberfroid et al. (3)). As a result thereof, inulin and oligofructose present highly interesting nutritional properties.

[0018] Firstly, inulin and oligofructose are considered as dietary fibres. They reach the large intestine unaltered, thus providing carbon energy to the microflora in the large intestine. In this manner, inulin and oligofructose are stimulating the growth of gut bacteria in the large intestine which has a beneficial effect on the gut function, including a bulking effect (i.e. increase of the bacterial biomass) which in turn results in an increased stool weight, an increased stool frequency and a relief of constipation (Roberfroid (4)).

[0019] Furthermore, it has been found that inulin and oligofructose have a strong bifidogenic effect because inulin and oligofructose selectively stimulate the growth and metabolic activity of Bifidobacteria and Lactobacilli. Besides, while the counts of intestinal Bifidobacteria are significantly increased by the oral intake of inulin or oligofructose, a concomitant significant reduction of the counts of undesirable or pathogenic bacteria, such as e.g. Clostridia and Escherichia, in the large intestine has been observed (Gibson et al. (5) and Wang (6)). The intake of inulin and oligofructose thus largely modifies and modulates the gut flora by selectively increasing colonisation of the large intestine by beneficial bacterial species, typically Bifidobacteria, while suppressing the growth of undesirable bacterial species, which in turn results in favourable prophylactic and therapeutic effects on intestinal disorders of the host.

[0020] WO 93/02566 discloses a reduced calorie chocolate confectionery composition that is obtained by partial substitution of the sugar and/or fat of a conventional composition by a fructan or fructan mixture. WO 93/02566 furthermore discloses in a generic manner that a mixture of inulin and fructo-oligosaccharides presents good dietary fiber effects in combination with bifido-stimulating effects and good promotion of intestinal flora proliferation, but is silent about possibly improved nutritional and health effects that may result form mixtures of inulin and fructo-oligosaccharides that present a particular inulin profile.

[0021] WO 96/03888 relates to a water continuous edible spread that presents good structural properties (in particular plasticity) and no sweet off-taste. Several spread compositions respectively with low-sugar inulin of av. DP 12 (Raftiline® LS) and long-chain inulin (Fibruline® LC of av. DP 20 and oligofructose of av. DP 25) are described. The disclosed experimental data indicate that a water continuous spread with the desired properties is obtained when the spread composition comprises at least 7 wt % oligofructose having an av. DP of at least 14 whereby the short oligofructose molecules are present in very small amounts or not at all. WO 96/0388 is silent about possibly improved nutritional and health effects that may be provided by compositions of short-chain and long-chain inulin molecules presenting a particular inulin profile.

[0022] In vivo experiments with healthy volunteers showed inulin (RAFRILINE® ST) and oligofructose (RAFTILOSE® P95) to be bifidogenic to the same extent (Gibson et al. (7)), while in vitro experiments revealed that inulin ((DP)≧10) is fermented in the large intestine about twice as slowly as oligofructose ((DP)<10) (Roberfroid et al. (3)).

[0023] From these observations it follows that oligofructose is almost completely fermented in the proximal part of the large intestine, i.e. the ascendent part, whereas inulin is likely to reach to a more or lesser extent also more distal parts of the large intestine, i.e. the transversal and descendent parts, where it is fermented.

[0024] In vitro tests revealed that agave inulin is about as easily fermented as oligofructose. Accordingly, it is assumed that agave inulin is also almost completely fermented in the proximal part of the large intestine of humans and mammals.

[0025] Moreover, it has been disclosed that oligofructose and inulin have preventive and therapeutic effects with respect to the genesis and growth of certain cancers such as colon cancer (WO 98/52578) and mammary cancer (EP 0 692 252).

[0026] The effects against mammary cancer seem to be related to an immuno-modulating effect, particularly a stimulating effect on the immune system, of oligofructose, inulin and/or their fermentation products, mainly short chain fatty acids (SCFA) (Namioka et al. (8)).

[0027] With respect to colon cancer (usually resulting from pre-neoplastic lesion formation in the distal part of the colon), it has been reported that long-chain inulin, i.e. inulin with a ($\overline{DP}$)≧20, is more effective in preventing the genesis of colon cancer and in inhibiting the growth of colon cancer, than oligofructose (with a ($\overline{DP}$)<10) and standard grade chicory inulin (with a ($\overline{DP}$) of about 10) (WO 98/52578).

[0028] Furthermore, it has been found in studies with healthy human volunteers who were slightly hyperlipidemic, that the consumption of oligofructose or inulin has beneficial effects on lipid metabolism since the consumption resulted in reducing the level of serum triglycerides and cholesterol (mainly LDL cholesterol) compared to a control placebo treatment (Brighenti et al. (9) and Jackson et al. (10)). Moreover, it has been demonstrated in rat experiments that the addition of oligofructose or inulin to a fat-rich diet reduced serum cholesterol as well as serum triglycerides by more than 50% compared to a control group (Kok et al. (11)).

[0029] Furthermore, positive effects of the consumption of oligofructose and inulin on the intestinal absorption of minerals, particularly calcium (Ca), magnesium (Mg) and iron (Fe), as well as on the bone mineral density (BMD), have been found in various studies. Shimura et al. (12), Levrat et al. (13), Rémésy et al. (14), Tagushi et al. (15) and Scholz-Ahrens et al. (16) reported studies with rats in which an increased absorption of calcium, and in some cases of other minerals, including magnesium, was demonstrated as a result of oral consumption of inulin or oligofructose. Ohta et al. (17) and Baba et al. (18) formulated the hypothesis that the positive effects of non-digestible carbohydrates on Ca and Mg absorption occur at the level of the large intestine. Up to then, it was generally accepted that mineral absorption occurred mainly via the small intestine. Deizenne et al. (19) reported that a diet supplemented with 10 wt % of either inulin (RAFTILINE®ST) or oligofructose (RAFTILOSE®P95) resulted in a strong absorption increase for magnesium and calcium and a moderate absorption increase for iron in healthy rats, and noted almost the same effect for inulin (RAFTILINE® ST) compared to oligofructose (RAFTILOSE® P95). Brommage et al. (20) disclosed a similar increase in Ca absorption in healthy rats fed a diet supplemented with 5 wt % oligofructose (RAFTILOSE® P95). Taguchi et al. (15) reported that in ovariectomised rats oligofructose (2.5 wt % and 5 wt % in the diet) increased mineral uptake, particularly Ca and Mg absorption, and increased bone density thus preventing bone loss caused by oestrogen deficiency. Using the same model, Scholz-Ahrens et al. (16) observed a dose-dependent effect of oligofructose (RAFTILOSE® P95) (at 2.5; 5 and 10 wt % in the diet) on calcium absorption and on bone mineralisation. In that study, oligofructose also significantly reduced the osteoporotic loss of the bone trabecular structure caused by ovariectomy. Furthermore, an increased Ca absorption with a concurring increased BMD in rats fed a diet containing 5 wt %, respectively 10 wt %, inulin (RAFTILINE® HP) was reported by Lemort et al. (21).

[0030] The findings that inulin and oligofructose can positively influence the absorption of minerals from the diet and affect the uptake of minerals in the bone tissue, leading to increased BMD, are of high importance for human health. Indeed, calcium uptake in the body, bone mineral density increase, as well as the possibility to prevent, to slow down or to curb bone mineral density reduction, are very important for human populations with a typical Western-type lifestyle and food pattern, since in these populations there occurs with increasing age, particularly in post-menopausal women, a dysbalance between mineral uptake and mineral resorption and excretion. Said dysbalance results in a reduction of BMD and in bone fragilisation, which in a pronounced stage is known as osteoporosis. In an advanced stage, osteoporosis leads in turn to a high incidence of bone fractures. Accordingly, it is very important to ensure the building up during the growth phase of children and adolescents of skeletal elements with a high BMD. Such skeletal elements will indeed resist longer to demineralisation caused by any factor, and this may thus postpone or even avoid bone fracture due to advanced osteoporosis. In view of the above, it is also most important to be able to reduce possible losses of bone mineral content in adults in order to prevent or to maximally delay undesirable osteoporosis-related conditions, and in particular to slow down the post-menopausal demineralisation leading to osteoporosis and eventually to bone fracture. Furthermore, it is very important to be able to remedy conditions of osteoporosis, in particular in case of the occurrence of osteoporosis-related bone fractures. At last, it is highly desirable to be able to stimulate and increase mineral uptake and formation of bone structure in case of necessity, for example in case of accidental bone fractures in children, adults and elderly people.

[0031] In view thereof, the disclosures regarding the increased mineral absorption in rats have received much attention from the medical world and several studies have been made in order to examine Ca absorption from the diet and to increase Ca uptake in the bone tissue, in order to increase or improve BMD and bone structure in humans. Ellegård et al. (2) determined the mineral balance in ileostomy volunteers who were administered 15 g/day of either inulin (RAFTILINE® ST) or oligofructose (RAFTILOSE® P95). The intake of neither inulin nor oligofructose was found to alter the mineral excretion from the small intestine, thus confirming that the effect of inulin and oligofructose on mineral absorption does not occur in the small intestine but essentially takes place in the large intestine (also termed the colon). Studies by Coudray et al. (22) with healthy male adults (metabolic balance method) showed a significantly increased Ca absorption with a dietary intake of 40 g inulin per day. In studies (dual stable isotopes method) with healthy male adolescents, Van den Heuvel et al. (23) found a significant increase in Ca uptake upon consumption of 15 g/day oligofructose (RAFTILOSE® P95).

[0032] The beneficially nutritional effects resulting from the intake of oligofructose and inulin apparently are the result of their fermentation in the large intestine. However, as reported by Roberfroid et al. (3), the fermentation rate of inulin is much slower than the one of oligofructose.

[0033] Furthermore, in vitro experiments (unpublished results) with human faecal slurries even indicated to the inventors that when long-chain inulin (i.e. inulin with (

$\overline{DP}$)≧20), was essentially free from oligofructose, i.e. inulin of (DP)<10), its fermentation hardly started.

[0034] The above observations, on the one hand the improved nutritional effects of inulin, particularly of long-chain inulin, and, on the other hand, the difficult and slow start of the fermentation and the resulting low fermentation rate of inulin, particularly long-chain inulin, in the large intestine, clearly lead to a technical problem which limits and even prevents the use of long-chain inulin to maximally generate nutritional benefits in humans and mammals.

[0035] Furthermore, in most of the nutritional studies disclosed so far, a daily consumption of relatively high amounts of oligofructose or inulin have been used, namely 15 g to 40 g/day in human studies and 2.5 wt % to 10 wt % and even 20 wt % of the diet in rat studies. Extrapolated to humans, a rat diet containing 2.5 wt % to 10 wt % oligofructose or inulin would correspond to an amount oligofructose or inulin of about 15 g to 60 g/day. Such relatively high daily amounts also constitute a further technical problem for the use of inulin for nutritional purposes, particularly for generating improved beneficially nutritional effects in humans, because, as is known, such relatively high doses may cause intestinal side effects, such as too much flatulence, too much intestinal pressure, intestinal cramps and even diarrhoea.

## Object of the Invention

[0036] It is an object of the present invention to provide a novel inulin product and compositions thereof presenting improved nutritional properties for humans, mammals and other vertebrates, compared to known inulin products, without imparting intestinal side effects.

[0037] It is another object of the present invention to provide a novel inulin product and compositions thereof which modulate the bacterial flora composition in the large intestine of humans, mammals and other vertebrates, and which modulate the fermentation pattern of inulin in said beings.

[0038] It is a further object of the present invention to provide the use of said novel inulin product and compositions thereof for the preparation of products and compositions for generating improved nutritional effects, in particular increased mineral absorption, in humans, mammals, and other vertebrates.

## Description of the Invention

[0039] In the search for improved inulin products, the inventors have surprisingly found a novel inulin product comprising a hardly fermentable inulin such as e.g. a long-chain inulin that, in spite of the above adverse indications for using such an inulin, nevertheless provides a solution to one or more of the mentioned and other problems.

[0040] According to one embodiment of the present invention, the inulin product of the invention consists of a mixture of an easily fermentable inulin component (hereinafter EFI) and a hardly fermentable inulin component (hereinafter HFI) in a specific weight ratio EFI/HFI ranging from 10/90 to 70/30.

[0041] By easily fermentable inulin (EFI) is meant herein linear as well as branched inulin-type products which are completely or almost completely fermented in the proximal part (the ascendent part) of the large intestine of humans and mammals. Typical EFI are short-chain inulin (i.e. inulin with a (DP)<10) and agave inulin (i.e. a branched inulin, typically of ($\overline{DP}$) of 14 to 16).

[0042] Short-chain inulin has preferably a (DP) ranging mainly from 2 to 7, with minor amounts, in total preferably less than 5%, more preferably less than 3%, inulin of (DP)=8 and (DP)=9 (% is wt % on total inulin). A preferred short-chain inulin is oligofructose obtained by enzymatic hydrolysis of chicory inulin.

[0043] By hardly fermentable inulin (HFI) is meant herein linear as well as branched inulin-type products of which the fermentation hardly starts in the proximal part of the large intestine and which are mainly fermented, though at a low rate, in the distal part (the transversal part and/or the descendent part) of the large intestine of humans and mammals. Typical HFI are long-chain inulin (i.e. linear as well as branched inulin with a ($\overline{DP}$)≧20), and inulin in a particular crystallographic form or a particular physical appearance form which does not enable easy and significant fermentation in the proximal part of the large intestine of humans and mammals.

[0044] Long-chain inulin has preferably a ($\overline{DP}$) of at least 23, more preferably of at least 25, even more preferably of at least 30, and contains in total preferably less than 5%, more preferably less than 3%, inulin of (DP)=9 and (DP)=10 (% is wt % on total inulin).

[0045] A preferred HFI is long-chain inulin from chicory (named herein long-chain chicory inulin) with a ($\overline{DP}$)≧20, preferably a ($\overline{DP}$)≧23, more preferably a ($\overline{DP}$)≧25, and another preferred HFI is inulin from bacterial origin.

[0046] In a preferred embodiment, the inulin product of the invention consists of a mixture of a EFI component that is free from agave inulin and consists of a short-chain inulin, and a HFI component which is a long-chain inulin, in a weight ratio EFI/HFI ranging from 10/90 to 70/30, and wherein the total content of inulin with (DP)=9 and (DP)=10 is maximally 5%, preferably maximally 3%, more preferably maximally 2%, most preferably maximally 1% (% is wt % on total inulin, determined by gas liquid chromatography (GLC) analysis according to De Leenheer et al. (1)).

[0047] In an other preferred embodiment, the inulin product of the invention consists of a mixture of a EFI component consisting of agave inulin, preferably natural agave inulin with a ($\overline{DP}$) ranging from about 14 to about 17, or any mixture of agave inulin with a short-chain inulin, and a HFI component which is a long-chain inulin, in a weight ratio EFI/HFI ranging from 10/90 to 70/30, in which product the total amount of inulin with (DP)=9 and (DP)=10 is maximally 5%, preferably maximally 3%, more preferably maximally 2%, most preferably maximally 1% (% is wt % on total inulin, determined by GLC according to De Leenheer et al. (1)).

[0048] In a preferred inulin product according to the invention, said weight ratio of EFI/HFI is preferably ranging from 20/80 to 65/35, more preferably from 35/65 to 65/35, and even more preferably from 40/60 to 45/55, typically about 50/50.

[0049] In a more preferred embodiment, the inulin product of the invention consists of a mixture of oligofructose as EFI

component and a long-chain chicory inulin as HFI component in an EFI/HFI weight ratio ranging from 10/90 to 70/30, wherein the total content of inulin with (DP)=9 and (DP)=10, is maximally 5%, preferably maximally 3%, more preferably maximally 2%, most preferably maximally 1% (% is wt % on total inulin, determined by GLC. In a highly preferred embodiment, the EFI/HFI weight ratio of the inulin product ranges from 35/65 to 65/35, most preferably from 40/60 to 45/55.

[0050] According to a further, highly preferred embodiment of the invention, the inulin product according to the invention is an industrial grade inulin product, which means an inulin product composed of a mixture of industrial grade short-chain inulin or agave inulin or any mixture thereof as EFI component and of industrial grade long-chain inulin as HFI component in a weight ratio EFI/HFI ranging from 10/90 to 70/30, preferably from 35/65 to 65/35, most preferably from 40/60 to 45/55, wherein the total content of inulin with (DP)=9 and (DP)=10 is maximally 5%, preferably maximally 3%, more preferably maximally 2% and most preferably maximally 1% (% is wt % on total inulin determined by GLC).

[0051] In the industrial grade inulin product according to the invention, the weight ratio EFI (short-chain inulin) component/HFI (long-chain inulin) component is defined on the basis of the real short-chain inulin and real long-chain inulin present in the respective components, without taking into account the amounts of glucose, fructose and sucrose which are possibly present. The real amount of inulin product of the invention in said industrial grade inulin product thus corresponds to the sum of the amounts of real short-chain inulin and real long-chain inulin present in the EFI and HFI components.

[0052] Accordingly, industrial grades of oligofructose can be used as EFI component in the industrial grade inulin product according to the invention, which may even contain in total maximally 20%, preferably maximally 15%, more preferably maximally 10%, even more preferably maximally 8%, most preferably maximally 5%, glucose, fructose and sucrose (%=wt % on total carbohydrates in the oligofructose product).

[0053] In still a further preferred industrial grade inulin product according to the invention, the oligofructose component consists of more than 43 wt % of inulin-type molecules of formula Fm wherein F indicates a fructosyl unit and m is the degree of polymerisation, ranging from 2 to 9, preferably mainly from 2 to 7.

[0054] Typically industrial grades of oligofructose which are suitable as EFI component of the industrial grade inulin product according to the invention are RAFTILOSE® L85, RAFTILOSE® L95 and RAFTILOSE® P95, which are all oligofructose grades obtained by enzymatic hydrolysis of chicory inulin. Suitable industrial grades of oligofructose can also be obtained by enzymatic in vitro synthesis from sucrose by known methods, for example according to patent U.S. Pat. No. 5,314,810. A suitable industrial grade of agave inulin is GAVEDIET®PR.

[0055] Industrial grade long-chain inulin with a $\overline{(DP)} \geqq 20$ which is suitable as HFI component of the industrial grade inulin product of the invention may contain inulin molecules with a (DP) from 10 to 20 up to about 45% (% is wt % on total carbohydrates). Possibly present inulin molecules with a (DP)<10 are calculated as part of the EFI component. In said industrial grade long-chain inulin, the content of glucose, fructose and sucrose is usually very low, typically less than about 2% (% is wt % on total carbohydrates).

[0056] A typically industrial grade inulin which is suitable as HFI component is long-chain chicory inulin with a $\overline{(DP)} \geqq 20$, preferably a $\overline{(DP)} \geqq 23$, such as RAFTILINE®HP.

[0057] The inulin product of the invention surprisingly presents significantly improved nutritional properties and its oral or enteral intake provides one or more significantly improved nutritional effects/benefits in humans, mammals and other vertebrates, compared to known inulin products, such as oligofructose, medium-chain inulin and long-chain inulin. Furthermore, the oral or enteral intake of the inulin product of the invention provides said improved nutritional effects/benefits in humans, mammals and other vertebrates, commonly at a lower daily dose than the daily dose which is needed of known inulin products to produce, if possible at all, such nutritional effects.

[0058] The mammals are particularly dogs, cats,horses, rabbits, pigs, piglets and calves.

[0059] The inulin product of the invention has the potential to quickly and significantly modify and modulate the composition of the bacterial flora in the large intestine, in the proximal part as well as in the distal part of the large intestine of humans, mammals and other vertebrates, which beings may be healthy, disfunctioned or diseased.

[0060] By disfunctioned humans, mammals and other vertebrates is meant herein non-diseased beings in which a bodily function is not functioning optimally, possibly leading to a higher risk for the development or leading to the development of a disease later on.

[0061] Without being bound by the following hypothesis, the inventors assume that the improved nutritional benefits of the inulin product of the invention result from the presence of an EFI component and a HFI component in the defined specific weight ratio, which is such that the specific amount of easily fermentable inulin in said inulin product selectively stimulates the growth and metabolic activity of Bifidobacteria and other beneficial bacteria in the proximal part of the large intestine of humans, mammals and other vertebrates, thus modifying and modulating the current bacterial flora into a flora composition much more consisting of beneficial bacteria and much less of undesirable bacteria than the current flora composition, and that these activated bacteria are dragged together with the specific amount of unaltered hardly fermentable inulin of the inulin product of the invention from said proximal part into the distal part of the large intestine. On arrival in the distal part, the activated bacteria, under pressure of the depletion of EFI (being consumed by the bacteria in the proximal part of the large intestine), trigger the fermentation of the HFI which, accordingly, will be quickly and completely fermented in the distal part (the transversal as well as the descendent part) of the large intestine. The inulin product of the invention thus provides on the one hand a HFI component which will reach almost unaltered the distal part of the large intestine where its fermentation is most beneficial, whereas on the other hand, through its EFI component, the inulin product of the invention ensures that the fermentation of said HFI in the

distal part of the large intestine is readily started by activated bacteria and is proceeding well to complete fermentation, which in turn results in providing one or more improved inulin-associated nutritional effects/benefits. The inulin product of the invention thus has the potential to modify and/or modulate the fermentation pattern of inulin in the large intestine, particularly in the distal part of the large intestine of humans, mammals and other vertebrates.

**[0062]** In a further embodiment, the present invention relates to a method for preparing an inulin product according to the invention, consisting in mixing the EFI component and the HFI component in the above defined specific weight ratio. The mixing can be carried out by conventional techniques, such as for example by dry mixing of the components or by wet mixing of the components, optionally followed by isolation of the formed inulin product in dry form via conventional techniques, e.g. via spray-drying. Wet mixing techniques include (a) mixing of the components dissolved, dispersed or suspended in a liquid, optionally followed by isolation of the formed inulin product via known techniques such as e.g. spray-drying, (b) mixing one of the components in dry form (preferably in powder form), in neat form or in solution, dispersion or suspension in a liquid, into the other component in neat form, in solution, dispersion or suspension in a liquid, the liquids being preferably the same, optionally followed by isolation of the formed inulin product by known techniques, typically by spray-drying, (c) preparing separately a solution, dispersion or suspension of each of the components in a liquid, followed by mixing them and isolation of the formed inulin product of the invention through co-drying techniques, especially co-spray-drying, and (d) agglomerating a dry mixture of said components in powder form by moistening with water in the liquid or vapour phase, followed by drying of the moist mixture in the presence of hot air, typically in an agglomerating chamber, followed by cooling and isolation of the formed particles. The particles can then be sieved to isolate an inulin product of the invention with a desired particle size while the particles outside the desired size can be recycled.

**[0063]** The inulin product of the invention is preferably manufactured by co-drying, preferably co-spray-drying, of both components in the specific weight ratio or by spray-drying one component while bringing the pulverised jet of said component during the spray-drying step into contact with the second component in particle form, in the desired specific weight ratio, in the presence of hot air in a drying chamber, thus forming co-dried particles or agglomerates. Isolation of the formed particles or agglomerates can be made conventionally.

**[0064]** Optionally, the mixing process, typically the mixing process which involves a spray-drying step, can include a conventional UHT (ultra-high-temperature) treatment step in order to produce an inulin product of acceptable microbiological quality.

**[0065]** The liquids used in the preparation of the inulin product of the invention should preferably not provoke hydrolysis of the components to a significant extent since otherwise the required specific weight ratio of the components might not be fulfilled any longer. The most suitable liquid is water which is a good solvent for short-chain inulin and agave inulin, as well as for long-chain inulin (at least at a temperature above about 80° C.).

**[0066]** The process conditions of the wet mixing process should be appropriate which means that the combination of the process parameters, including kind of liquid, pH of the solution, dispersion or suspension, temperature, and retention time (i.e. the time the components and/or the formed inulin product remain in said conditions), are selected in such a manner that no, or at least no significant, hydrolysis or degradation of the components or of the formed inulin product occurs.

**[0067]** In a further embodiment the present invention relates to compositions containing an effective amount of the inulin product of the invention, and one or more edible or pharmaceutically acceptable components. Typical compositions include food, feed, drinks, functional food, functional feed, medicaments and pharmaceuticals (including prophylactic compositions and therapeutic compositions), and intermediates thereof.

**[0068]** By functional food or feed is meant food or feed containing a food or feed ingredient that may provide a health benefit beyond the traditional nutrients it contains (definition according to the Institute of Medicine of the National Academy of Sciences (USA; 1994).

**[0069]** Said edible or pharmaceutically acceptable components are preferably selected from the group consisting of sugars (for example: glucose, fructose, sucrose, lactose, galactose, maltose, isomaltulose), polyols (for example: sorbitol, lactitol, maltitol, isomalt, mannitol, xylitol), maltodextrins, sweeteners, hydrogenated glucose syrups, food or feed additives, food or feed ingredients, food or feed intermediates, food or feed products, liquids, drinks, sources of minerals, particularly sources of calcium, of magnesium and of iron, pharmaceutically acceptable excipients, therapeutically active substances, medicaments and pharmaceutical compositions containing one or more active ingredients.

**[0070]** By effective amount is meant herein an amount of the inulin product of the invention which provides said improved nutritional effects/benefits in humans, mammals and other vertebrates when the composition is orally or enterally taken, preferably regularly taken at a daily dose.

**[0071]** A particularly advantageous and preferred composition according to the present invention comprises the inulin product of the invention in the presence of an edible or pharmaceutically acceptable, bio-available source of one or more minerals, particularly a source of calcium and/or magnesium and/or iron, such as for example dairy products and salts and complexes of calcium, magnesium and iron.

**[0072]** Typically the bio-available amount of a mineral in said source of minerals that is present in a daily dose of the composition of the invention equals an amount which corresponds to the recommended daily dose (RDI value) for said mineral. However, said composition may also contain less or more of said bio-available mineral than the recommended daily dose.

**[0073]** The compositions according to the invention can be prepared by conventional techniques, including, for example, mixing an inulin product of the invention with at least one edible or pharmaceutically acceptable component, or, alternatively, by mixing the EFI component and the HFI component in the specified weight ratio according to the invention, together with one or more of said edible or

pharmaceutically acceptable components, optionally followed by bringing the obtained composition in a desired form by conventional techniques. The composition of the invention may appear as a solid, a semi-solid such as a cream or paste, a gel, a liquid, a dispersion, a suspension or an emulsion, in any desired form.

**[0074]** The composition may appear, for example, in the form of all kinds of food, feed, drink, functional food and functional feed, e.g. as bread, cookies and biscuits, cheese and other dairy products, chocolate, jam, pudding and other dairy desserts, spreadable products, frozen desserts and ice-cream; in the form of a pharmaceutical composition and medicament, e.g. as a powder, an aggregate, a granulate, a tablet, a coated tablet, a lozenge, a capsule, a drink, a syrup, a composition for tube feeding, for enteral intake, for oral administration and for enteral administration.

**[0075]** Furthermore, the inulin product of the invention and composition thereof can be in the form of a consumer product, being a product or composition presented in the form and/or package which allows its direct use by the consumer, for example in the form of tablets, granules or powder preferably packed in a unit dose.

**[0076]** In a further aspect, the present invention relates to the use of an inulin product or a composition according to the present invention as a food, a feed, a drink, a consumer product, a functional food, a functional feed, a pharmaceutical, a medicament, or an intermediate thereof.

**[0077]** In a further aspect, the present invention relates to the use of an inulin product or a composition according to the present invention, by oral and/or enteral intake or administration, preferably of a daily dose, for modifying and modulating the bacterial flora composition in the large intestine, particularly in the distal part of the large intestine, and/or for modifying and modulating the fermentation pattern of inulin, in healthy, disfuntioned or diseased humans, mammals and other vertebrates, as well as for providing one or more improved inulin-associated nutritional effects/benefits in humans, mammals and other vertebrates, which beings may be healthy, disfuntioned or diseased.

**[0078]** In a further embodiment, the present invention relates to the inulin product and a composition according to the invention, for use as a food, feed, drink, consumer product, composition, functional food, functional feed, pharmaceutical, medicament, or intermediate thereof, in particular for modifying and modulating the bacterial flora composition in the large intestine, particularly in the distal part of the large intestine, for modifying and modulating the fermentation pattern of inulin, as well as for providing one or more improved inulin-associated nutritional effects/benefits, in humans, mammals and other vertebrates, which beings may be healthy, disfuntioned or diseased.

**[0079]** In still a further embodiment, the present invention relates to the use of an inulin product and a composition according to the invention for the manufacture of a composition, food, feed, drink, consumer product, functional food, functional feed, pharmaceutical, medicament, or intermediate thereof, particularly for modifying and modulating the bacterial flora composition in the large intestine, particularly in the distal part of the large intestine, for modifying and modulating the fermentation pattern of inulin, as well as for providing one or more improved inulin-associated nutritional effects/benefits in healthy, disfuntioned or diseased humans, mammals and other vertebrates.

**[0080]** Said improved inulin-associated nutritional effects/benefits include dietary fibre effects, particularly in the colon, more particularly in the distal part of the colon, including the generation of beneficial metabolites such as short chain fatty acids (SCFA's) and the generation of bacterial biomass, the reduction of the colonic pH, a prebiotic action and/or a bifidogenic effect, particularly in the distal part of the large intestine, including an increase of the counts of Bifidobacteria with a concurrent reduction of the counts of non-desirable and/or pathogenic bacteria, which in turn will benefit the prevention and treatment of intestinal disfunctions, disorders and diseases.

**[0081]** Furthermore, said improved nutritional effects/benefits also include a modulation of the lipid metabolism, a stimulation of the immune system, the reduction of the risk of cancer, and preventive and therapeutic effects against cancer, particularly against mammary cancer and colon cancer.

**[0082]** Further effects/benefits include improved absorption of minerals in the body, particularly of calcium and magnesium, improvement of the bone mineral density and of the bone structure in healthy, disfunctioned or diseased humans, mammals and other vertebrates, and the possibility to prevent, delay, curb or significantly reduce the bone demineralisation process and osteoporosis in humans, particularly in post-menopausal women, in gastrectomised humans, in elderly persons and in diseased humans, particularly in humans suffering from osteoporosis.

**[0083]** Moreover, said effects/benefits also enable the building up of a strong skeleton in growing children, growing adolescents, in growing mammals and other vertebrates, and to increase in humans the peak bone mass, which in turn enables to prevent or postpone bone demineralisation and osteoporosis later in life, particularly in post-menopausal women.

**[0084]** Furthermore, the inventors surprisingly found that the amount of EFI present in the inulin products of the invention exerts such a pronounced activating effect on the intestinal flora that the amount of HFI present in said products is readily and completely fermented in the distal part of the large intestine. This property of said inulin products of the invention results in the fact that the improved nutritional effects/benefits can be obtained by a lower daily dose of said inulin products compared to the daily dose of known inulin products which is required to obtain a similar effect, if possible at all. The said improved nutritional effects/benefits in humans, e.g. improved mineral absorption, are indeed already obtained with a daily dose in adults of as little as about 4 g inulin product of the invention, either as the inulin product per se (included also the industrial grade inulin product) or in a composition (g is gram of the real inulin product according to the invention).

**[0085]** The daily dose of real inulin product of the invention suitable for generating said improved nutritional effects/benefits in adults preferably ranges from about 4 g to about 12 g, corresponding to about 50 mg to about 150 mg/day/kg body weight, more preferably from about 6 g to about 10 g, and is typically about 8 g, and for babies and children the daily dose preferably ranges from about 40 mg to about 400

mg/day/kg body weight. Said small daily dose of inulin product of the invention results in considerable additional benefits for humans since it increases the comfort of the inulin intake compared to the rather large corresponding quantities of known inulin products (ranging from about 15 g to about 40 g per day) that are required for generating similar effects/benefits, if possibly at all. Besides, as a result of said small daily taken quantity of inulin, the humans will not encounter the intestinal side effects which are often associated with the intake of rather large quantities of inulin, such as flatulence, intestinal pressure, bloating, intestinal spasms and/or diarhoea.

**[0086]** Although the particular improved nutritional effects/benefits of the inulin product of the present invention have been explicitly disclosed herein with respect to humans and mammals, it has to be noted that the oral and/or enteral intake of the inulin product of the present invention also generates one or more of said improved nutritional effects/benefits, in other vertebrates, in spite of the possibly different digestive system of the latter animals compared to the systems of humans and mammals. The said vertebrates include fish, for example: salmon and turbot; amphibians; reptiles; and birds, for example: ostriches and poultry, particularly chicken and turkey.

**[0087]** The invention is further illustrated by the examples below.

**[0088]** **FIG. 1**: represents a dionex chromatogram of an inulin product according to the invention consisting of a mixture of EFI (oligofructose with a (DP) mainly from 2 to 7) and HFI (chicory inulin with a (DP) of about 25) in a weight ratio 45/55.

## EXAMPLE 1

### Rat Study to Evaluate the Effect of the Intake of an Inulin Product According to the Invention Compared to Oligofructose and Long-chain Inulin on the Absorption of Calcium

**[0089]** Calcium absorption was measured in four groups of Wistar male rats (groups of 9 or 10 rats; age of 6 weeks; weight of 160-180 g):

**[0090]** group 1: control group, receiving a diet of standard semi-synthetic food corresponding to the recommendations of the American Institute of Nutrition with mineral content according to AIN 1976;

**[0091]** group 2: group receiving said standard semi-synthetic food containing industrial grade oligofructose of (DP) mainly from 2 to 7;

**[0092]** group 3: group receiving said standard semi-synthetic food containing industrial grade long-chain chicory inulin with a (DP) of about 25;

**[0093]** group 4: group receiving said standard semi-synthetic food containing an inulin product according to the invention consisting of industrial grade oligofructose of (DP) mainly from 2 to 7 and industrial grade long-chain chicory inulin with a (DP) of about 25, in a weight ratio real EFI/real HFI of 45/55, prepared by co-spray-drying, corresponding to the product of **FIG. 1**.

**[0094]** After gradual adaptation of the rats to the diet during three weeks, the rats were kept for a fourth week in metabolic cages and received the respective diet containing 10 wt % of the tested oligofructose or inulin product (100 g food+100 g water per day). Food intake was monitored and the last four days of the fourth week the faeces and urine were collected to determine the digestive absorption of calcium. Calcium in urine samples was determined by atomic absorption spectrometry. Samples of the diet and of the lyophilised and grounded faeces were calcinated at 500° C., and the ashes were taken up in nitric acid/hydrogen peroxide, and after dilution with milli-Q water, calcium was determined by atomic absorption spectrometry.

**[0095]** The digestive absorption was calculated by the following formula:

daily digestive absorption =

quantity orally taken − quantity excreted via the faeces;

% digestive absorption = 100 ×

{(quantity orally taken − quantity excreted via the faeces)/quantity orally taken}

**[0096]** The results are presented in Table 1 below. The data of Table 1 show that compared to the control group, and taking into account that the urinary excretion of calcium did not differ amongst all 4 groups, calcium absorption was increased in all test groups, but only the increase of calcium absorption in group 4 (by about 20%) was found to be statistically significant versus the control group.

TABLE 1

Effect of oral intake of oligofructose or inulin on the intestinal absorption of calcium in the rat.

| | Digestive calcium absorption (%) | | | |
|---|---|---|---|---|
| | Group 1** (control) | Group 2** (oligofructose) | Group 3** (long-chain inulin) | Group 4 (inulin product of invention) |
| M ± SD* | 47.9 ± 5.5 | 52.7 ± 6.0 | 54.1 ± 5.6 | 58.1 ± 7.4 |
| min–max | 39.1–55.9 | 44.0–63.1 | 45.0–61.3 | 50.1–61.2 |

*Mean value ± standard deviation;
**comparative

## EXAMPLE 2

### Human Study to Evaluate the Effect of the Intake of an Inulin Product According to the Invention Compared to Oligofructose on the Calcium Absorption in Young Adolescent Girls

**[0097]** Calcium absorption was measured in adolescent, healthy girls (11 to 14 years old; of 44 kg mean body weight ). Ethical approval and informed consent were obtained in all cases. Only subjects were elected for the study with habitual calcium intakes between 500 mg and 1400 mg/day. Subjects were excluded from the study if they had a chronic gastrointestinal disease, renal failure, or disorders of calcium homeostasis, if they were taking prescription medication,

smoking, were on a contraceptive pill or had a weight greater than the 90th percentile for age. The subjects were studied using a randomised, double-blind, cross-over design. The subjects were randomised in two separate groups to receive two packets of 4 g servings of oligofructose or inulin product according to the invention daily for 3 weeks and two packets of 4 g servings of placebo daily for 3 weeks. The studies were separated by a 2-week wash-over period. The subjects received oligofructose or inulin product of the invention and placebo in a random order, and the investigators were blinded to the treatment assignment.

**[0098]** Two identical protocols were carried out simultaneously. In Protocol I (n=30) the test product was industrial grade oligofructose with (DP) mainly from 2 to 7. In Protocol II (n=29) the test product was an inulin product according to the invention, composed of a mixture of industrial grade oligofructose with (DP) mainly from 2 to 7 and industrial grade long-chain chicory inulin with a (DP) of about 25, in a weight ratio of 45/55 (weight ratio on total real short-chain inulin and real long-chain inulin content), prepared by co-spray-drying, corresponding to the product of **FIG. 1**. In both protocols the placebo was packed and presented in an identical manner to the oligofructose/inulin. At the end of each 3-week adaptation period (to 8 g/day), calcium absorption was measured using a previously validated dual tracer stable isotopes technique. Furthermore, a baseline urine sample was collected from the subjects. The subjects consumed a low-calcium breakfast and a glass of calcium fortified orange juice to which was added one 4 g packet of oligofructose, inulin product or placebo, and 10 mcg of $^{46}$calcium. Immediately after breakfast 1.5 mg of $^{42}$calcium was infused intravenously over 2 to 3 minutes. The mid-day meal contained approximately 400 mg calcium either as calcium fortified orange juice, milk or yoghurt. The evening meal contained another serving of calcium fortified orange juice, 10 mcg of $^{46}$calcium and another 4 g packet of oligofructose, inulin product or placebo. The subjects consumed daily approximately 1300 mg calcium during the 8 week-study. A 48 hour urine collection was started immediately after isotope administration. Calcium absorption was measured by the ratio of the cumulative fractional excretion of the oral and intravenous isotopes in the 48 hour urine collection. Samples were purified using an oxalate precipitation method and isotope ratios were measured by thermal ionization magnetic sector mass spectrometry.

**[0099]** Compliance was assessed by a count of opened and unopened packets and any packet not accounted for was assumed to be unopened. The compliance for oligofructose and for the inulin product of the invention was very good as is shown by the data presented in Table 2 below.

TABLE 2

Compliance

| compliance | oligofructose** | inulin product of invention |
|---|---|---|
| Mean ± SD* | 95% ± 7 | 94% ± 12 |

*Mean value ± standard deviation;
**comparative

**[0100]** The results of the calcium measurements are given in Table 3 below.

TABLE 3

Calcium absorption in healthy adolescent girls

| Protocol carbohydrate | Calcium absorption % (as Mean ± SD)* | p-value versus placebo (sucrose) |
|---|---|---|
| Protocol I | | |
| sucrose** | 30.9 ± 10.0 | |
| oligofructose** | 31.8 ± 9.3 | 0.75 |
| Protocol II | | |
| sucrose** | 32.3 ± 9.8 | |
| inulin product of the invention | 38.2 ± 9.8 | 0.007 |

*Mean value ± standard deviation;
**comparative

**[0101]** The results in Table 3 indicate that there was no significant difference (p=0.89) in calcium absorption between Protocol I and Protocol II with sucrose (placebo), and also that oligofructose did not significantly alter calcium absorption. With the inulin product according to the invention, however, a significant increase in calcium absorption was obtained versus the placebo and also vis à vis oligofructose, i.e. from 31.8% to 38.2%, which corresponds to a relative increase of calcium absorption of 20%.

**[0102]** Moreover, the study of the urinary calcium excretion showed that, as seen from the data presented in Table 4 below, there were no significant differences in urinary calcium excretion between any of the study groups.

**[0103]** From the above experiment it can be concluded that, at the currently recommended intake of calcium (about 1300 mg/day for adolescent girls), the intake of an amount as little as 8 g/day inulin product according to the invention significantly increased the calcium absorption, without a compensatory increase in urinary calcium excretion, whereas the intake of 8 g/day oligofructose by the same population under the same experimental conditions did not significantly increase calcium absorption.

TABLE 4

Urinary calcium excretion in healthy adolescent girls

| Protocol carbohydrate | Urinary calcium excretion mg/day (M ± SD)* | p-value versus placebo (sucrose) |
|---|---|---|
| Protocol I | | |
| sucrose** | 71 ± 48 | |
| oligofructose** | 79 ± 50 | 0.75 |
| Protocol II | | |
| sucrose** | 65 ± 54 | |
| inulin product of the invention | 71 ± 50 | 0.57 |

*Mean value ± standard deviation;
**comparative

## EXAMPLE 3

Rat Study Evaluating the Effect of the Intake of an Inulin Product of the Invention Compared to Oligofructose on the Absorption of Magnesium

**[0104]** Magnesium absorption was measured in Wistar male rats (groups of 9 or 10 rats; age of 6 weeks; weight of 160-180 g) with:

**[0105]** group 1: control group, receiving a diet of standard semi-synthetic food corresponding to the recommendations of the American Institute of Nutrition with mineral content according to AIN 1976;

**[0106]** group 2: group receiving said standard semi-synthetic food containing industrial grade oligofructose of (DP) mainly from 2 to 7;

**[0107]** group 3: group receiving said standard semi-synthetic food containing an inulin product according to the invention consisting of industrial grade oligofructose of (DP) mainly from 2 to 7 and industrial grade long-chain chicory inulin with a (DP) of about 25, in a weight ratio real short-chain inulin/real long-chain inulin of 45/55, prepared by co-spray-drying, corresponding to the product of **FIG. 1**.

**[0108]** After three weeks of gradual adaptation to the diet, the rats were kept for a fourth week in metabolic cages and received their respective diet which contained 10 wt % of the tested oligofructose or inulin product (100 g food+100 g water per day). Food intake was monitored and the last four days of the fourth week the faeces and urine were collected to determine the digestive absorption of magnesium. Magnesium was determined in the samples of urine, faeces and feed, by atomic absorption spectrometry and the digestive magnesium absorption was calculated as indicated in Example 1. The results are shown below in Table 5.

TABLE 5

Effect of oral intake of oligofructose or inulin product on the intestinal absorption of magnesium in the rat.

| | Digestive magnesium absorption (%) | | |
|---|---|---|---|
| | Group 1** (control) | Group 2** (oligofructose) | Group 3 (inulin product of invention) |
| Mean ± SD* | 48.8 ± 5.3 | 71.3 ± 4.5 | 76.7 ± 6.7 |
| min–max | 39.3–57.0 | 65.0–78.6 | 67.6–90.4 |

*Mean value ± standard deviation;
**comparative

**[0109]** The data of Table 5 show that, compared to the control group, magnesium absorption was statistically significantly increased in groups 2 and 3, in particular in group 3 with a relative increase of magnesium absorption of about 57%, and that the increase in magnesium absorption was more pronounced with the inulin product of the invention than with oligofructose.

## EXAMPLE 4

Evaluation of the Effect of the Intake of an Inulin Product of the Invention Compared to Oligofructose and Long-chain Inulin on Lipid Metabolism in the Rat

**[0110]** The effect of oligofructose and inulin products on lipid metabolism was measured in Zucker male rats. Zucker rats have a mutation of the leptine receptor which makes that these rats rapidly develop fat tissue instead of lean meat tissue and present as further characteristics hypertriglyceridemia, insulin resistance and hepatic steatose. The following groups of rats (groups of 7 rats; age of 5 weeks) were involved in the study:

**[0111]** group 1: control group, receiving a diet of standard feed;

**[0112]** group 2: group receiving a diet of said standard feed containing oligofructose of (DP) mainly from 2 to 7;

**[0113]** group 3: group receiving a diet of said standard feed containing long-chain chicory inulin with a (DP) of about 25;

**[0114]** group 4: group receiving a diet of said standard feed containing an inulin product according to the invention consisting of oligofructose of (DP) mainly from 2 to 7 and long-chain chicory inulin with a (DP) of about 25, in a weight ratio real short-chain inulin/real long-chain inulin of 45/55.

**[0115]** After one week of adaptation to the diet (control diet or diet containing respectively 5 wt % of oligofructose, long-chain inulin or inulin product according to the invention), the rats received the study diet containing 10 wt % of the respective tested product for 6 weeks and were then sacrificed. Then, according to standard techniques, the parameters fat tissue weight, liver weight, concentration of liver triglycerides and activity of the enzyme fatty acid synthase in the liver were determined. The results are presented in Table 6 below.

TABLE 6

Effect of oligofructose or inulin products on biometric and biochemical parameters in Zucker rats

| Group | Fat tissue weight (g) * | Liver weight (g) * | Liver triglycerides (mg/g liver) * | Activity of liver fatty acid synthase (FAS (mU/mg protein) * |
|---|---|---|---|---|
| 1 (control)** | 5.94 ± 0.23 | 18.32 ± 0.40 | 452.44 ± 72.05 | 32.31 ± 1.88 |
| 2 (oligofructose)** | 5.70 ± 0.23 | 17.28 ± 0.67 | 414.97 ± 50.12 | 22.4 ± 2.6 |
| 3 (long-chain inulin)** | 6.06 ± 0.28 | 18.17 ± 1.32 | 500.66 ± 62.68 | 25.3 ± 2.6 |

TABLE 6-continued

Effect of oligofructose or inulin products on biometric and biochemical parameters in Zucker rats

| Group | Fat tissue weight (g) * | Liver weight (g) * | Liver triglycerides (mg/g liver) * | Activity of liver fatty acid synthase (FAS (mU/mg protein) * |
|---|---|---|---|---|
| 4 (inulin product of invention) | 4.99 ± 0.19 | 14.83 ± 0.60 | 286.64 ± 47.73 | 13.7 ± 0.52 |

* Mean value ± standard deviation;
**comparative

**[0116]** From the data of Table 6 it clearly follows that the inulin product according to the invention has the most pronounced effect on lipid metabolism compared to oligofructose and long-chain inulin.

## EXAMPLE 5

### Evaluation of the Effect of an Inulin Product of the Invention on Azoxymethane (AOM) Induced Carcinogenesis in Rat Colon

**[0117]** Carcinogenesis is known to be a complex multi-step process that commonly occurs in three stages, named initiation, promotion and progression. Initiation is defined as exposure of normal cells to carcinogenic agents, such as certain chemicals, resulting in changes of the genomic level which promote selective growth of the cells. Promotion involves clonal expression of the initiated cells that is generally associated with altered morphological and/or phenotypic changes. Progression involves genotypic and phenotypic changes, associated with malignancy and metastasis.

**[0118]** Aberrant crypt foci (ACF) are putative precursor lesions from which adenomas and carcinomas may develop in the colon. Inhibitors of ACF formation have been shown to reduce the incidence of colon tumours in laboratory animals (Wargovich et al., (24)). Based upon these findings, ACF induction in animal models can be used to evaluate compounds for their potential preventive and therapeutic properties against colon cancer.

**[0119]** Example 5 describes an experiment wherein the effect of an inulin product of the invention on AOM induced ACF formation in rat colon has been evaluated in comparison with known inulin products. The experiment was run as follows. The animals involved were male Fisher 344 weanling rats that were, after one week of acclimatisation on control diet, divided into the desired number of groups and assigned control food based on AIN93G diet or experimental food being control food but containing the evaluated product (10 wt %) at the expense of corn starch. Feed and water were provided ad libitum. Weekly body weights and daily feed intakes were recorded. After two weeks on the assigned diet, all animals received a subcutaneous injection of AOM (Sigma, St. Louis Mo., USA) in saline at 16 mg/kg body weight at 7 and 8 weeks of age. At the end of the feeding period which was eight weeks after the last AOM injection, the animals were sacrificed using $CO_2$ euthanasia. The colons were removed, flushed with potassium phosphate buffer and then fixed overnight with 10% buffered formalin. The colons were cut into proximal and distal portions of equal length, which in turn were cut into 2 cm long segments. The segments were examined, after staining with 0.5% methylene blue, under a light microscope, for ACF or tumours, and the total number of ACF as well as the number of crypts per focus were scored. The data were analysed using the SAS statistical program and means were separated using the Tukey's Studentized Range Test. The composition (in g/kg) of the diets is shown in Table 7.

TABLE 7

Composition of the diets used in the experiment

| | control diet** amended to contain (g/kg) | | |
|---|---|---|---|
| Animal group | corn starch | inulin product# | type of inulin product# |
| Group 1* (Control group) | 397.5 (=control diet) | 0.0 | — |
| Group 2* | 297.5 | 100.0 | RAFTILINE ® HP |
| Group 3* | 297.5 | 100.0 | RAFTILINE ® ST |
| Group 4* | 297.5 | 100.0 | RAFTILOSE ® P95 |
| Group 5 | 297.5 | 100.0 | Inulin product of invention |

*comparative test
**Control diet (in g/kg): corn starch (397.5); inulin product (0.0); casein (85% protein) (200); dextrinized corn starch (90–94% tetracharides) (132); sucrose (100); soybean oil (no additive) (70); Fiber (Solka-Floc ®, 200FCC of FS&D, St. Louis, MO, USA or equivalent) (50); mineral mix (AIN 93G-MX) (35); vitamin mix (AIN-93 VX) (10); L-cystine (3); choline bitartrate ((41.1% choline) (2.5).
#products obtained from ORAFTI ® (Belgium): RAFTILINE ® HP (long-chain chicory inulin with a $\overline{(DP)}$ of about 25); RAFTILINE ® ST (standard grade chicory inulin with $\overline{(DP)}$ of about 12); RAFTILOSE ® P95 (industrial grade oligofructose of (DP) mainly from 2 to 7); Inulin product of invention: consisting of industrial grade oligofructose of (DP) mainly from 2 to 7 and industrial grade long-chain chicory inulin with a $\overline{(DP)}$ of about 25, in a weight ratio real short-chain inulin/real long-chain inulin of 45/55, prepared by co-spray-drying, corresponding to the product of FIG. 1.

**[0120]** Results:

**[0121]** No statistically significant difference was found in body weight gains and in mean daily feed intake between rats fed control diet and experimental diets. A small group of rats (blanco control group) receiving no AOM but only saline and fed the control diet showed no evidence of ACF formation in the colon. Results concerning the induction by AOM of ACF in rat colon are shown in Table 8 below.

TABLE 8

Effect of inulin products on ACF induction in rat colon by AOM

| Group | number of animals | ACF in proximal part | ACF in distal part | ACF total |
|---|---|---|---|---|
| Group 1 * (Control) | 12 | 39.92 ± 0.82 [a] | 114.17 ± 1.57 [a] | 155.42 ± 1.64 [a] |
| Group 2 * (RAFTILINE ® HP) | 10 | 13.30 ± 1.45 [c] | 70.20 ± 1.18 [c] | 83.50 ± 1.67 [c] |
| Group 3 * (RAFTILINE ® ST) | 10 | 14.20 ± 0.57 [c] | 93.90 ± 1.75 [b] | 109.50 ± 2.15 [b] |

TABLE 8-continued

Effect of inulin products on ACF induction in rat colon by AOM

| Group | number of animals | ACF in proximal part | ACF in distal part | ACF total |
|---|---|---|---|---|
| Group 4 * (RAFTILOSE ® P95) | 10 | 24.80 ± 1.32 [b] | 92.10 ± 1.35 [b] | 116.90 ± 1.24 [b] |
| Group 5 (Inulin product of invention) | 12 | 14.90 ± 0.94 [c] | 63.00 ± 2.05 [d] | 75.90 ± 1.54 [d] |

[a b c d] Means ± SEM within the column with different letters are significantly different ($p < 0.05$) by Tukey's Studentized Range Test.
* comparative test

[0122] From Table 8 it follows that by AOM treatment ACF were induced in the proximal as well as in the distal part of the rat colon, but predominantly in the distal part.

[0123] From Table 8 it further follows that ACF induction by AOM in the proximal part as well as in the distal part of the rat colon is reduced by various inulin products, including RAFTILINE® HP, RAFTILINE® ST, RAFTILOSE®P95, but significantly better by the inulin product according to the invention, as also shown in Table 9 below in which said reduction is presented in percent compared to the control group.

TABLE 9

Reduction in ACF induction in rat colon by diets containing inulin products

| Group | Reduction by diets containing inulin products of ACF induction in rat colon by AOM (% compared to control group) proximal part | distal part | total |
|---|---|---|---|
| Group 1 * (Control group) | — | — | — |
| Group 2 * (RAFTILINE ® HP) | 66.7 | 38.5 | 46.3 |
| Group 3 * (RAFTILINE ® ST) | 64.4 | 17.8 | 29.5 |
| Group 4 * (RAFTILOSE ® P95) | 37.9 | 19.3 | 24.8 |
| Group 5 (Inulin product of invention) | 62.7 | 44.8 | 51.2 |

* comparative test

[0124] From these results it clearly follows that the inulin product according to the invention inhibits in a much stronger manner than short-chain inulin, standard grade chicory inulin, and long-chain chicory inulin the induction of AOM induced ACF in rat colon.

[0125] Accordingly, the inulin products of the present invention are considered to be much more effective in the prevention and reduction of the risk of developing cancer as well as in the treatment of cancer, particularly colon cancer, in humans, mammals and other vertebrates, than short-chain inulin, standard grade inulin and long-chain inulin.

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1. Inulin product consisting of a mixture of an easily fermentable inulin (EFI) component and a hardly fermentable inulin (HFI) component, characterised in that the weight ratio EFI/HFI ranges from 10/90 to 70/30, the EFI component is free from agave inulin and consists of a short-chain inulin with a degree of polymerisation (DP)<10, the HFI component consists of a long-chain inulin with a number average degree of polymerisation (DP)≧20, and the total content of inulin with (DP)=9 and (DP)=10 is maximally 5% (% is wt % on total inulin, determined by gas liquid chromatography (GLC)).

2. Inulin product consisting of a mixture of an easily fermentable inulin (EFI) component and a hardly fermentable inulin (HFI) component, characterised in that the weight ratio EFI/HFI ranges from 10/90 to 70/30, the EFI component consists of agave inulin or any mixture of agave inulin with a short-chain inulin with a (DP)<10, the HFI component is a long-chain inulin with a (DP)≧20, and the total amount of inulin with (DP)=9 and (DP)=10 is maximally 5% (% is wt % on total inulin, determined by GLC).

3. Inulin product according to any one of claims **1** or **2**, wherein the total content of inulin with (DP)=9 and (DP)=10 is maximally 2%.

4. Inulin product according to any one of claims **1** or **2**, wherein the total content of inulin with (DP)=9 and (DP)=10 is maximally 1%.

5. Inulin product according to any one of claims 1 to 4 wherein the weight ratio of the EFI/HFI components ranges from 35/65 to 65/35.

6. Inulin product according to any one of claims 1 to 5 wherein the HFI component consists of long-chain chicory inulin.

7. Inulin product according to any one of claims 1 to 6, characterised in that it is an industrial grade inulin product consisting of a mixture of industrial grade oligofructose containing maximally 20% in total of glucose, fructose and sucrose (% is wt % on total carbohydrates) or industrial grade agave inulin, or any mixture of said oligofructose and said agave inulin as EFI component, and industrial grade long-chain inulin as HFI component, in a weight ratio real short-chain inulin/real long-chain inulin contained in said industrial grade components as defined in any one of claims **1**, **2** or **5**.

8. Inulin product according to claim 7, wherein the HFI component is industrial grade long-chain chicory inulin.

9. Inulin product according to any one of claims **7** or **8** wherein the short-chain inulin is industrial grade oligofructose containing maximally 8% in total of glucose, fructose and sucrose (% being wt % on total carbohydrates).

10. Method for preparing an inulin product defined in any one of claims 1 to 9 consisting in dry mixing of the EFI component and the HFI component in the specified weight ratio or consisting in wet mixing of the EFI component and the HFI component in the specified weight ratio, optionally followed by isolation of the obtained inulin product in dry form, said isolation step optionally including a spray-drying step.

11. Composition containing an inulin product defined in any one of claims 1 to 9, and one or more edible or pharmaceutically acceptable components.

12. Composition according to claim 11 wherein the one or more edible or pharmaceutically acceptable components are selected from the group consisting of sugars, polyols, hydrogenated glucose syrups, maltodextrins, sweeteners, food ingredients, feed ingredients, food additives, feed additives, food intermediates, feed intermediates, food products, feed products, edible liquids, drinks, bio-available sources of minerals, pharmaceutically acceptable excipients, pharmaceutically active substances, therapeutically active substances, pharmaceutical compositions and medicaments.

13. Composition according to claim 12 wherein the bio-available sources of minerals contain a source of calcium and/or a source of magnesium and/or a source of iron.

14. Composition according to any one of claims 11 to 13 which is a food, feed, drink, consumer product, functional food, functional feed, pharmaceutical composition or medicament.

15. Method for preparing a composition defined in any one of claims 11 to 14, comprising mixing an inulin product defined in any one of claims 1 to 9, or mixing the EFI component and the HFI component defined in any one of claims 1 to 9 in the weight ratio defined in any one of claims **1**, **2** or **5**, with at least one other component defined in any one of claims 11 to 13, optionally followed by bringing the composition in the desired form.

16. Inulin product defined in any one of claims 1 to 9 or composition defined in any one of claims 11 to 13, for use as a food, feed, drink, consumer product functional food, functional feed, pharmaceutical or medicament.

17. Use of an inulin product defined in any one of claims 1 to 9 or of a composition defined in any one of claims 11 to 13 for the manufacture of a composition, food, feed, drink, consumer product, functional food, functional feed, pharmaceutical, medicament, or intermediate thereof.

18. Use according to claim 17, for the manufacture of a composition, food, feed, drink, consumer product, functional food, functional feed, pharmaceutical or medicament for modifying or modulating the bacterial flora composition in the large intestine or in the distal part of the large intestine of humans, mammals or other vertebrates.

19. Use according to claim 17 for the manufacture of a composition, food, feed, drink, consumer product, functional food, functional feed, pharmaceutical or medicament for modifying or modulating the fermentation pattern of inulin in the large intestine or in the distal part of the large intestine of humans, mammals or other vertebrates.

20. Use according to claim 17, for the manufacture of a composition, food, feed, drink, consumer product, functional food, functional feed, pharmaceutical or medicament for providing one or more improved inulin-associated nutritional effects/benefits in humans, mammals or other vertebrates compared to the effects provided by known inulin products or compositions thereof.

21. Use according to claim 20 wherein said improved inulin-associated nutritional effects/benefits are selected from the group consisting of dietary fibre effects, modulation of gut function, prebiotic action and/or bifidogenicity, increased absorption of minerals, increased absorption of calcium and/or of magnesium and/or of iron, bone mineral density increase, bone mineral content increase, peak bone mass increase, improvement of the bone structure, reduction of bone mineral density loss, reduction of loss of bone structure, modulation of lipid metabolism, stimulation of the immune system, prevention or reduction of the risk of cancer, prevention or reduction of the risk of colon cancer and prevention or reduction of the risk of breast cancer.

**22**. Use according to claim 21, for the manufacture of a composition, food, feed, drink, consumer product, functional food, functional feed, pharmaceutical or medicament, for improving the absorption of calcium and/or magnesium in healthy humans.

**23**. Use according to claim 17 for the manufacture of a pharmaceutical or medicament for preventing or reducing bone demineralisation or the risk of osteoporosis, or for treating osteoporosis in humans.

**24**. Use according to claim 23 wherein the human is a post-menopausal woman or an elderly person.

**25**. Use according to claim 17 for the manufacture of a pharmaceutical or medicament for preventing cancer, reducing the risk of cancer, or for the treatment of cancer.

**26**. Use of an inulin product defined in any one of claims 1 to 9 or of a composition defined in any one of claims 11 to 14 for modifying or modulating the bacterial flora composition in the large intestine or in the distal part of the large intestine of humans, mammals or other vertebrates.

**27**. Use of an inulin product defined in any one of claims 1 to 9 or of a composition defined in any one of claims 11 to 14 for modifying or modulating the fermentation pattern of inulin in the large intestine or in the distal part of the large intestine of humans, mammals or other vertebrates.

**28**. Use of an inulin product defined in any one of claims 1 to 9 or of a composition defined in any one of claims 11 to 14 for providing one or more improved inulin-associated nutritional effects/benefits in humans, mammals or other vertebrates, compared to the effects provided by known inulin products.

**29**. Use according to claim 28 wherein said improved nutritional effects/benefits are the ones defined in claim **21**.

* * * * *

# Appendix 3

## Petition to add to the National List the substance "Oligofructose enriched with Inulin Documented for Calcium Absorption"

**January 12, 2007**

# EVALUATION OF THE FOOD SAFETY ASPECTS OF INULIN AND OLIGOFRUCTOSE

## GRAS Determination

Conducted by a committee of experts
On food safety convened by
Kolbye Associates
7313 Helmsdale Road
Bethesda, MD 20817

September, 1992

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# I. INTRODUCTORY OVERVIEW

As indicated in the Food, Drug and Cosmetic Act (21 CFR 170.30) GRAS food substances are exempt from the requirements of pre-marketing clearance for food additives. GRAS means generally recognized as safe. GRAS status can be established either through scientific procedures based upon published literature similar to the quality and quantity of scientific evidence that would be required for FDA approval of a food additive regulation; or a general recognition of safety can be based on history of common use in food in the U.S.A. or elsewhere prior to 1958. This latter case does not require the same quality and quantity of scientific testing data as a food additive; but is based on generally available data and information concerning use and safety, and can be determined independently by experts qualified by training and experience in evaluating food safety.

The final rule of eligibility for classification as GRAS, as amended for common use in food in section 170.3, requires a documented history of consumption of the food substance by a significant number of consumers prior to 1958. Further, the data and documentation of the use in food of the substances in question must be widely available in the published literature. Affirmation as GRAS is based largely on the history of use in food prior to 1958 or is determined by scientific evaluations that rely on publicly available scientific data. The information must be of sufficient quality to develop a consensus in the scientific community by experts qualified by training and experience in food safety to judge that the substance in question is safe for its intended use in food.

It is the considered opinion of the scientific experts in food safety who authored this report that Beneo® inulin and Beneo® oligofructose are GRAS, based upon the following reasoning:

1) Inulin has been consumed in food by a significant number of consumers prior to 1958; since it is a natural component of many commonly occurring grains and vegetables, e.g. wheat, artichokes, onions, leeks, chicory, etc. which have been natural ingredients of the human diet for centuries.
2) Oligofructose is the mixture of lower chain chemical oligomers of fructose that are present in inulin. Hence oligofructose has been also routinely present as natural ingredient of the human diet prior in 1958. It may be derived also from inulin by simple hydrolysis.
3) Inulin has been safely used in humans at intravenous dose levels of 10g in 100ml of saline since 1931 in the "Inulin Clearance Test" for renal glomerular filtration.
4) Acute, subacute and chronic feeding studies with fructo-oligosaccharides were preformed in rats and mice of both sexes; teratology, reproduction, genotoxicity and carcinogenicity studies were also completed without any adverse observed treatment-related effects.
5) Oligofructose has been administered clinically to normal and diabetic human subjects for as long as 8 weeks without untoward effects.

In the context, all the available information on Beneo® inulin and Beneo® oligofructose, as contained in the cited reference material, has been reviewed and its interpretation and assessment are described in this document.

Nutritional and Health Benefits of Inulin and Oligofructose



# Inulin and Oligofructose: Safe Intakes and Legal Status[1]

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ABSTRACT Inulin and oligofructose are a significant part of the daily diet of most of the world's population. Daily intakes for the U.S. and Europe have been estimated at up to 10 g, specifically 1–4 g for the 97th percentile in the U.S. Because both inulin and oligofructose are macroingredients, it is difficult to apply classical toxicology tests. Although some high dose animal tests have been performed, none have revealed any toxic effects. The safety of inulin and oligofructose for use in foods was evaluated by many legal authorities worldwide. As a result, both inulin and oligofructose are accepted in most countries as food ingredients that can be used without restrictions in food formulations. In the U.S., a panel of experts performed a generally accepted as safe (GRAS) Self-Affirmation Evaluation in 1992 and concluded similarly. At high doses, increased flatulence and osmotic pressure can cause intestinal discomfort. These doses vary widely from person to person and also depend on the type of food in which inulin or oligofructose is incorporated. With regard to labeling, both inulin and oligofructose are gradually being accepted as "dietary fibers" in most countries around the world. The mention of their "bifidogenic effect" on food labels has also been legally accepted in several countries. J. Nutr. 129: 1412S–1417S, 1999.

KEY WORDS: • *inulin* • *oligofructose* • *safety* • *labeling* • *acceptability*

Inulin was defined by Rose in the early 1800s as the carbohydrate substance that he isolated from the root of *Inula helenum* (Rose 1804). We now know that it is present in a wide range of plants, including common vegetables and fruits (Van Loo et al. 1995). Inulin is a polydisperse substance based on fructose polymers (De Leenheer et al. 1994).

Today, only inulin from chicory roots is commercialized as a purified food ingredient. The chicory roots that are used are of the same species (*Cichorium intybus*) that has been used for many years to produce the coffee substitute. At the moment, no genetically modified organism-derived chicory roots are used.

Among the several commercial inulin types available, all have a very high purity; they differ with regard to their powder characteristics and carbohydrate composition (**Table 1**). Standard inulin, as it is extracted from chicory roots, always contains a small amount of sugars (up to 10%). These sugars are present in the root and are not a result of processing. Low sugar and high performance inulin are obtained by chromatographic or physical removal of the mono-, di- and oligosaccharide fractions.

Oligofructose was introduced as a synonym for fructo-oligosaccharides by Orafti in 1989. It is a word that, by analogy with polydextrose, can be used easily for food-labeling purposes. Today, both names are used for labeling. However, in most cases, oligofructose refers to the partial hydrolysate of inulin. For this process, an inulinase enzyme is used. The resulting products can have different carbohydrate compositions, as reflected in **Table 2**. All of these industrial products are free of gluten, fat, protein and phytic acid, for example, and contain only very small (negligible) amounts of some minerals and salts. They are also free of pesticides, toxins and allergens. Thanks to their plant origin, together with the use of modern processing techniques, commercial inulin and oligofructose products can easily meet today's high microbiological standards for food ingredients.

## *Safe intakes*

***Natural occurrence and history of use.*** Both inulin and oligofructose are present in the daily diet of many of the world's populations (Van Loo et al. 1995). This presence is not a matter of trace amounts; several grams per day may be ingested through the normal diet (**Table 3**). This fact is the cornerstone of the safety evaluation of both inulin and oligofructose. On the one hand, it shows that mankind has been exposed to both substances for centuries. On the other hand, the fact that specific meals and even some diets can contain considerable amounts of inulin or oligofructose (up to 20 g) provides a history of exposure to such high amounts through the diet.

As far as we know, historical literature provides no specific reports doubting the safety of inulin-containing vegetables. Although this is no proof of safety, it is a comforting fact to know that, throughout so many centuries, nobody has suggested any doubt over the safety of inulin-containing foods. On the contrary, many of these foods have been hailed as stimulants of good health (chicory, garlic and leek), more

[1] Presented at the conference Nutritional and Health Benefits of Inulin and Oligofructose held May 18–19, 1998 in Bethesda, MD. This symposium was supported in part by educational grants from the National Institutes of Health Office of Dietary Supplements, the U.S. Department of Agriculture and Orafti Technical Service. Published as a supplement to *The Journal of Nutrition*. Guest editors for the symposium publication were John A. Milner, The Pennsylvania State University, and Marcel Roberfroid, Louvain University, Brussels, Belgium.

**TABLE 1**

*Typical composition of some commercial inulin powders*[1]

| | Raftiline ST | Raftiline LS | Raftiline HP |
|---|---|---|---|
| Dry substance (d.s.) | ≥95% | ≥95% | ≥95% |
| Inulin on d.s. | ~92% | ~99.5% | ~100% |
| Sugars[2] on d.s. | ~8% | ~0.5% | ~0% |
| Oligosaccharides on d.s. | ~30% | ~30% | <3% |
| Dietary fiber[3] on d.s. | ~92% | ~99.5% | ~100% |
| Carbohydrate content on d.s. | >99.5% | >99.5% | >99.5% |
| Ash (sulphated) on d.s. | <0.2% | <0.2% | <0.2% |

1 ST, standard; LS, low sugar; HP, high performance.
2 Glucose, fructose and sucrose.
3 As determined by AOAC Fructan method 997.08.

specifically for diabetics (Külz 1874, Lewis 1912, Strauss 1911).

***Use as food ingredient.*** Inulin and oligofructose are macronutrients. They are used either as supplements to foods or as macronutrient substitutes. As supplements to foods, they are added mainly for their nutritional properties. Adding inulin or oligofructose increases the dietary fiber content of the food. Such additions are usually in the range of 3–6 g per portion, in extreme cases up to 10 g. In other applications, inulin or oligofructose are added to allow a specific nutritional claim such as that regarding the bifidogenic activity. In these foods, typical levels are 1–6%, leading to ~3–8 g per portion.

As macronutrient substitutes, inulin and oligofructose are used mainly to replace fat andsugars, respectively. The fat-replacing potential of inulin was discovered and patented by Orafti in 1992. Using a specific processing technique, inulin is combined with water to produce the same texture and mouth-feel as fat. This is possible only in water-based foods such as dairy products and table spreads, and not in dry foods such as most snacks, bakery and confectionery products. Typically, 1 g of fat is replaced by a 0.25 g of inulin. Consequently, fat replacement in most foods will lead to inulin concentrations of ~2–6 g per portion.

Oligofructose has technical properties that are comparable to those of sugar and glucose syrups, yet nutritionally speaking, it has totally different properties. The sweetness of (pure) oligofructose is ~30% compared with sugar. Consequently, it is difficult to use oligofructose alone as a sugar substitute; most often, it must be combined with intense sweeteners to obtain the desired sweetness level.

**TABLE 2**

*Typical composition of some commercial liquid (L) and powder (P) oligofructose products*

| | Raftilose L60 | Raftilose L95 | Raftilose P95 |
|---|---|---|---|
| Form | Syrup | Syrup | Powder |
| Dry substance (d.s.) | ≥75% | ≥75% | ≥95% |
| Oligofructose on d.s. | ~60% | ~95% | ~95% |
| Sugars[1] on d.s. | ~40% | ~5% | ~5% |
| Oligosaccharides on d.s. | ~60% | ~95% | ~95% |
| Dietary fiber[2] on d.s. | ~60% | ~95% | ~95% |
| Carbohydrate content on d.s. | >99.5% | >99.5% | >99.5% |
| Ash (sulfated) on d.s. | <0.2% | <0.2% | <0.2% |

1 Glucose, fructose and sucrose.
2 As determined by AOAC Fructan method 997.08.

**TABLE 3**

*Estimations of the average inulin consumption*[1]

| g/d per capita | | 50th percentile | 90th percentile |
|---|---|---|---|
| Europe | 3–11 | | |
| North America | 1–4 | 0.8–3 | 2–8 |

1 Source: Van Loo et al. (1995).

Further, the use of oligofructose (and inulin) is not possible in most soft drinks and fruit jams. In such acid foods with a long shelf life, both substances are slowly hydrolyzed into fructose. Therefore, oligofructose is used as a sugar substitute mainly in dairy products and bakery products, at levels that cause no intestinal discomfort. In practice, amounts of 2–6 g per portion are used frequently.

On the basis of these considerations, a committee of experts (Kolbye et al. 1992) concluded that even for a consumer at the 90th percentile, increased exposure to inulin and oligofructose is likely to be of negligible biological significance.

***Toxicity studies.*** The long history of mankind's safe use of inulin-containing foods is reflected by the fact that very little formal toxicity testing in laboratory animals has been reported on inulin or its oligosaccharide hydrolysis products.

A number of animal toxicity studies with Neosugar have been published. Neosugar has the same chemical structure as inulin, but has shorter chain length (up to four fructose units) and is produced by enzymatic synthesis from sucrose. No specific safety issues were raised in these studies (Clevenger et al. 1988, Sleet and Brightwell 1990, Takeda and Niizato 1982).

Numerous publications in peer-reviewed clinical journals document careful studies with inulin and oligofructose in normal subjects and patients with disease states (Roberfroid 1993). These individuals of different ages have provided additional assurances of the safety of inulin and oligofructose. For example, inulin has been used to measure glomerular filtration rate by intravenous injection since 1931. This has become a standard procedure without a recorded history of toxic effects (Price et al. 1978). In addition, man's history of the food uses of inulin has not shown evidence of untoward effects. A committee of experts (Kolbye et al. 1992), based on a review of these studies, concluded, "*There is no reason to believe the oligofructoses or their metabolites would have a toxic potential from expanded use in foods; on the contrary, recent findings document the beneficial nutritional effects of these purified, chemically identified, derivatives of inulin in the gastrointestinal tract of man.*"

***Safety of the inulinase enzyme.*** Oligofructose is produced from inulin by partial enzymatic hydrolysis (Norman and Hojer-Pedersen 1989). This process uses an inulinase enzyme isolated from the carbohydrase complex of *Aspergillus niger*. The enzyme was toxicologically tested by the producer and classified as safe for use in the production of foods.

Enzymes from *Aspergillus niger* are considered by JECFA[2] (Joint FAO/WHO Expert Committee on Food Additives) to represent no hazard to human health when used in the production of food. The JECFA evaluation resulted in "acceptable daily intake (ADI) not specified" (35th meeting, 1989).

2 Abbreviations used: ADI, acceptable daily intake; a.o., xxxx; DP, degree of polymerization; GRAS, generally recognized as safe; JECFA, Joint FAO/WHO Expert Committee on Food Additives; NOEL, no-effect level.

These enzymes are widely used in the food industry, e.g., for fruit juice production.

In the U.S., the carbohydrase complex of *Aspergillus niger* is covered by a generally regarded as safe (GRAS) Affirmation Petition (GRASP 3G0016) filed by the ad hoc Enzyme Technical Committee in 1973. The Authorities in Denmark have evaluated the safety of the inulinase and accepted the use of the enzyme for the production of oligofructose (1990). The Conseil Supérieur d'Hygiène Public in France has also evaluated and recommended the acceptance of the use of the enzyme for the production of, among others, oligofructose (1990).

***Intestinal acceptability.*** *Principles.* Intestinal acceptability of nondigestible components is determined mainly by two phenomena. First is the osmotic effect, which leads to an increased presence of water in the colon. Smaller molecules exert a higher osmotic pressure and bring more water into the colon. This is probably the reason why sorbitol, for example, has a higher laxative potential than oligofructose (Hata and Nakajima 1984). Second is the fermentation effect, which is caused by the fermentation products, mainly short-chain fatty acids and gases. Slowly fermenting compounds appear to be easier to tolerate than their fast fermenting analogs. This can explain why inulin is easier to tolerate than oligofructose.

It is difficult to distinguish between an acceptable and a nonacceptable side effect of fermentation. Flatulence, for instance, is a well-known and often accepted side effect of the intake of vegetables. Dietary fibers, in general, are known and rewarded for their properties of stool softening; the step to a laxative effect is thus small.

The intestinal acceptability of a food can be judged only by the person who eats it. Diarrhea is certainly a symptom of nonacceptability, but soft stools may be an acceptable or even desired phenomenon. A dose of indigestible compounds that does not cause diarrhea can create other unwanted side effects such as flatulence and intestinal pressure. These parameters are much more difficult to measure objectively. Moreover, the same amount of flatulence can be acceptable to one person while being too much for another person.

For all of these reasons, the traditional concept of no-effect level (NOEL) for diarrhea is not very meaningful in the case of substances such as inulin and oligofructose. The 50% effective dose values for fructo-oligosaccharides have been proposed to be ~30g/d (Briet et al. 1995). A new approach, based on a personal judgement of discomfort, was developed by Orafti.

For this purpose, a food is considered unacceptable if it causes one of the following symptoms: too much flatulence, too much intestinal pressure, too much intestinal noise, too many intestinal cramps or diarrhea, as observed and evaluated by the test person himself.

Meaningful tests can be done only if the volunteers are unadapted to the product because only this reflects the reality in the consumer market. The test doses should be taken in a predetermined amount of time. The resulting "levels causing discomfort" appear at doses that are considerably lower than the traditionally calculated NOEL. This approach is therefore much more severe than the "laxative dose" approach found in much of the literature; in that approach, often only diarrhea is considered and the volunteers have passed an adaptation period.

***Test results.*** Orafti's tests and experience show that, regarding the sensitivity to (totally) fermentable carbohydrates, three categories of people can be distinguished: *1) nonsensitive persons* can consume 30 g/d or more of the compound almost without undesirable reactions as defined above; *2) sensitive persons* can consume 10 g/d of the compound without undesirable reactions but might experience undesirable reactions with doses of ≥20 g/d; *3) very sensitive persons* can already experience undesirable reactions at doses of ≤10 g/d.

**TABLE 4**

*Average distribution of the sensitivity of adult volunteers to fully fermentable carbohydrates, when ingested in one dose (one-shot) or in two doses per day (two-shot) without adaptation*

| Distribution of adults | I Nonsensitive | II Sensitive | III Very sensitive |
|---|---|---|---|
| One-shot | | | |
| Solid food | 89% | 10% | 1% |
| Liquid food | 71% | 25% | 4% |
| Two-shot | | | |
| Solid food | 94% | 5% | 1% |
| Liquid food | 85% | 13% | 2% |

The distribution of the sensitivity of the adult population is given in **Table 4** (Absolonne et al. 1995). The values in this table were calculated from the average reactions of a panel of nearly 100 adult volunteers to ingestion of three nondigestible or poorly digestible but totally fermentable compounds of the type disaccharide sugar alcohols or low-molecular-weight oligosaccharides. Indeed, it was found that the intestinal acceptability of these indigestible carbohydrates is quite comparable and that it is reasonable to calculate averages that are valid for the whole group.

This sensitivity distribution seems to be independent of sex or age, but tends to be slightly dependent upon body weight. Little or no general information is available concerning the acceptability of indigestible carbohydrates for children. A test with oligofructose (Cadranel and Coussement 1995) showed that daily doses of 3, 6 and 9 g of oligofructose in drinks or confectionery products cause no significant undesirable side effects in children from 10 to 13 y old.

***Experiences.*** Several hundred different food products containing added inulin or oligofructose are on the market today. The most successful applications occur in dairy products, such as fermented milks, milk, milk drinks, cheeses, and desserts, bakery products, spreadable products, chocolate, meal replacers, bars, cereals and ice creams. This provides a solid base of experience. Most foods contain doses of 2–4 g of inulin or oligofructose per portion. Many others contain higher amounts. In all of these cases as far as we know, acceptability problems with consumers have never caused the manufacturers to reconsider the formulation or labeling of the products.

***Conclusions and recommendations.*** It can be concluded that intestinal acceptability of nondigestible fermentable carbohydrates differs from person to person. Many people can consume ≥10 g without noticeable side effects, whereas some people experience intestinal discomfort that they consider too much after ingestion of even small amounts of nondigestible fermentable carbohydrates. Moreover, the reactions are influenced by the type of food (differing mainly between solid foods and liquid foods). It is therefore neither possible nor relevant to define no-effect levels for these substances.

Inulin, in general, performs slightly better than oligofructose, which in turn performs slightly better than most sugar alcohols. Inulin rarely causes diarrhea. The values that can be recommended as formulation doses, based on both the tests

**TABLE 5**

*Summary of elements that were taken into consideration in the safety evaluation of inulin and oligofructose*[1]

| |
|---|
| Definitions |
| Production process data |
| Food application data |
| History of long-term use before 1958 |
| Estimates of intake in the United States |
| Estimated consumption of added inulin and oligofructose by the U.S. population |
| Metabolism, nutritional and physiologic effects |
| Safety of comparable carbohydrates |
| Food intake data |
| Human studies |
| Animal toxicity data |

[1] Source: Kolbye et al. (1992).

with volunteers and the experience in the food industry, range from 5–8 g per portion for oligofructose and 10 g for inulin. These doses are not to be taken as NOEL values, which are significantly higher.

***Other nutritional side effects.*** Dietary fibers can have other unwanted side effects, such as a negative influence on vitamin or mineral absorption, allergic reactions, and an undesirable influence on the gut flora and their metabolism. No such negative effects have been found for inulin and oligofructose. On the contrary, recent research suggests that the effects on mineral absorption and gut flora might well be positive.

### *Legal status*

***Legal classification and acceptance.*** Inulin and oligofructose are legally classified as food or food ingredients, and not as additives, in all countries in which they are used. Although this seems evident if one considers the nutritional properties and the use of both substances, it has not been easy to obtain confirmation of this legal status from many of the legal authorities in the world. As a consequence, neither inulin nor oligofructose are listed as accepted food additives in the standard positive lists from the European Union or from Codex Alimentarius. EU Directive EC 95/2 explicitly lists inulin as a substance that is not an additive. The EU Standing Committee meeting of June 1995 confirmed that oligofructose is a food ingredient.

In Europe, both inulin and oligofructose were brought to market long before the Novel Foods Regulation (EC 258/97) came into force. Since 1987, Orafti has applied for authorization as a food ingredient for both substances in all European countries separately. In most countries, the files were submitted to the Superior Health Council (or the corresponding government body) for advice. None of the European countries has ever expressed reservations with regard to the safety of inulin or oligofructose. In all countries, both substances are accepted for food use without limitations. No ADI were fixed.

In the U.S., a committee of experts convened by Orafti declared both inulin and oligofructose as Generally Recognized As Safe in 1992 (Kolbye et al. 1992). The committee was composed of Albert C. Kolbye, Herbert Blumenthal, Barbara A. Bowman, John H. Byrne, C. Jelleff Carr, John C. Kirschman, Marcel B. Roberfroid and Morris A. Weinberger. The evaluation took all of the elements of **Table 5** into account. The conclusion was as follows: *Our opinion regarding the safety of inulin and oligofructose is based on reasoned judgement, primarily on the fact that inulin and oligofructose are natural components of many of our present foods that have been safely consumed by humans over millennia.*

*In addition, available scientific evidence clearly indicates that inulin and oligofructose are not hydrolysed in the stomach or small intestine, but are fermented completely into harmless metabolites in the colon, where they are specific substrates for the growth of Bifidobacteria. We now know that Bifidobacteria are desirable organisms in the human colon. Most convincing are the findings in patients with disease states and normal subjects of different ages fed oligofructose.*

*Inulin and oligofructose intake is self-limiting because of a gaseous response in the colon that prevents over-usage. Available animal toxicity studies are consistently free of any suggestions of adverse effects to be expected from such proposed levels of use in foods.*

*The exact chemical structures and compositions of inulin and oligofructose have been established and fall into the non-toxic classification. This represents an advantage of direct knowledge as compared to many other naturally occurring food components with unknown chemical composition and structure.*

*Inulin and oligofructose are dietary fibers by definition and by their nutritional properties. These substrates have not always been classified as 'dietary fiber', and classical analytical methods for dietary fiber analysis do not measure them. However, we conclude that the most appropriate classification and labelling for inulin and oligofructose is that of 'dietary fiber.'*

*Accordingly, we find there is no scientific evidence in the available data and literature on the food uses of these substances that demonstrates or suggests reasonable grounds to suspect a hazard to the public when used at levels that are current or that might reasonably be expected to be used in the future.*

*Our position regarding the safety of inulin and oligofructose is based on the long human experience of consuming inulin containing foods as well as evaluation of available scientific evidence relating to inulin and its hydrolysis products. Since inulin and oligofructose have been natural components of many foods consumed safely by humans over millennia, there is no reason to suspect a significant risk to the public health when used in foods.*

*Therefore, we conclude that these food substances are generally recognized as safe, both by long-established history of use in foods and by the opinion of experts qualified by scientific training and experience in food safety after a thorough review of the available scientific evidence.*

### *Labeling: ingredients list*

The labeling laws of most countries require that a specific name be used for the ingredients list. For chicory inulin, the name inulin is a logical and legally accepted choice. For oligofructose, either fructo-oligosaccharides or oligofructose can be used, the latter being a more consumer friendly choice. About 70% of chicory inulin molecules have a degree of polymerization (DP) >10. Oligosaccharides are defined as having a DP between 2 and 10 (IUB-IUPAC 1982). Oligofructose was defined by AOAC as having a DP between 2 and 10 (Hoebregs 1997). Therefore, it is not acceptable to label inulin as "oligofructose."

The commercial products contain fractions of mono- and disaccharides (Tables 1 and 2). These sugars may need separate labeling. Native inulin and standard oligofructose products always contain some sugars, which can be considered as a normal part of the inulin or oligofructose. Therefore, it has been legally accepted that these sugars do not have to be labeled specifically in most practical cases. Of course,

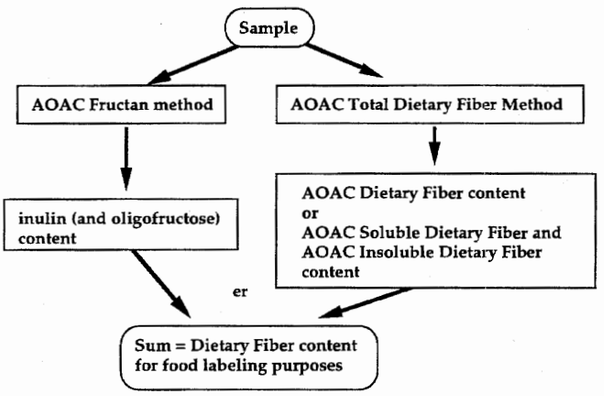


**FIGURE 1** Schematic presentation of AOAC methods for dietary fiber determination including inulin and oligofructose.

those sugars do have to be labeled separately in the nutrition labeling.

### *Analytical determination*

Inulin and oligofructose can be analyzed using the AOAC Fructan Method nr. 997.08 (Hoebregs 1997). This method measures the total of inulin plus oligofructose in any food product. The method is very specific for both substances and has proven to be accurate and reliable. Oligofructose can be measured separately using HPLC or gas chromatography techniques (Van Loo et al. 1995).

### *Nutrition labeling*

Inulin and oligofructose behave as dietary fibers in the human body; therefore, it is logical to classify these substances as dietary fiber. This principle has already been accepted by almost all European countries and is now being evaluated in most other countries. Both substances comply with the Codex Alimentarius definition of dietary fiber which is "edible plant and animal material not hydrolyzed by the endogenous enzymes of the human digestive tract as determined by the agreed upon method" [Codex Guidelines on Nutrition Labelling CAC/GL 2–1985 (Rev. 1 - 1993)]. They also meet the AOAC definition as "remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man" (Trowell 1975).

In many countries, dietary fiber is defined for labeling purposes as the substances measured by a specifically prescribed analytical method. Most often, the AOAC methods are the standard. These methods do not measure inulin or oligofructose, and neither do the Englyst methods (Van Loo et al. 1995). Therefore, the specific AOAC Fructan method must be used. This method can be combined with the AOAC Total Dietary Fiber methods (**Fig. 1**). It seems most logical to include inulin and oligofructose in the "soluble dietary fiber" group.

The caloric value of inulin and oligofructose has been determined to lie between 1 and 1.5 kcal/g (Roberfroid et al. 1993). This "scientific" value, however, is often in conflict with the "legal" caloric values as they are prescribed for dietary fiber, for example, in Europe (0 cal) or the U.S. (4 kcal/g for soluble fiber, 0 for insoluble fiber). Requests have been submitted to adapt the food laws to the scientific and nutritional value for inulin and oligofructose.

### *Health claims*

In Europe, Japan and several other countries, the nutritional properties of inulin and oligofructose are used to formulate health claims on food products and food supplements. An overview of these is given by Coussement (1997). In most countries including the United States and the European countries, such claims should not suggest the cure or prevention of disease, should not be misleading and should be based on sound science.

At the moment, claims regarding the dietary fiber effects and the stimulation of *Bifidobacteria*, all based on inulin or oligofructose, are legally being made in many countries. In some countries, a specific authorization from the legal authorities has been obtained for specific claims. Such claims are also called "nutrient-function" claims or "positive" claims.

In the United States, the DSHEA (Dietary Supplement Health and Education Act) allows four "statements of nutritional support" under certain conditions. The stimulation of *Bifidobacteria* by inulin or oligofructose can be classified among such claims.

### *Future labeling*

Inulin and oligofructose as novel dietary fibers might have significant consequences for nutrition labeling systems. In most countries, these substances have pushed the experts to reconsider the definitions of a.o. carbohydrates, complex carbohydrates and dietary fiber (Lee and Prosky 1995). Furthermore, the standard nutrition labeling might not accommodate the appearance of these nondigestible oligosaccharides. Suggestions have been made that the classification of carbohydrates be reconsidered (Cummings et al. 1997).

In the United States, the NLEA (Nutrition Labeling and Education Act) in principle allows "disease-related" claims on the condition that an official authorization from the FDA or a confirmation from a National Institute of Health has been obtained. This situation might allow more claims for inulin and oligofructose, in particular relating to osteoporosis, heart disease or colon cancer, on the condition that the present research indications are confirmed by further research. In Europe, such claims on food products would require a fundamental change in the labeling directives; however, the first suggestions have been made.

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**U. S. Food and Drug Administration**
**Center for Food Safety and Applied Nutrition**
**Office of Food Additive Safety**
**May 5, 2003**



**Agency Response Letter**
**GRAS Notice No. GRN 000118**

Claire L. Kruger, Ph.D.
Environ
4350 North Fairfax Drive
Suite 300
Arlington, Virginia, 22203

Re: GRAS Notice No. GRN 000118

Dear Dr. Kruger:

The Food and Drug Administration (FDA) is responding to the notice, dated November 4, 2002, that you submitted on behalf of Imperial Sensus, LLC (Imperial Sensus) in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on November 7, 2002, filed it on November 14, 2002, and designated it as GRAS Notice No. GRN 000118.

The subject of the notice is inulin from the root of the chicory plant (*Cichorium intybus*). The notice informs FDA of the view of Imperial Sensus that inulin is GRAS, through scientific procedures, for use in food in general, including meat and poultry products, as a bulking agent. Imperial Sensus lists 43 proposed food categories that would contain inulin at varying use levels. Based on these proposed uses, Imperial Sensus estimates that dietary intake of inulin at the 90th percentile level would be approximately 6 grams per day for infants less than one year of age, approximately 15 grams per day for infants one year of age, and approximately 20 grams per day for the general population (i.e., two years of age and older).

Imperial Sensus' notice includes the findings of a panel of individuals who evaluated the data and information that serve as the basis for Imperial Sensus' GRAS determination. Imperial Sensus considers these individuals to be qualified by scientific training and experience to evaluate the safety of substances added to food.

Imperial Sensus describes published information about inulin, which is a polysaccharide that occurs naturally in plants such as chicory, Jerusalem artichokes, some cereal grains, and onions. It contains a chain of fructose units that are linked in a beta 2-1 configuration, usually with a single terminal glucose molecule. The degree of polymerization (DP) (i.e., the number of fructose units in the chain) ranges from two to greater than 60, depending

on the plant source, the time of harvest and the duration and conditions of post-harvest storage.

Imperial Sensus describes published information about other "fructan" polysaccharides - i.e., polysaccharides that contain a chain of fructose units. These fructan polysaccharides include fructooligosaccharide (FOS; DP ranging from three to five) and oligofructoses (DP ranging from two to twenty). Because the fructose units in inulin, FOS, and oligofructoses all contain the characteristic beta 2-1 linkage, Imperial Sensus assesses the safety of inulin using data and information regarding all three of these fructan polysaccharides.

Imperial Sensus describes the manufacture of inulin, which is extracted from the root of the chicory plant. The harvested roots are washed via a process similar to that used in the sugar beet industry. The washed roots are sliced and fed into a scalding apparatus that renders the plant cell walls semi-permeable. After scalding, the chicory root slices are fed into a diffusion tower, where inulin is extracted in a continuous countercurrent process. The raw inulin juice emerging from this process is cooled and further purified and concentrated. Spray drying of this concentrated juice results in a final concentration of greater than 95 percent dry matter. Imperial Sensus provides specifications for this spray dried product.

Imperial Sensus describes published information about the presence of inulin as the energy reserve in a number of plants consumed as food world-wide, including chicory, dahlia, Jerusalem artichoke, murnong, and yacon. Imperial Sensus notes that these sources of inulin have been consumed either as dietary staples or as sustenance crops in times of hardship. Imperial Sensus describes published information about approximately two dozen inulin-containing food sources, including the percent of inulin contained in the edible part of the plant. Imperial Sensus notes that the inulin content of edible plants ranges from less than one percent (e.g., in many cereal grains) to more than 20 percent (e.g., in Jerusalem artichokes and other tubers). Imperial Sensus also cites a publication regarding the consumption of Jerusalem artichokes by some populations as a substitute for white potatoes and estimates that consumption of inulin by these populations may have reached 25 to 32 grams per day. In the U.S., the most commonly consumed inulin-containing foods are cereal grains, bananas, garlic, onions, and tomatoes. Based on published reports, Imperial Sensus estimates that the average intake of inulin and oligofructose in the U.S. is 2.6 grams per day, primarily from consumption of wheat and onions. In Europe, Imperial Sensus estimates that the intake of inulin and oligofructose may be as high as 10 grams per day.

Imperial Sensus cites published information to support its view that the beta 2-1 linkage between fructose units of inulin is largely resistant to digestion by mammalian digestive enzymes of the small intestine and that ingested inulin will pass largely intact to the colon, where it is subject to fermentation by the resident microbial flora. This fermentation results in the production of gases such as hydrogen, carbon dioxide, and methane, as well as short-chain fatty acids. The short-chain fatty acids are either utilized locally as an energy source by the resident flora, taken up systemically via the

colonocytes and transported to the liver for caloric utilization by the host, or excreted in the feces.

Imperial Sensus describes results from published *in vitro* genetic toxicity studies with a commercially available fructooligosaccharide and concludes that these studies demonstrate that this fructooligosaccharide lacks any significant genotoxic potential. Imperial Sensus also discusses published human studies and reviews related to human tolerance of inulin and related fructans and concludes that regular consumption of 40 to 70 grams per day of inulin by healthy adults appears to result in no significant adverse effects, especially when the consumption is divided over the course of the day.

Based on the information provided by Imperial Sensus, as well as other information available to FDA, the agency has no questions at this time regarding Imperial Sensus' conclusion that inulin is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of inulin. As always, it is the continuing responsibility of Imperial Sensus to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in the notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

**Potential Labeling Issues**

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FFDCA), a food is misbranded if its labeling is false or misleading in any particular. Section 403(r) of the FFDCA lays out the statutory framework for a health claim. In describing the intended use of inulin and in describing the information that Imperial Sensus relies on to conclude that inulin is GRAS under the conditions of its intended use, Imperial Sensus raises potential labeling issues under these provisions of the FFDCA. These labeling issues consist of Imperial Sensus' assertion that inulin has a variety of physiological effects that Imperial Sensus views as beneficial. If products that contain inulin bear any claims about such benefits on the label or in labeling, such claims are the purview of the Office of Nutritional Products, Labeling, and Dietary Supplements (ONPLDS) in the Center for Food Safety and Applied Nutrition (CFSAN). The Office of Food Additive Safety (OFAS) neither consulted with ONPLDS on these labeling issues nor evaluated the information in Imperial Sensus' notice to determine whether it would support any claims made about inulin on the label or in labeling.

**Use in Meat and Poultry Products**

During its evaluation of GRN 000118, FDA consulted with the Labeling and Consumer Protection Staff of the Food Safety and Inspection Service (FSIS) of the USDA. Under

the Federal Meat Inspection Act and the Poultry Products Inspection Act, FSIS is responsible for determining the efficacy and suitability of food ingredients in meat and poultry products as well as prescribing safe conditions of use. Suitability relates to the effectiveness of the ingredient in performing the intended purpose of use and the assurance that the conditions of use will not result in an adulterated product, or one that misleads consumers.

FSIS has previously evaluated the use of inulin as a water binder, emulsifier, stabilizer and texturizer at a level between two and five percent in processed meat food products.[1] FSIS advised that, based on the current use of inulin in the production of meat products, FSIS would not object to the proposed use of inulin in non-standardized meat or poultry products at four percent of the total product formula. In regard to the use of inulin in standardized meat and poultry products, FSIS is currently in the process of completing rulemaking that would permit the use of any safe and suitable binder in the production of meat and poultry products where standards of identity and other Federal regulations already permit the addition of binders, e.g., hot dogs. Until this rulemaking is complete, inulin cannot be used to formulate meat and poultry products with a standard of identity in Title 9 of the Code of Federal Regulations, parts 319 and 381.

Sincerely,
/s/
Alan M. Rulis, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

cc: Dr. Robert Post, Director
Labeling and Consumer Protection Staff
Office of Policy, Program Development and Evaluation
Food Safety and Inspection Service
1400 Independence Ave., SW, Suite 602, Annex
Washington, DC 20250-3700

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[1]In a letter dated May 9, 1997, from FSIS to FDA, FSIS requested consultation with FDA regarding a request, from Imperial-Suiker Unie, that FSIS advise Imperial-Suiker Unie of the acceptability of the use of inulin as a water binder, emulsifier, stabilizer and texturizer at a level between 2 and 5 per cent in processed meat food products. In a letter dated May 14, 1999, FDA informed FSIS that FDA had completed its evaluation of the information submitted by Imperial-Suiker Unie as well as other information available to the agency. Based on its evaluation, FDA determined that, at that time, the agency would not challenge Imperial-Suiker Unie's conclusion that inulin is GRAS under the proposed conditions of use




November 29, 2000

Susan Luscutoff, Ph.D.
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Food and Drug Branch
California Department of Health Services
MS 357
601 North 7th Street
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Dear Dr Luscutoff,

At the suggestion of our attorney, Brian Donato, I am writing to provide you with additional, detailed scientific information supporting approval of the structure-function claim "helps boost calcium absorption" for use on the labels of Stonyfield Farm Yogurt products. The claim in question is based on the fact that these products contain Raftilose® Synergy 1, a proprietary type of inulin developed by Orafti. Before addressing the information included here, let me first review some of the nomenclature that is used.

**Fructan** is the general scientific name for molecules composed primarily of fructose. Linear fructans are referred to as **inulin**. Inulin is the second most abundant plant storage carbohydrate next to starch and is found in a variety of edible fruits and vegetables such as wheat, onions, leeks, garlic, asparagus, artichokes, and bananas. Inulin is actually a natural mixture of molecules which vary in length (or degree of polymerization, DP) from 2 to 60 fructose units. The molecules in inulin that have a DP of 10 or less are referred to as **oligofructose** or **fructooligosaccharides (FOS)**. So oligofructose or FOS are actually the shorter chain portion of inulin. Sometimes in the scientific literature, and especially in any regulatory literature, the trade names of these materials may be used. Oraftis' brand name for inulin is **Raftiline®** (ine for inulin) and our brand name for oligofructose is **Raftilose®** (ose for oligofructose). Finally, **Osteoboost™**, a consumer friendly brand name which may be used in place of our Raftiline inulin and Raftilose oligofructose products has been developed.

101 Lindenwood Drive – Malvern, PA 19355 – Tel.: 610 889 9828 – Fax: 610 889 9821

**The following literature substantiates approval of the requested claim:**

A. A fully referenced literature review prepared by Orafti of the literature supporting Raftiline® and Raftilose's® ability to enhance calcium absorption entitled "Improvement of Calcium Absorption" is included for your reference. This document was also included in my original submission to Lee Jensen.

B. A letter from Dr. Connie Weaver, a pre-eminent scientist in the area of calcium nutrition. This letter has previously been provided to you by Brian Donato at Hyman Phelps and McNamara. Dr. Weaver conducted an independent review of the literature and subsequently evaluated the evidence for the structure-function claims for Raftiline® and Raftilose®. As you can see, she concluded "these claims are supported by competent and reliable scientific evidence provided they are used on food or supplements that also contain calcium." The specific claim which Stonyfield wishes to use, "helps boost calcium absorption", is addressed on page 1 of this letter. Further, Dr. Weaver in her conclusion states that " Raftiline and Raftilose have remarkably increased calcium absorption in children and adults...A large gap exists between calcium intakes and calcium requirements for most of the U.S. population over the age of 11 (Dietary Reference Intakes 1997). Improving calcium absorption efficiency is another strategy to improve calcium nutrition among the population in addition to increasing calcium intakes."

C. Copies of the three double blind palcebo controlled human studies demonstrating increases in calcium absorption. The first is "Effect of soluble or partly soluble fibre supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men" by Coudray et al.(1997).

The second is "Oligofructose stimulates calcium absorption in adolescents" by van den Heuvel et al. 1999. I have also enclosed, for the sake of completeness, van den Heuvels 1998 publication "Non-digestible oligosaccharides do not interefere with calcium and non-heme iron absorption in healthy young men". These two publications need to be taken into consideration together, since the 1998 publication reports no effect of increased calcium absorption with oligofructose consumption, but the 1999 publication does report an increase in absorption. Van den Heuvel hypothesizes that differences in the calcium absorption measurement technique may be responsible for the differences between the two studies. A double stable isotope technique that depends on measurement of isotopes in urine was used in both studies, but in the first study the urine was only collected for 24 hours, while in the second study it was collected for 36 hours.

Since the mechanism by which oligofructose increases calcium absorption is believed to take place in the colon, the shorter urine collection time in the 1998 study may not have allowed enough time to see the colonic effect. Refer to van den Heuvels 1999 paper for a complete discussion. Also please note that at the time Dr. Weaver reviewed the literature for her claims evaluation, the second van den Heuvel paper was in press, and thus listed as 1998.

The third clinical supporting the claim is a recently completed but not yet published study by Dr. Steve Abrams at the USDA Children's Nutrition Research Center & Baylor College of Medicine. A confidential report of this study entitled "The effect of Raftilose® Synergy1 on calcium absorption in adolescent girls" is enclosed. Raftilose® Synergy1 is an inulin product that is enriched with oligofructose, and is the exact product which Stonyfield is using in their yogurt. **Dr. Luscutoff, I must ask you to treat the report by Dr. Abrams confidentially and for internal use only, as any external use could jeopardize its publication,** which should occur in the next few months. Please note the second and third paragraph of Dr. Abrams discussion, where he talks about the public health benefit of inulin based on its ability to increase calcium absorption.

"Although a (an absorption) benefit was seen at all calcium absorption levels, our data suggest that the maximum benefit may occur in those subjects with the lowest calcium absorption values. This suggests that RAFTILOSE® Synergy1 might be most effective in subjects at the highest risk for low bone mineral mass. This effect, generalized to the entire population may be of significant public health benefit."

Orafti works with well-recognized experts in order to assure substantiation of the benefits of our products. To that end, we have consulted with the appropriate experts in regards to the calcium claim associated with our inulin ingredient. We are satisfied that the claim is scientifically valid and trust that once you have reviewed the materials, you will concur.

We recognize that your time is valuable. However, Brian Donato and I would appreciate an opportunity to discuss any questions you may have about the materials we have provided to you or any you may have in connection with your own review of this matter. To that end, Brian will be calling you to arrange for a telephone conference.

Thank you for your consideration in this matter.

Sincerely,

Mark T. Izzo

Mark T. Izzo, PhD
Director of Technology and Regulatory Affairs

CC: Lee Jensen
California Dept of Food and Ag, Dairy Foods Control Branch
1220 N Street Room A170, Sacramento Ca, 95814

Kasi Reddy
Stonyfield Farm, 10 Burton Drive, Londonderry, NH 03053 (603) 437-4040

Brian Donato, Esq.
Hyman, Phelps and McNamara
2603 main street Suite 650, Irvine, Ca 92614

**From:** "Loscutoff, Susan (DHS-FDB)" <SLoscuto@dhs.ca.gov>
**To:** 'Mark Izzo' <MIzzo@orafti-us.com>, "Waddell, Jim (DHS-FDB)" <JWaddell@dhs.ca.gov>
**Date:** 5/2/02 2:07PM
**Subject:** RE: Stonyfield Calcium Claim

I have no objection to Stonyfield including a claim on their yogurt that inulin added to the yogurt increases calcium absorption.

-----Original Message-----
From: Mark Izzo [mailto:MIzzo@orafti-us.com]
Sent: Thursday, May 02, 2002 9:53 AM
To: Loscutoff, Susan (DHS-FDB)
Cc: bbordessa@cdfa.ca.gov
Subject: Stonyfield Calcium Claim

Dear Susan,

As we discussed on the phone, the new person to communicate your approval of the claim to is Mr. Bill Bordessa, Chief of the Milk and Dairy Foods Control Branch. His e-mail is bbordessa@cdfa.ca.gov. I have made Stonyfield aware of your decision so thay may now submit the label.

thanks for your help

kind regards

Mark

Mark T. Izzo, Ph.D.
Director of Science and Technology
Orafti North America
101 Lindenwood Drive
Malvern, PA 19355
610-889-9828 (phone)
619-889-9821 (fax)

Please note that my email has changed to mizzo@orafti-us.com. Thank you.

**CC:** <bbordessa@cdfa.ca.gov>

# Appendix 4

## Petition to add to the National List the substance "Oligofructose enriched with Inulin Documented for Calcium Absorption"

**January 12, 2007**

## Nutrition Facts

Serving Size 1 Cup (227g)
Servings Per Container about 4

| Amount Per Serving | |
|---|---|
| **Calories** 250 Calories from Fat 70 | |
| | **% Daily Value*** |
| **Total Fat** 8g | **12%** |
| Saturated Fat 5g | **25%** |
| *Trans* Fat 0g | |
| **Cholesterol** 30mg | **10%** |
| **Sodium** 120mg | **5%** |
| **Total Carbohydrate** 36g | **12%** |
| Dietary Fiber 3g | **12%** |
| Sugars 32g | |
| **Protein** 8g | **16%** |

Vitamin A 6% • Vitamin C 0%
Calcium 35% • Iron 0%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower based on your calorie needs:

| | Calories: | 2,000 | 2,500 |
|---|---|---|---|
| Total Fat | Less than | 65g | 80g |
| Sat Fat | Less than | 20g | 25g |
| Cholesterol | Less than | 300mg | 300mg |
| Sodium | Less than | 2,400mg | 2,400mg |
| Total Carbohydrate | | 300g | 375g |
| Dietary Fiber | | 25g | 30g |
| Protein | | 50g | 65g |

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

OUR FAMILY RECIPE: CULTURED PASTEURIZED ORGANIC WHOLE MILK, NATURALLY MILLED ORGANIC SUGAR, INULIN*, ORGANIC NATURAL VANILLA FLAVOR, PECTIN, CONTAINS SIX LIVE ACTIVE CULTURES INCLUDING L. ACIDOPHILUS, BIFIDUS, L. CASEI AND L. REUTERI.

* Studies have shown that 8g per day of inulin (a natural dietary fiber) increases calcium absorption. Each serving of this yogurt contains 3g of inulin.

**Our Organic Guarantee:** made without the use of antibiotics, synthetic growth hormones, and toxic pesticides.

0 52159 00002 8

## SKINNY DIPPIN' WITH NEWMAN'S OWN

**Just Peachy Salsa Dip**

***A summer snack favorite with just the right touch of sweetness.***

Blend 1 cup of Stonyfield Farm French Vanilla Whole Milk yogurt with 1 cup of Newman's Own PEACH Salsa.

**Lemon-Alded Fruit Dip**

***Sweet and tangy, the perfect dip for cut summer fruit.***

Blend 1 cup of Stonyfield Farm French Vanilla Whole Milk yogurt with 1 cup Newman's Own Lemonade.

*Visit Stonyfield.com and Newmansown.com for more delicious recipes that combine the nutritional goodness of Stonyfield Farm yogurt with the great all natural taste of Newman's Own products.*

GRADE A
L. REUTERI LIVE ACTIVE CULTURE
USDA ORGANIC

**We give 10% of our profits to efforts that help protect and restore the earth.**

STONYFIELD FARM LONDONDERRY, NH 03053 1-800-PRO-COWS (776-2697) M-F 9-5 EST
Certified Organic by OAI

3g FIBER

Helps Boost Calcium Absorption*

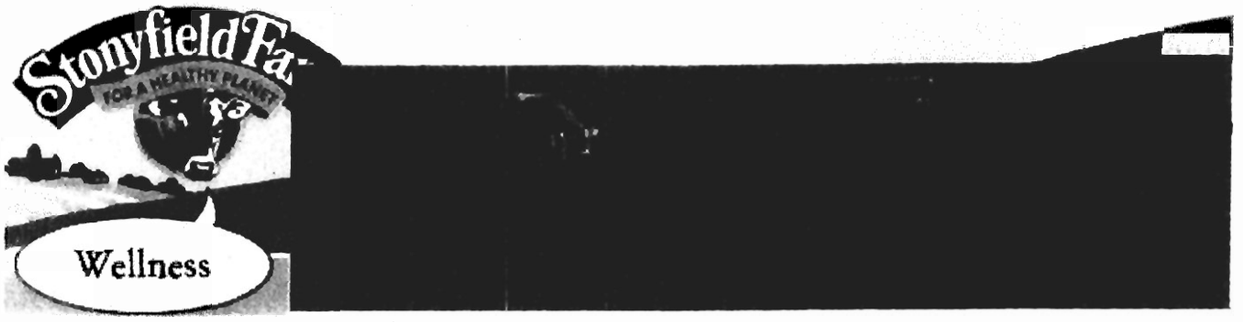
Stonyfield Farm ORGANIC

whole milk

yogurt FRENCH VANILLA

cream on top

32 OZ (2Lb) 907g




HOME

## *New Enriched Inulin Boosts Calcium Absorptio*

Stonyfield uses a new specially enriched form of inulin in their yogurt, which has been clinica boost calcium absorption at low use levels.

Eleven animal studies have proven inulin effective in increasing both calcium absorption and density. Further human studies have confirmed the absorption effect and have show that at increases the body's absorption of calcium by approximately 58%. In addition, 15g/day of oli component of inulin) has been proven to increase the body's calcium uptake by 26%.

Now, a new double blind placebo-controlled study has demonstrated that 8g/day of a new sp inulin increases calcium absorption by 20%.

**Study Design**

Twenty-nine girls aged eleven to fourteen participated in a study led by Professor Steven A. Dr. Ian Griffin, M.D. and colleagues at the USDA/ARS Children's Nutrition Research Center College of Medicine in Houston, Texas. The girls consumed a calcium-fortified diet during tw periods. They were given a daily calcium dose of about 1300-mg, which is the recommende their age category.

During one of the three-week periods, the girls were given a diet containing 8g/day of enrich the other three-week period, they were given a diet containing a placebo (8g/day sucrose). N scientists performing the study nor the girls themselves knew which diet they were consumin period and enriched inulin period were separated by a two-week washout interval. At the enc week period, calcium absorption and urinary calcium excretion were monitored.

**Results**

In a population of healthy girls consuming their current recommended intake of calcium, 8 g/ "enriched inulin" increased calcium absorption by approximately 20% - a significant increase increase in calcium absorption due to this new form of inulin was approximately 90 mg/day, be clinically important during the period of rapid adolescent bone growth. Adolescence is a k lives for building bone mass. For example, if we assume that this additional calcium is retain the two years of maximal adolescent bone mineral accumulation, the net increase in bone m would be a total of 65g. Assuming an average peak bone mineral mass of 1200 g, this would 5.5% increase in peak bone mineral mass for individuals consuming enriched inulin. This eff to the entire population may be of significant public health benefit.

Including this new enriched inulin in Stonyfield yogurt is another example of Stonyfields com bringing you the best nutritional benefits naturally.

For additional information see the attached references.

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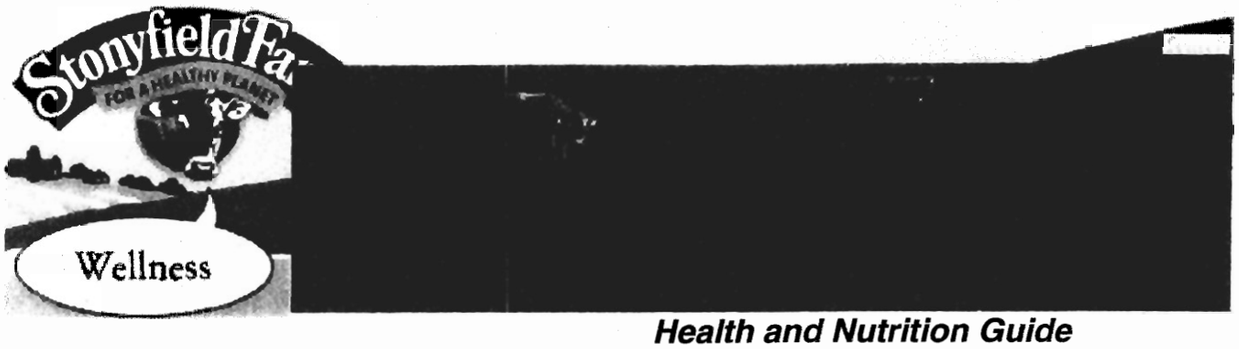


## Health and Nutrition Guide

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HOME

Stonyfield Farm was founded on the belief that yogurt should not only be great tasting, but healthy as well—for you and the planet. All of our yogurts are made with only the finest all natural and organic ingredients and environmentally friendly processes.

### What exactly is yogurt?

Yogurt is milk that has been inoculated with two live active cultures: *Lactobacillus bulgaricus (L. bulgaricus)* and *Streptococcus thermophilus (S. thermophilus)*. During incubation, these cultures break down the milk sugar (lactose) and produce lactic acid, which gives the milk the tangy taste we associate with yogurt. Yogurt is a highly nutritious food choice because it's a good source of calcium and protein, and it's rich in vitamins and minerals that are necessary for good health.

## What makes Stonyfield Farm yogurt so special?

- At Stonyfield Farm, we choose every ingredient and step in the yogurt-making proces care—with regard for the taste and healthfulness of our products as well as the healtl
- We use only 100% natural ingredients—never any artificial flavors, sweeteners, pres fillers like modified food starch or gelatin.
- We add SIX live active cultures, each with unique health benefits.
- We add inulin, a natural dietary fiber that helps boost calcium absorption.
- We're opposed to the use of synthetic bovine growth hormone (rBGH) and pay our d premium to not treat their cows with it.
- All of the ingredients in our organic products are made without the use of antibiotics, hormones and toxic pesticides or fertilizers.
- Each year we give 10% of our profits to efforts that help protect and restore the earth

### Live Active Cultures—the proactive approach to good health.

In addition to *L. bulgaricus* and *S. thermophilus*, Stonyfield Farm adds four more live active c our yogurts. (Other major brands typically add just one or two more.) These cultures, known are beneficial bacteria that naturally reside in your digestive tract. Studies have shown that p benefit your health in a variety of ways:

- Enhance digestion
- Fortify the body's natural defenses and prevent common digestive ailments
- Decrease the presence of harmful bacteria, including those that cause food-borne illr Coli, Salmonella, Staphylococcus, Listeria and the yeast Candida
- Improve absorption of nutrients

***Lactobacillus acidophilus***

*L. acidophilus* provides various health benefits in the gastrointestinal tract. Several studies in *acidophilus* helps lower cholesterol by interfering with cholesterol re-absorption in the intestir

***Bifidobacteria (Bifidus)***

*Bifidus* stimulates the immune system, helps prevent common digestive ailments and suppoi growth and development of the digestive tract.

***Lactobacillus casei***
*L. casei* enhances positive bacterial balance in the intestine. It enhances the immune syster growth of diarrheaproducing organisms, alleviates constipation, reduces hypertension, inhibits the growth of carcinogenic tumors, and suppresses diseaseproducing microorganism

***Lactobacillus reuteri***
Stonyfield Farm is the only U.S. yogurt brand to offer *L. reuteri* in its products. *L. reuteri* inhib harmful bacteria such as Salmonella, E. Coli, Staphylococcus, Listeria and the yeast Candid prophylactic effect on both viral and bacterial diarrhea. Research shows that *L. reuteri* helps immune system and enhances the body's resistance to gastrointestinal disease.

## *Inulin—nature's calcium absorption booster and more!*

All of our yogurts now contain inulin, a natural dietary fiber present in many common fruits ar such as artichokes, asparagus, onions, garlic, raisins and bananas. The inulin used in Stony yogurts comes from chicory roots.

Inulin has the health benefits normally associated with dietary fiber plus a whole lot more! Inu type of fiber known as a prebiotic, which means it has been scientifically proven to increase t beneficial live active cultures, and prevent the growth of harmful bacteria in the digestive trac

Inulin also helps boost calcium absorption! Studies have shown that 8 grams per day of the t Stonyfield Farm yogurts increases calcium absorption by as much as 20%!

Another unique benefit of inulin is its ability to boost the body's natural defenses. A study cor Johns Hopkins University School of Medicine found that children in a daycare setting who co had significantly lower incidence of fever with cold symptoms, less antibiotic use, and fewer ( absences than a similar group of children not consuming inulin.

Together inulin and our six live active cultures provide health benefits no other yogurt brand can match!

- Significantly increases the level of beneficial cultures in your intestinal tract, which he body's natural defenses and prevent common digestive ailments
- Decreases the presence of harmful bacteria, including those that cause food-borne ill Coli, Salmonella, Staphylococcus, and Listeria
- Improves the absorption of minerals and synthesis of vitamins

## *Calcium—vital for strong bones, helpful for weight loss!*

Calcium is essential for building and maintaining strong bones. In fact, 97% of your body's ca in your bones! Approximately 40-45% of peak adult bone mass is actually built up during the After adolescence and early adulthood, calcium in the bones can be lost, but not added. If yc consume enough calcium, your body adjusts to make up for the shortage, often by taking it fi So when you're in your 30's and older, your bone mass can actually decrease. This occurs e women who are postmenopausal and can lead to osteoporosis, a debilitating disease that ca breakable bones.

Calcium also serves important nerve, muscle, and blood clotting functions. These functions a that calcium will be pulled from the bone if necessary to make sure they happen appropriatel you have to get calcium daily or your bones will become less dense.

Experts agree that it's best to obtain calcium from food rather than supplements. Supplemen substitute for a nutritionally adequate diet. Foods containing calcium also contain other valua a balanced, natural form.

Dairy foods possess the most concentrated sources of calcium in a diet, and are better abso sources of calcium. Each 6 oz. serving of Stonyfield Farm yogurt provides 25-35% of your da requirement! All of the calcium in Stonyfield Farm yogurts comes from only 100% natural ing with small amounts in fruit), unlike other brands that add calcium phosphate and tricalcium p increase the calcium amount. And we're the only yogurt brand to add inulin, a natural dietary been shown to increase calcium absorption by as much as 20%!

The USDA Dietary Guidelines for Americans recommend three servings of milk products per promote bone mineral density and to add to a nutrient-dense diet. If you are cutting calories i lose weight, don't cut dairy foods from your diet. Eating three servings of dairy a day in a red naturally provides calcium and protein needed for strong bones and supports weight loss.

The National Academy of Sciences outlines the following guidelines for adequate daily intake

| Age (years) | Calcium (mg per day) |
|---|---|
| 1-3 | 500mg |
| 4-8 | 800mg |
| 9-18 | 1,300mg |
| 19-50 | 1,000mg |
| 51+ | 1,200mg |
| Pregnant/Lactating Women | 1,300mg |

### *Protein—an important nutrient.*

Protein could be said to be the most important nutrient for your body. Protein is key to your ir as well as every chemical reaction in your body from energy production to digestion to growt repair. Protein also makes up your body's muscle, blood and plasma. Throw in hormones an pretty obvious how important protein is.

We need foods with protein to obtain amino acids—the building blocks that make up muscle, hormones, antibodies, cellular membranes, DNA and enzymes. There are 9 essential and 11 amino acids. Essential means
that your body can't manufacture them, so you need to obtain them from the foods you eat.

The National Academy of Sciences Recommended Dietary Allowances (RDA) of protein are:

| Age (years) | RDA of Protein (grams per day) |
|---|---|
| 1-3 | 16 |
| 4-6 | 24 |
| 7-10 | 28 |
| 11-14 | 45-46 |
| **Males** | |
| 15-18 | 59 |
| 19-24 | 58 |
| 25+ | 63 |
| **Females** | |
| 15-18 | 44 |
| 19-24 | 46 |
| 25+ | 50 |

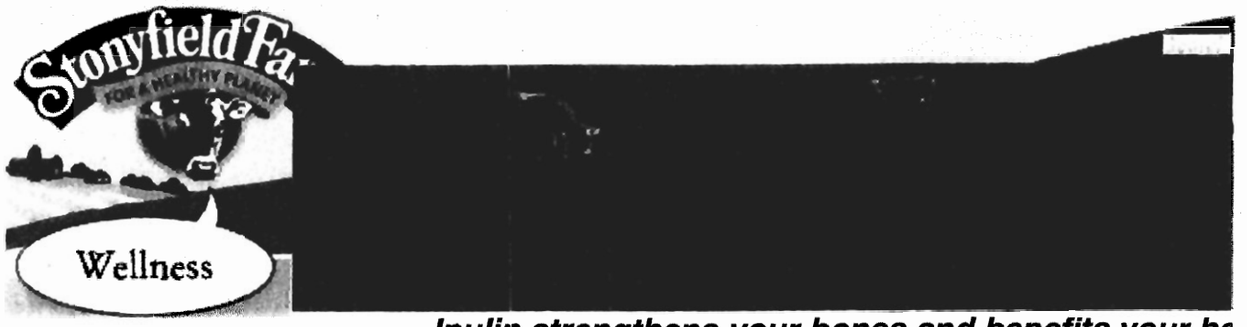
At any age, the ideal protein is one that contains all the amino acids in the required amounts Animal foods, such as milk, meat, and fish, provide all the essential amino acids in the right p humans. Grains and legumes are also good sources of protein. Plant proteins (with the exce have low levels of one or more essential amino acids, but by combining plant foods that have complementary proteins, they can be used just as effectively as animal proteins.

Stonyfield Farm yogurt is a good source of protein. In fact the USDA recognizes this and allo substituted as a protein in its school lunch program. Six ounces of Stonyfield Farm yogurt pr of protein—a significant portion of your daily dietary need!

### *Fiber—an essential part of your diet.*

Experts recommend 25 to 30 grams of dietary fiber every day (or at least 10 to 13 grams per Most Americans consume only 10 to 15 grams per day. Fiber is essential for good digestive also has a number of other known health benefits:

- *Promotes good digestive health.* Fiber is an indigestible material in foods that stimulates the intestine to move its contents along, preventing c




HOME

## *Inulin strengthens your bones and benefits your he*

The entire Stonyfield Farm yogurt line contains *inulin*, a natural dietary fiber found in over 35 foods like artichokes, asparagus, onions, garlic, raisins and bananas. The inulin used in Stor yogurts comes from chicory roots.

### *Inulin helps boost calcium absorption and is clinically proven to in bone density* [1,2,3]

Previous studies demonstrate that the inulin used in Stonyfield Farm yogurts increa absorption by as much as 20 percent. A newly published study in the *American Jou Clinical Nutrition* shows that inulin increased calcium retention or bone density in ad 15 percent. This increase in calcium absorption and bone density resulted after sup the diet with 8 grams of inulin.

### *Inulin is a fiber that promotes good digestive health* [4]

As a prebiotic fiber, inulin is food for the probiotics. This causes the probiotic *Bifidol* increase. As they grow, they secrete acids. This change in the pH in the colon helps the growth of harmful bacteria like E. Coli, Salmonella and Campylobacter.

The addition of inulin in the diet is known to improve regularity and reduce constipat

### *Inulin helps keep kids well* [5]

In a study conducted at Johns Hopkins, 123 infants were fed an infant cereal supple inulin or a placebo for 6 months. The children consuming the cereal with inulin show incidence of fever, less antibiotic use, fewer doctor visits, less vomiting and fewer d absences.

### *Inulin can be beneficial in the management of diabetes* [6]

Inulin as an undigested fiber has no effect on blood glucose levels, nor does it affec levels. It has a zero Glycemic Index. Inulin in foods provides both bulk and sweetne causing a rise in glucose levels after meals. It's appropriate for diabetics.

Adding inulin to a food with a low Glycemic Index, like yogurt, can be ideal for gluco

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Learn more about inulin and digestive health.

Learn more about inulin and calcium absorbtion.

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# Appendix 5

## Petition to add to the National List the substance "Oligofructose enriched with Inulin Documented for Calcium Absorption"

**January 12, 2007**

*Critical Reviews in Food Science and Nutrition*, 41(5):353–362 (2001)



# Inulin and Oligofructose as Dietary Fiber: A Review of the Evidence*

*Gary Flamm,[1] Walter Glinsmann,[2] David Kritchevsky,[3] Leon Prosky,[4] and Marcel Roberfroid[5]*

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* The authors express their appreciation to Dr. Barbara Schneeman, University of California, for her review of this manuscript and the opportunity for discussion of key points of the manuscript.

**Referee: Dr. Joanne Slavin, Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenues, St. Paul, MN 5108**

**ABSTRACT:** This critical review article examines the composition and source of inulin and oligofructose, the physiological effects of their consumption, and how these materials relate to the concept of dietary fiber. Inulin and oligofructose are fructans extracted on a commercial basis from the chicory root. Inulin has been defined as a polydisperse carbohydrate material consisting mainly, if not exclusively, of beta (2-1) fructosyl-fructose links ranging from 2 to 60 units long. Native chicory inulin has an average degree of polymerization (DP) of 10 to 20, whereas oligofructose contains chains of DP 2 to 10, with an average DP of 4.

While a universally accepted definition for dietary fiber does not exist, it is generally agreed that this term includes saccharides (+ lignin) that are not hydrolyzed or absorbed in the upper part of the gastrointestinal tract. These materials reach the colon, where they may be totally fermented, partially fermented, or remain unfermented. In addition, fibers contribute to fecal bulking.

Inulin and oligofructose are not digested in the upper part of the gastrointestinal tract or are they absorbed and metabolized in the glycolytic pathway, or directly stored as glycogen like 'sugars' or starches. None of the molecules of fructose and glucose that form inulin and oligofructose appear in the portal blood. These materials are quantitatively fermented by the microflora of the colon; further, it has been demonstrated that this fermentation leads to the selective stimulation of the growth of the bifidobacteria population.

After reviewing their chemistry, origin, and physiological effects, it is the opinion of the authors that inulin and oligofructose are dietary fiber. They share the basic common characteristics of dietary fibers, that is, saccharides of plant origin, resistance to digestion and absorption in the small intestine, and fermentation in the colon to produce short-chain fatty acids that are absorbed and metabolized in various parts of the body. Moreover, this fermentation induces a bulking effect.

## I. INTRODUCTION

Inulin and oligofructose are fructans extracted on a commercial basis from the chicory root. These materials are widely used in Europe as dietary fiber in a variety of foods. This critical review article examines the composition and source of inulin and oligofructose, the physiological effects of their consumption, and how these materials relate to the concept of dietary fiber. However, before considering the above points, it is useful to consider some background information on the general class of carbohydrates known as dietary fiber.

### A. Dietary Fiber

While a universally accepted definition for dietary fiber does not exist, it is generally agreed

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that this term includes saccharides (+ lignin) that are not hydrolyzed or absorbed in the upper part of the gastrointestinal tract. Traditionally, the area of dietary fiber has included various brans, pectins, gums, and celluloses. Dietary fibers reach the colon intact, where they may be totally fermented, partially fermented, or remain unfermented. Fibers also contribute to fecal bulking in a number of ways. They can contribute directly via their own mass and/or the mass of the water they attract. In addition, they can influence fecal bulking in an indirect manner, by being fermented by colonic microflora thus stimulating their growth and resulting in an increase in microbial biomass. When fermented (partly or totally), they are metabolized to hydrogen, methane, carbon dioxide, and short-chain fatty acids (SCFAs). These SCFAs are absorbed and further metabolized in the colonocytes, the liver cells, or the peripheral tissues. The caloric value of the nondigestible but fermented carbohydrates has been estimated at 1.5 to 2 kcal/g.[1,2,3] However, if they are nondigestible and totally resistant to colonic fermentation that value is reduced to 0 kcal/g.

Besides these characteristic behaviors in the gastrointestinal tract, dietary fibers have miscellaneous systemic physiological effects, mainly on lipid metabolism and bioavailability of minerals, and they may help to reduce the risk of diseases such as colon cancer and cardiovascular disease.[4,5,6,7,8,9] However, not all dietary fibers have the same effects, which depend on their physiochemical nature. No specific pattern of gastrointestinal or systemic physiological effects has yet been identified to characterize what is a dietary fiber. Moreover, it is very unlikely that such a pattern will ever be identified because the term dietary fiber refers to a chemically heterogeneous family of products (from various brans to pectins, gums, cellulose) that have different chemical and physical properties.

For the purpose of communicating nutrition information to the consumer, the term dietary fiber is of great value because it clearly distinguishes between this nondigestible class of carbohydrates and digestible, glycemic carbohydrates such as sugars and starches. The importance of distinguishing between these two classes of carbohydrates stems from the need for a balance between the two in a healthy diet. The digestible carbohydrates that are hydrolyzed and absorbed in the small intestine and then systemically metabolized are an important source of energy in the diet. Dietary fiber, as those portions of plants that are resistant to the digestive processes, are beneficial to maintain regularity in the short term, and potentially afford protection against chronic diseases in the long term. A further benefit of dietary fiber is its ability to provide caloric dilution in a diet that is often too energy dense.

## II. INULIN AND OLIGOFRUCTOSE: IDENTITY AND OCCURRENCE

### A. Chemistry and Biochemistry

Inulin and oligofructose are fructans. Fructan is a general name used for any carbohydrate in which one or more fructosyl-fructose links constitute the majority of glycosidic bonds. Inulin has been defined as a polydisperse carbohydrate material consisting mainly, if not exclusively, of beta (2-1) fructosyl-fructose links. It is of plant origin.[10,11] Because, in the plant, it is synthesized from sucrose by repeated fructosyl transfer from a fructosyl donor, inulin usually has a terminal glucose unit. Indeed, the enzymes generally considered to be involved in plant fructan synthesis are sucrose-sucrose fructosyltransferases (EC 2.4.1.99) that catalyze fructosyl transfer from one sucrose molecule to another, leading to 1-kestose (glucosyl-1,2-fructosyl-1,2-fructose). Chain elongation is mediated by 1F-fructan-fructan-fructosyltransferase (EC 2.4.1.100), leading to inulin.[12]

### B. Plant Sources

Fructan-containing plant species are found in a number of mono- and di-cotyledonous families, such as Liliaceae, Amaryllidaceae, Gramineae, and Compositae. Various fructan-containing plant species are often eaten as vegetables (e.g., asparagus, garlic, leek, onion, artichoke, Jerusalem artichoke, scorzonera, chicory roots).[13] However, only a limited number of species are suitable for indus-

trial food and nonfood applications.[10] Despite the high fructan content of the aerial parts of many Gramineae, particularly of young seedlings (up to 70% of their dry weight), grasses and cereals do not lend themselves for industrial extraction and processing of fructans. Conversely, in Liliaceae, Amaryllidaceae, and Compositae, fructans (most exclusively inulin) are usually stored in bulbs, tubers, and tuberous roots, which because of the absence of interfering components can be easily extracted and processed to purified products.

## C. Inulin and Oligofructose

The two species that are used currently by the food industry to produce inulin both belong to the Compositae: the Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus*), the latter being by far the most commonly used source today.[14] In chicory inulin, both GpyFn (alpha D-glucopyranosyl-[beta D-fructofuranosyl]*n*-1-D-fructofuranoside) and FpyFn (beta D-fructopyranosyl-[beta D-fructofuranosyl]*n*-1-D-fructofuranoside) compounds are considered to be included under the same nomenclature and the number of fructose units varies from 2 to more than 70 units (Figure 1). The presence of FpyFn compounds in native inulin extracts has been demonstrated regularly using either gas chromatography (GC) or high-pressure anion exchange chromatography (HPAEC).[15] Native chicory inulin (i.e., extracted from fresh roots, taking precautions to inhibit the plant's own inulinase activity as well as acid hydrolysis) has an average degree of polymerization (DP) of 10 to 20. For native Jerusalem artichoke the average DP is 6.[16] Native inulin has a very small degree of branching (approximately 1%).[15] The various fructose monomers in the GpyFn forms of inulin are all present in the furanose form. Only in the FpyFn forms is the reducing fructose in the pyranose form.[13,14,15,16] Native inulin is processed by the food industry to produce either short-chain fructans, namely oligofructose (DP 2 to 10; average DP 4), as a result of partial enzymatic (endoinulinase, EC 3.2.1.7) hydrolysis or long-chain fructans by applying industrial physical separation techniques.[13]

## D. Presence in the Diet

Inulin-type fructans are present in significant amounts in several edible fruits and vegetables. Average daily consumption has been estimated to be 1 to 4 g in the U.S.[17] and 3 to 11 g in Europe, the most common sources being wheat, onion, banana, garlic, and leek.[13] Chicory inulin and oligofructose are officially recognized as natural food ingredients and classified as dietary fiber in almost all European countries.

## E. Analysis of Inulin-Type Fructans

An analytical method has been developed recently to quantify inulin and oligofructose in plants and food products.[18] As a result of a multicenter validation ring test, this method has been adopted as the official AOAC method 997.08 "Fructans in food products, ion exchange chromatography method" and published in the 4th supplement (1998) to *the Official Methods of Analysis,* 16th edition.[19] The method allows the quantitative determination of fructans in foods and food products, integrated into the AOAC total dietary fiber measurement. The method relies on the enzymatic treatment of the sample with an inulinase enzyme. Inulin and oligofructose are extracted from the sample with boiling water. One aliquot is kept untreated as the initial sample. A second aliquot of the extract is hydrolyzed using an amyloglucosidase enzyme. A sample of the hydrolysate is kept as the second sample, and the rest is further hydrolyzed using an inulinase (Fructozyme SP 230). Glucose, fructose, and sucrose are quantified in the three samples either by capillary gas chromatography (CGC), high-performance liquid chromatography (HPLC), or preferably high-performance anion exchange chromatography–pulse amperometric detection (HPAEC-PAD). Inulin is then calculated by subtracting the sugars in the first and second aliquots from the third. Because oligofructose is not recovered by the AOAC-TDF methods and only a small fraction of inulin is recovered by the AOAC-TDF methods, a correction can be made for inulin that would be double counted. On the one hand, inulinase can be added to the enzyme complex in

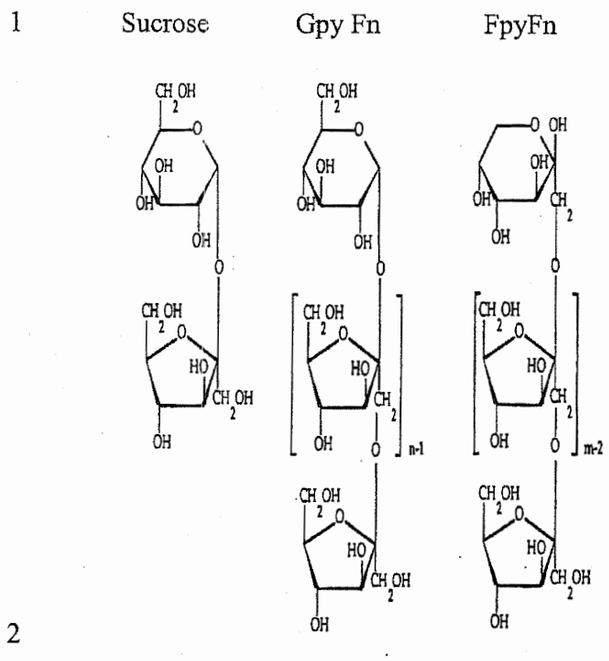


**FIGURE 1.** Basic chemical structure of sucrose (GF) and the fructans GpyFn and FpyFn.

the TDF method. This removes all fructans from the sample. This method was optimized by Quemener et al.[18] Another approach is to determine the fructan content in precipitates of the TDF methods, and then to subtract this amount from the TDF amount.

## III. METABOLISM AND PHYSIOLOGICAL EFFECTS OF INULIN AND OLIGOFRUCTOSE

### A. Nondigestibility in the Upper Gastrointestinal Tract

Because of the beta configuration of the anomeric C2 in their fructose monomers, inulin, and oligofructose are resistant to hydrolysis by human digestive enzymes (alpha glucosidase; maltase-isomaltase; sucrase) that are specific for glycosidic linkages. As indicated above, these carbohydrates have been classified as 'nondigestible' oligosaccharides.[20,21] Both *in vitro* and *in vivo* data support this classification.

When incubated in the presence of a homogenate of different segments (duodenum, jejunum, ileum) of rat or human small intestine, inulin and oligofructose remain unchanged for up to 1 or 2 h.[22,23] In addition, as concluded by Nilsson et al.,[24,25] 'the stomach hydrolysis is likely to be of limited physiological significance', these products proceed undigested through the upper part of the gastrointestinal tract into the colon.

That this is indeed the case has been confirmed by *in vivo* studies in humans.[26,27,28] Knudsen and Hessov[27] and Ellegärd et al.[28] have used the ileostomy model to study the action of the upper gastrointestinal tract on inulin. This model provides a valuable technique to study digestive physiology in man and has often been used to quantify nutrients exiting the small intestine, in particular the carbohydrates.[29,30] Both studies show that 86 to 88% of the ingested dose (10, 17, or 30 g) of inulin and oligofructose are recovered in the ileostomy effluent, supporting the conclusion that inulin and oligofructose are practically indigestible in the small intestine of man. The percentage of recovery in the ileostomy effluent is of the same order as the recovery of pectin but slightly

lower than for cereal[31] and potato fibers.[30] The small loss of inulin and oligofructose during the passage through the small intestine could be due to fermentation by the microbial population colonizing the small intestine in individuals with ileostomies. This microbial population is known to be up to 100 times greater in the ileostomists than in individuals with intact GI tracts.[31] Knudsen and Hessov[27] have measured lactic acid and short-chain carboxylic acids, the end products of the anaerobic fermentation of carbohydrate in the ileostomy effluents before and after inulin intake. There was no difference in total acids exiting the small intestine during periods of inulin consumption vs. control periods. However, there was a shift in the nature of the fermentation products, with a decrease in SCFA and an increase in lactic acid, perhaps because of changes in the preferred fermentation substrate from starch and nonstarch polysaccharide to inulin. This theory is supported by Livesey,[32] who pointed out that bacterial colonization of the small intestine and subsequent fermentation are problems encountered when using the ileostomy model to determine energy value of carbohydrates.

Inulin and oligofructose resist digestion in the upper part of the gastrointestinal tract, and, further, there is no evidence that they are absorbed to any significant extent.[26,33,34] Thus, it has been proposed to classify them as 'colonic food', that is, a 'food entering the colon and serving as substrate for the endogenous bacteria, thus indirectly providing the host with energy, metabolic substrates and essential micronutrients'.[35]

## B. Fermentation in the Large Bowel

That inulin and oligofructose are fermented by bacteria colonizing the large bowel is supported by a large number of *in vitro* and *in vivo* studies that, in addition, confirm the production of lactic and short-chain carboxylic acids as end products of the fermentation.[36,37] Furthermore, it has been demonstrated that in human *in vivo* studies this fermentation leads to the selective stimulation of growth of the bifidobacteria population, making inulin and oligofructose prototype prebiotics.[38,39,40] The first line of evidence that inulin and oligofructose are fermented in the large bowel is the demonstration that these carbohydrates are metabolized when incubated with human faecal samples in anaerobic batch cultures. Because such fermentation is known to produce various acids, changes in the culture pH is an easy way to demonstrate this assumption and also, by using pure cultures, to identify which bacteria have the potential to perform such a metabolic process. Moreover, by estimating the size of the drop in culture pH over a given period of incubation, it is also possible to compare different substrates on a semiquantitative basis. Such data have been reviewed by Roberfroid et al.[41] In summary, inulin and oligofructose are well fermented when incubations are performed using human fecal flora as inoculum. They are both rapidly and completely metabolized by human fecal microflora. *In vivo* studies have confirmed the fermentation of inulin and oligofructose by colonic microbiota. They are not recovered as inulin or oligofructose in the feces and, due to an increase in the biomass, they induce a bulking effect both in rats[42] and in humans[35] that is equivalent to that of gums and pectins.

## C. Production of Short-Chain Fatty Acids

During their passage through the gastrointestinal tract, inulin and oligofructose never produce fructose or glucose.[23] Rather, their colonic fermentation produces short-chain fatty acids and lactate plus gases as products of their anaerobic metabolism. Concerning the pattern of production of short-chain fatty acids, *in vitro* fermentation and animal studies have demonstrated that supplementing the diet with inulin and oligofructose decreases the cecal pH and increases the size of the cecal pool of short-chain fatty acids. Due to the selective fermentation of these chicory fructans, the relative proportion of the three main SCFA is changed. Typically, the molar ratio of butyrate in rats fed inulin or oligofructose is increased when compared with rats fed a typical starch-rich standard diet without added oligosaccharides.[43,44,45]

In summary, as is the case with other dietary fibers, inulin and oligofructose are resistant to digestion in the upper part of the intestinal tract and subsequently are fermented in the colon. They also have a bulking effect due to the increase in the microbial biomass that results from their fermentation. From a quantitative point of view, that bulking effect, expressed as the increase in the daily fecal mass, has been reported to vary between 1.5 and 2 g/g of ingested inulin or oligofructose,[38,47] which is of the same order of magnitude as pectin. Another typical dietary fiber effect is the increase in stool frequency, as was observed recently when inulin was incorporated into the diet of healthy but chronically slightly constipated human volunteers.[48]

## D. Caloric Value

Several studies have estimated the caloric value of inulin and oligofructose in human subjects using various methodologies.

Molis et al.[26] fed oligofructose and then measured the amount that exits the small intestine using intestinal aspiration, as well as the amounts excreted in feces and urine. With these measurements the authors determined the amount absorbed and metabolized from the small intestine (reported as 11%) and the amount fermented in the colon (reported as 89%). Caloric value was determined by multiplying small intestine utilization by 4 kcal/g (the accepted energy value for digested carbohydrate) and by assuming the amount fermented in the colon yields 50% of the energy of a digestible carbohydrate. Using this analysis a caloric value of 2.3 kcal/g (9.5 kJ/g) was reported. However, the energy capture specifically from fermentation of oligofructose has been shown by Roberfroid et al.[49] to be only 38% of that of digestible carbohydrate, not the general value of 50% as assumed in this study. Further, it is recognized that intestinal aspiration is a technique that may overestimate the amount of material absorbed in the small intestine;[32] therefore, the small intestinal factor in this analysis could drop as low as zero. When caloric value is calculated using these factors, a value of 1.5 kcal/g (assuming no small intestinal caloric contribution) to 1.8 kcal/g (assuming the 11% small intestinal absorption) is obtained.

In an earlier study, Hosoya et al.[50] used a radiorespirometric method to determine a caloric value of 1.5 kcal/g. $C^{14}$ radiolabeled oligofructose was fed to subjects and the radioactivity was tracked in urine, feces, breath, and flatus. The proportion of labeled $C^{14}$ in feces, urine, and flatus was taken as the proportion of ingested oligofructose that had no caloric value, because it was excreted from the body. The proportion of labeled $C^{14}$ in the breath was the amount of oligofructose converted to $CO_2$ either as a direct consequence of fermentation or as a result of the body metabolizing SCFA that was produced by fermentation. To distinguish between these two, *in vitro* data were generated by incubating fecal slurries from the subjects with labeled oligofructose to track radioactive incorporation into fermentation products. From this *in vitro* work, the proportions of $CO_2$, volatile fatty acids (VFA), and bacterial biomass produced by fermentation of oligofructose was determined. Combining these data, the authors determined the amount of respired $CO_2$ that resulted directly from fermentation vs. that which resulted from the metabolism of SCFA to yield energy. The amount of SCFA was then calculated, and by multiplying this by the caloric value for SCFA a caloric value of 1.5 kcal/g was arrived at for oligofructose.

In addition to these two studies, Castiglia and Vermorel[51] and Castiglia-Delavaud et al.[52] conducted energy balance experiments using whole-body indirect calorimetry methods in which these authors reported caloric values for inulin ranging form 2.1 to 2.8 kcal/g. However, this technique carries quite a bit of variability, only being able to estimate within 0.5 kcal/g precision as described by Livesey.[32] The variable results of the Castiglia studies seem to support Livesey's conclusion, as both studies used the same number of subjects, and same methods, but yielded different results.

In summary, because only a part of the energy of these dietary carbohydrates is salvaged, their available energy content is only 40 to 50% that of a digestible carbohydrate, giving them an energy value of 1.5 to 2.0 kcal/g.

### E. Other Physiological Effects

Besides the properties reviewed above, concerning their role as fiber in the diet, inulin and oligofructose have been shown to induce interesting physiological/nutritional effects that they share with some (but not all) dietary fibers. These effects relate to hypotriglyceridemia, hypoinsulinemia, improved calcium bioavailability, hypocholesterolemia, and reduction of risk of colon cancer in experimental models. These effects have been reviewed recently.[53,54,55]

### F. Safety

As noted above, inulin and oligofructose are natural food ingredients present in edible plants and already part of the traditional diet. Based on USDA data, it was estimated that the average per capita daily intake of these fructans ranges between 1 and 4 g/day, with the 90th percentile consuming 2 to 8 g/day.[17] Their current and proposed uses in multiple food categories could lead, in principle, to increased consumer exposure to dietary fiber in general, and inulin/oligofructose in particular. This may have some desirable effects on colonic function and health.

The daily reference value (DRV) for fiber (soluble and/or insoluble) is 25 g/day. The average daily intake of inulin/oligofructose, naturally present in food, is estimated between 1 to 4 g/day in the U.S. and 3 to 11 g/day in Europe. The extensive available data indicate that safety, as traditionally defined, is not an issue in the case of inulin and oligofructose.[56] This is supported by the critical review of the toxicological studies showing that fructooligosaccharides do not increase morbidity, mortality, or cause reproductive or target organ toxicity. These fibers are not mutagenic, carcinogenic, or teratogenic.[56,57]

Numerous animal and human investigational studies have been performed to identify the physiologic and potential health benefits of these fructans, while simultaneously assessing possible intolerance. The only biological effects observed are attributed to their action as nondigestible, fermentable carbohydrates causing self-limited gastrointestinal (GI) distress. Symptoms range from flatulence (the most common), to borborygmi and bloating, to laxation (as the most severe). As demonstrated by various studies, the ensuing GI symptoms are dose dependent.[58,59] Results indicate that these fructans are well tolerated at up to 20 g/day, while diarrhea can develop with intakes of 30 g/day or more.[56]

The deleterious effects of a high intake of dietary insoluble fiber (specifically those containing phytate) on mineral and calcium balance have been described in the literature.[60,61] However, soluble fibers such as inulin and oligofructose do not contain phytate. Further, inulin and oligofructose have been shown to enhance calcium absorption, while not influencing the balance of other minerals.[28,62,63,64,28]

## IV. CONCLUSION

After reviewing their chemistry, origin, and physiological effects, it is the opinion of the authors that inulin and oligofructose are dietary fiber. Inulin and oligofructose fit well within the current concept of the class of materials referred to as dietary fiber. They are not digested in the upper part of the gastrointestinal tract or are they absorbed and metabolized in the glycolytic pathway or directly stored as glycogen like 'sugars' or starches. This agrees very well with the accepted definition of dietary fiber put forth by Trowell et al.[65] None of the molecules of fructose and glucose that form inulin and oligofructose appear in the portal blood. These materials share the basic common characteristics of dietary fibers, that is, saccharides of plant origin, resistance to digestion and absorption in the small intestine, and fermentation in the colon to produce short-chain fatty acids that are absorbed and metabolized in various parts of the body. Moreover, this fermentation induces a bulking effect.

Finally, given that inulin and oligofructose clearly elicit dietary fiber effects, it is important to recognize that foods containing these materials do in fact contain fiber and should be consumed accordingly.

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# Net energy value of non-starch polysaccharide isolates (sugarbeet fibre and commercial inulin) and their impact on nutrient digestive utilization in healthy human subjects

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The energy value of NSP has been expressed as their metabolizable energy (ME) content. The aim of the present study was to determine whether differences in ME and net energy (NE) contents were similar for insoluble and soluble NSP. Nine healthy young men were offered three diets according to a Latin-square design (3 × 3) with three repetitions: diet C (control), diet B (control + 50 g sugarbeet fibre/d) and diet I (control + 50 g commercial inulin/d). After a 16 d adaptation period to NSP isolate, food intake was controlled (duplicate meal method) and faeces and urine were collected for 8 d. A period of 60 h was devoted to measurement of energy expenditure (EE) by whole-body indirect calorimetry. NSP-isolate ingestion induced significant increases in the number of defecations and stool weight resulting from increases in water, DM and microbial mass excretion. After deduction of microbial N, differences in faecal N excretion between diets were not significantly different. Urinary N excretion was slightly decreased by sugarbeet fibre or commercial inulin ingestion but the N balances for the diets were not significantly different. Diet energy, N and lipid apparent digestibilities decreased by only 1–2 %. Commercial inulin was entirely fermented and fermentability of sugarbeet fibre averaged 0·886 (SD 0·117). Sugarbeet fibre and commercial inulin ME values averaged 10·7 (SD 1·2) and 13·0 (SD 2·3) kJ/g DM respectively. NSP-isolate ingestion caused significant (sugarbeet) and non-significant (inulin) increases in daily EE. The maintenance NE contents of sugarbeet fibre and inulin averaged 5·0 (SD 5·0) and 11·9 (SD 1·3) kJ/g DM respectively. Differences in maintenance NE contents of NSP isolates were much greater than differences in ME values.

**Non-starch polysaccharides: Sugarbeet fibre: Inulin: Indirect calorimetry**

In addition to the well-known beneficial effects of NSP ingestion on satiety and regulation of digestive transit (Cummings *et al.* 1978), epidemiological studies have shown increased incidence of pathologies such as cardio-vascular diseases and colon cancer in Western countries, particularly in western Europe (Trowell, 1972; Southgate, 1988), where NSP intake has decreased greatly during the last few decades (Burkitt, 1987). The regulatory effects of volatile fatty acids (VFA), produced by soluble-NSP fermentation, on colonic mucous membrane cellular development have been demonstrated by Breuer *et al.* (1991) among others. These results have highlighted the beneficial effect of NSP in the prevention of colon cancer.

The potential benefit of greater NSP intake has led to several studies in human subjects on the effect of soluble-fibre ingestion on digestive utilization of dietary components. Thus, ingestion of fruit, vegetable and cereal fibre (+57·5 g/d) resulted in significant decreases in apparent digestibilities of energy, crude protein and lipids (Göranzon *et al.* 1983; Wisker *et al.* 1988; Miles, 1992). From these measurements, the energy value of NSP was expressed in terms of metabolizable energy (ME). However, this energy unit does not take into account energy lost as fermentation heat, or differences in efficiency of glucose and VFA energy utilization, or the probable increase in metabolic rate of the large intestine. The net energy (NE) values of NSP for

**Abbreviations:** EE, energy expenditure; ME, metabolizable energy; MEm, maintenance ME requirement; NE, net energy; VFA, volatile fatty acids.

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man can be estimated from their measured digestibilities and from estimates of the energy lost in their use for synthesis of microbial biomass, $H_2$ and $CH_4$, estimates of the energy lost as heat of fermentation and from the efficiency with which the VFA, products of fermentation, may be utilized (Livesey, 1992). However, all these losses, as well as the probable increase in metabolic rate of the large intestine, can be determined by whole-body indirect calorimetry (Van Es *et al.* 1986).

Because of the beneficial effects of NSP in human nutrition and increasing attention paid to them by the food industry, the aims of the present study were to determine (1) the maintenance NE values of partly insoluble (sugarbeet fibre) and soluble (inulin) NSP isolates and their effect on nutrient digestive utilization in healthy human subjects and (2) whether differences in maintenance NE and ME contents were similar. Energy balances were carried out in subjects adapted to NSP-isolate ingestion, by using whole-body indirect calorimetry which requires relatively high levels of NSP-isolate intake. The best ways to incorporate the isolates into food and the highest tolerable doses were defined from the results of a digestive tolerance study (Seguenot, 1990).

## Subjects and methods

### *Subjects*

Nine healthy young men, without any medical history of renal, vascular, digestive, endocrine or currently evolving disease, 21·5 (SD 2·5) years of age, and weighing 69·3 (SD 5·0) kg, were enlisted after a normal physical examination. Those who had a BMI higher than 25 kg/m$^2$ were also excluded. Each subject received a complete explanation of the purpose and procedures of the investigation and signed an informed consent form. The study protocol was approved by the regional Medical Faculty Ethical Committee (CCPPRB no. AU38). During the study, the subjects lived at home and had lunch and dinner at the Human Nutrition Laboratory. Extra food items, such as alcoholic and energy-containing beverages, were not permitted.

### *Methods*

*Experimental design.* The nine subjects were offered three diets according to a Latin-square design (3×3) with three repetitions: a control diet (22 g of NSP/d; diet C); a sugarbeet diet (diet B) and an inulin diet (diet I) corresponding to diet C +50 g/d sugarbeet fibre or commercial inulin respectively. Each experimental period, lasting for 28 d, comprised 2 d (days 1 and 2) with the control diet, 14 d (days 3–16) with a progressive adaptation to the NSP isolates up to a maximum of 50 g/d; 12 d (days 17–28) with a constant intake of NSP isolates. The balance period covered 8 d (days 21–28) and involved total collection of faeces and urine; the last 2 d (days 27 and 28) were devoted to measurement of energy expenditure (EE) using whole-body calorimetry. Food intake was determined by the duplicate meal method.

*Experimental diets.* Four daily balanced low-fibre diets (Table 1) were composed by the dietitian of the Human Nutrition Laboratory. They were distributed in rotation to subjects during each balance period. The ME supply to each subject was calculated from the results of a dietary inquiry, and it was adjusted to appetite over 2 d (days 17 and 18) before the first balance period.

Two types of NSP isolate were studied: sugarbeet fibre and chicory inulin (commercial product containing 620 g pure inulin/kg) produced by the Agro-industries, Recherches et Développements (ARD) Society. Their technological properties allowed their incorporation into

**Table 1.** Diets. The quantities of the different components shown here are indicative of the actual consumed quantities which were weighed exactly for each subject during each dietary period

| Day 1 | | Day 2 | |
|---|---|---|---|
| Lunch | Dinner | Lunch | Dinner |
| Grated carrots 100 g<br>Dressing 10 g<br>Chicken 120 g<br>Pasta 200 g<br>Margarine 10 g<br>Yoghurt 135 g<br>Compote 100 g<br>Biscuits 30 g<br>Bread 120 g | Vegetable soup 200 g<br>Fish 120 g<br>Rice 200 g<br>Margarine 10 g<br>Cheese 30 g<br>Fruit 200 g<br>Bread 120 g | Cucumber 80 g<br>Cream 10 g<br>Beef 120 g<br>Mashed potatoes 250 g<br>Margarine 10 g<br>Fruit yoghurt 120 g<br>Canned fruit 150 g<br>Bread 120 g | Tomato soup 200 g<br>Eggs (2) 100 g<br>Lentils 150 g<br>Blue cheese 120 g<br>Fruit 150 g<br>Orange juice 200 g<br>Bread 120 g |
| **Day 3** | | **Day 4** | |
| Lunch | Dinner | Lunch | Dinner |
| Peeled tomatoes 100 g<br>Dressing 10 g<br>Veal 120 g<br>Carrots 300 g<br>Margarine 10 g<br>Yoghurt 135 g<br>Dessert cream 100 g<br>Bread 120 g | Vegetable soup 200 g<br>Fish 120 g<br>Potatoes 200 g<br>Butter 10 g<br>Cheese 27 g<br>Biscuits 30 g<br>Orange juice 200 g<br>Bread 120 g | Red beets 100 g<br>Dressing 10 g<br>Beef 120 g<br>Rice 200 g<br>Margarine 10 g<br>Cream cheese 120 g<br>Fruit 150 g<br>Bread 120 g | Vegetable soup 200 g<br>Omelette 120 g<br>Courgette gratin 300 g<br>Cheese 30 g<br>Compote 100 g<br>Biscuits 30 g<br>Bread 120 g |

diets, particularly in bread (Seguenot, 1990). Each NSP isolate (50 g) was added to the diet each day in two ways: 30 g in bread before kneading and 20 g sprinkled on liquid foods by the volunteers.

*Sample treatment.* Representative food samples were prepared during the last 10 d of each experimental period. Duplicate meals and bread were homogenized, freeze-dried and analysed separately. Urine was collected in plastic bottles and weighed daily during the last 8 d of each control period. Representative samples (50 ml/l) were pooled in acid-washed plastic bottles. Faeces were collected in plastic pots, homogenized for the 8 d balance period, freeze-dried and stored at −18° until analysis.

*Analytical methods.* The DM content of dietary and faecal samples was determined after drying at 80° for 48 h. The gross energy content of dietary samples, faeces and urine was analysed using an adiabatic bomb calorimeter (Gallenkamp, London, UK) calibrated with benzoic acid. Total N content of diets, faeces and urine was analysed using the macro-Kjeldahl method. Lipids of foods and faeces were extracted by the Folch technique (Folch *et al.* 1957), and fatty acids were extracted by lipid saponification with 1·8 M-KOH, acidification with 6 M-HCl and extraction by hexane.

The scheme for carbohydrate analysis is presented in Fig. 1. Starch was analysed in samples of duplicate meals, bread and faeces according to the method of Faisant *et al.* (1995) which includes resistant starch. Glucose was determined enzymically (Merckotest 14365; Merck, Darmstadt, Germany). Before analysis of cell-wall carbohydrates starch was removed from duplicate meal and bread samples using the method of Thivend *et al.* (1972) modified to prevent cell-wall-carbohydrate degradation. In brief, 100 ml distilled water was added to 5 g ground freeze-dried sample, boiled for 5 min and cooled. Then 135 ml acetate buffer (0·3 M, pH 5·0), 12 ml amyloglucosidase (*EC* 3.2.1.3; Merck, 75 IU/mg, 24 g/l) and 0·25 ml Termamyl (Novo 120 L; Novo Nordisk, Fontenay-sous-bois, France) were added. Hydrolysis was carried out in a shaking water-bath for 2 h at 60°. After filtering through a sintered crucible (porosity 2) residue 1 was dried at 60°. The latter was defatted by Soxhlet refluxing with toluene–methanol (2:1, v/v) and filtered through a sintered crucible (porosity 2). Hemicellulose and cellulose in this residue 2 were determined according to Jarrige (1961) after sequential acid hydrolysis and filtering through sintered crucibles (porosity 3), and analysed colorimetrically in the hydrolysates for reducing sugars using xylose and glucose as standards (Besle *et al.* 1981). The cell-wall carbohydrate content of samples containing no (sugarbeet fibre) or little (faeces) starch was determined similarly, except for the long initial step of starch removal. Sugarbeet fibre was washed three times with distilled water at 40°. The hemicellulose content of faeces was corrected by deducting the very low glucose content since starch was totally hydrolysed by refluxing with 1 M-$H_2SO_4$ for 3 h.

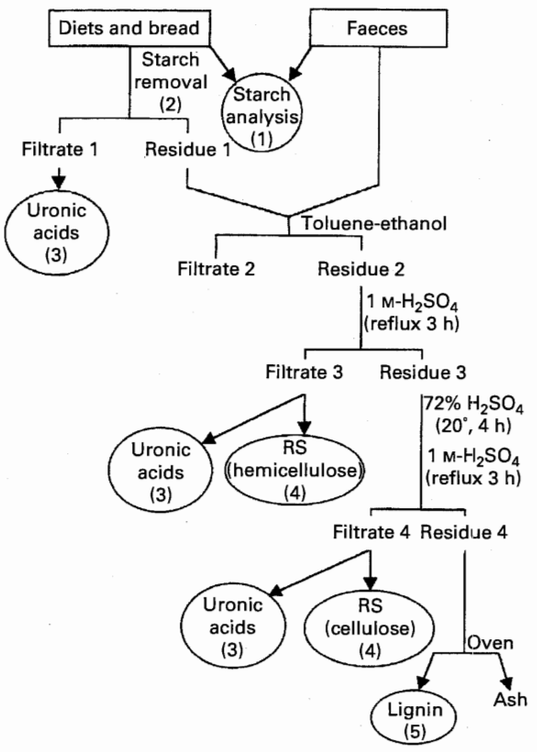


**Fig. 1.** Flow diagram showing the various stages of carbohydrate analysis. (1), Faisant *et al.* (1995); (2), Thivend *et al.* (1972), modified; (3), Blumenkrantz & Asboe-Hansen (1973); (4), Besle *et al.* (1981); (5), Jarrige (1961). RS, reducing sugars.

Uronic acids were determined in the water extracts and in the filtrates according to the method of Blumenkranz & Asboe-Hansen (1973). Inulin was hydrolysed from inulin powder, inulin bread, duplicate meals and faeces samples with 0·3 M-$HClO_4$ (80°, 1 h; Beutler, 1984) and analysed enzymically for fructose (Boehringer Mannheim 139106; Boehringer Mannheim, Meylan, France). All the results were expressed as polymers, i.e. taking off one molecule of water per monomeric unit.

Faecal bacteria were extracted as follows: freeze-dried faeces samples were suspended in saline (9 g NaCl/l) solution (100 g washed sample/320 ml) and homogenized three times for 1 min using a Waring blender. The homogenate was pummeled for 5 min in a Colworth Stomacher 400 (A. J. Steward & Co. Ltd., London, UK) using a sterile polyethylene bag (180×300 mm) and squeezed through seven layers of surgical gauze. Solid residue was rinsed in saline (100 g/100 ml) and squeezed, as described earlier, through surgical gauze at the end of the procedure. The filtrate was centrifuged at 500 ***g*** for 30 min at 4° (Kontron H401: rotor A 6.14; Kontron, Saint-Quentin en Yvelines, France). The supernatant fraction was retained and the solid fraction was submitted to the same treatment as the initial sample. The two supernatant fractions were pooled and centrifuged at 30 000 ***g*** for 45 min at 4° for isolation of bacteria. The latter were freeze-dried and weighed. The faecal bacterial mass was determined from the purine-base content of the extracted bacteria and faeces samples. Purine bases were precipitated with $AgNO_3$ at pH 2 and quantified by spectrophotometry at 260 nm (Zinn & Owens, 1986).

*Energy expenditure measurements.* Whole-body indirect calorimetry was used to determine EE. The two open-circuit calorimetric chambers used (11 $m^3$ each) were airtight (inflatable seals), continuously ventilated by atmospheric air, and equipped with an air-conditioning system controlling air temperature (± 0·5°) and relative humidity (± 2 %). $O_2$ consumption and $CO_2$ production were measured continuously using differential gas analysers: $CO_2$, 0–1 %; $O_2$, 21–20 % (Mahiak, Hamburg, Germany). At the end of each balance period, subjects spent 2·5 d in the calorimeters under cardiac supervision, one evening and one night for adaptation to the chamber environment and 2 d for EE measurement. During each measurement period volunteers followed precisely a standardized activity programme with four 20 min periods of exercise on a cycle ergometer (Ergomeca, Sorem, Toulon, France). All physical variables such as air temperature, relative humidity, flow and composition, as well as heart rate were recorded every minute. The validity of gas exchange measurements was checked by infusions of $CO_2$ and $N_2$ into the chambers for 8 h after equilibrium (Vermorel *et al.* 1995). Recovery averaged 99·5 (SD 0·6) % for $CO_2$ and $O_2$.

*Calculations.* Computation of nutrient and energy intakes was as previously described (Vernet & Vermorel, 1993). Apparent digestibility of dietary energy was calculated as: ((gross energy intake − gross energy content of faeces)/gross energy intake). Analogous equations were used for N, lipid and fatty acid apparent digestibilities, and NSP (cellulose + hemicellulose + uronic acids) fermentability. Dietary ME was calculated as: digestible energy − urinary energy, because of lack of reliable measurements of $H_2$ and $CH_4$ production. Digestible energy values ($DEV_{NSP}$) and ME values ($MEV_{NSP}$) of NSP were calculated as follows:

$$DEV_{NSP} = \frac{\left[GEI_{NSP\ diet} \times \left(\frac{DEI}{GEI}\right)_{NSP\ diet}\right] - \ldots}{NSP} \quad \frac{\ldots \left[(GEI_{NSP\ diet} - GEI_{NSP}) \times \left(\frac{DEI}{GEI}\right)_{control\ diet}\right]}{}, \tag{1}$$

$$MEV_{NSP} = \frac{\left[GEI_{NSP\ diet} \times \left(\frac{MEI}{GEI}\right)_{NSP\ diet}\right] - \ldots}{NSP} \quad \frac{\ldots \left[(GEI_{NSP\ diet} - GEI_{NSP}) \times \left(\frac{MEI}{GEI}\right)_{control\ diet}\right]}{}, \tag{2}$$

where $DEV_{NSP}$ and $MEV_{NSP}$ are expressed in kJ/g DM; NSP is expressed as g DM/d; GEI is gross energy intake (kJ/d); DEI is digestible energy intake (kJ/d); and MEI is ME intake (kJ/d).

EE was calculated using the Brouwer (1965) formula (3·866 $O_2$ (litres) + 1·200 $CO_2$ (litres) − 1·430 urinary N (g)). Retained energy was calculated as: ME − EE. Maintenance ME requirement (MEm) values for retained energy = 0 were calculated individually from retained energy assuming that ME efficiency was 0·95 for maintenance (negative energy balance) and 0·90 for fattening (positive energy balance) (Van Es *et al.* 1984). Differences in MEm between the experimental diets and the control diet were considered to result from differences in efficiency of NSP-isolate ME utilization for maintenance. The maintenance NE value of NSP ($NEV_{NSP}$) was calculated as follows:

$$NEV_{NSP} = \frac{ME_{NSP} - \Delta\ MEm}{NSP}, \tag{3}$$

where $ME_{NSP}$ is the ME supplied by NSP (kJ/d). Thus, the NE content of NSP isolate was ME content minus the algebraic difference in MEm between experimental and control diets.

*Statistical analysis.* Data were analysed statistically according to a Latin-square design (3 × 3) with three repetitions. Comparison between experimental diets was done by ANOVA using the general linear models procedure of Statistical Analysis Systems (1987), according to the following model: $\mu + \alpha$ diet + $\beta$ repetition + $\delta$ subject (repetition) + $\varepsilon$. The 'LS MEAN' statement was used to calculate the adjusted means, and the 'CONTRAST' statement to compare the three diets. For each experimental diet the data are presented as adjusted values, because of lack of one subject on diet I, with standard errors of the mean.

## Results

### *NSP isolate and diet composition*

Sugarbeet fibre (15·13 kJ gross energy/g DM) was composed of (g/kg DM): ash 22, crude protein 65, cellulose 233, hemicellulose 272, uronic acids 176, lignin 29, soluble components (proteins, sugars) 203. Commercial inulin (16·65 kJ gross energy/g DM) consisted of (g/kg DM): inulin (fifteen fructose units) 620 and sugars (mono-, di- and trisaccharides) 380. During the cooking of the bread 93 % of the incorporated inulin was hydrolysed. Consequently daily inulin supply was only 22 g instead of 50 g. On average, 0·34, 0·37 and 6·44 g cellulose, 2·22, 2·39, and 9·19 g hemicellulose and 0·17, 0·18 and 4·79 g uronic acids were supplied daily by control, inulin and sugarbeet breads respectively.

All the volunteers completed the study, except one who missed the inulin treatment because of slight diarrhoea. The daily amounts of ingested nutrients and gross energy are given in Table 2. The combined cellulose, hemicellulose and uronic acid intakes were similar for diets C and I but 143 % higher for diet B: 20·0, 19·6 and 48·5 g/d respectively.

### *Faecal weight and faecal microbial excretion*

Daily ingestion of 50 g sugarbeet fibre did not cause digestive disorders in any of the nine volunteers, except a feeling of flatulence in some of them. Ingestion of commercial inulin caused diarrhoea in one subject. However, NSP-isolate ingestion resulted in an increase in the number of

**Table 2.** Experimentally determined daily nutrient and gross energy intakes of subjects consuming a control diet and diets containing sugarbeet fibre or inulin*

(Mean values and standard deviations for nine subjects per diet)

| Diet... | Control | | Sugarbeet | | Inulin | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Gross energy (kJ/d) | 11436 | 1408 | 11505 | 1585 | 11761 | 1477 |
| DM (g/d) | 560·5 | 65·5 | 564·8 | 68·5 | 585·7 | 66·6 |
| Protein (g/d) | 113 | 16 | 111 | 15 | 110 | 16 |
| (% energy) | 23·5 | | 23·0 | | 22·3 | |
| Fat (g/d) | 69 | 15 | 68 | 16 | 64 | 14 |
| (% energy) | 24·5 | | 24·0 | | 22·0 | |
| Total fatty acids (g/d) | 54 | 10 | 54 | 14 | 51 | 12 |
| Hemicellulose (g/d) | 10·4 | 1·8 | 21·7 | 2·5 | 10·3 | 1·8 |
| Cellulose (g/d) | 4·05 | 0·82 | 14·03 | 2·04 | 3·93 | 0·88 |
| Uronic acids (g/d) | 5·57 | 1·24 | 12·85 | 1·51 | 5·38 | 1·21 |

* For details of diets, see Table 1 and pp. 344–345.

**Table 3.** Wet and dry stool weights, faecal bacterial excretion and faecal bacterial nitrogen excretion of subjects consuming a control diet and diets containing sugarbeet fibre or inulin†

(Values are adjusted least square means for nine subjects during an 8 d period, with their pooled standard errors)

| Diet... | Control | Sugarbeet | Inulin | SEM |
|---|---|---|---|---|
| Wet faecal weight (g/d) | $129^{a}$ | $202^{b*}$ | $204^{b**}$ | 16 |
| Dry faecal weight (g/d) | $27·7^{a}$ | $37·6^{b*}$ | $37·1^{b*}$ | 2·3 |
| Faecal bacterial weight (g DM/d) | $13·0^{a}$ | $20·3^{b**}$ | $18·6^{b**}$ | 1·2 |
| Faecal bacterial nitrogen (g/d) | $0·400^{a}$ | $0·621^{b**}$ | $0·782^{c***}$ | 0·047 |
| Faecal nitrogen (g/d) | $1·72^{a}$ | $1·99^{b*}$ | $1·98^{b*}$ | 0·07 |

$^{a,b,c}$ Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
Mean values were significantly different from control: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.
† For details of diet and procedures, see Tables 1 and 2 and pp. 344–346.

defecations (+20% and +15% for diets B and I respectively) and a 57% increase in stool weight ($P < 0·05$) with the two diets (Table 3). The latter resulted from increases in both water and DM excretion ($P < 0·05$). Furthermore, there were large ranges in stool weights (from 59·0 to 281·5 g/d, 62·9 to 393·8 g/d and 71·7 to 297·2 g/d for diets C, B and I respectively) and DM contents, even after correction for differences in food intake whatever the diet.

Daily microbial mass excretion increased by 56 and 43% with diets B and I respectively ($P < 0·01$, Table 3). The difference in bacterial energy excretion between diets B and C amounted to 35% of the increase in fermented NSP energy, assuming that the gross energy content of bacteria was 21·3 kJ/g DM as for rumen bacteria (Hussein *et al.* 1995). Furthermore, while the N content of the microbial mass was similar for diets C and B, it was 38% higher with diet I. Finally, microbial N contributed 23·2, 31·2 and 39·5% to total faecal N excretion with diets C, B and I respectively.

*Apparent digestibility of diets and nitrogen retention*

Fermentability of dietary NSP averaged 0·738 (SD 0·087), 0·825 (SD 0·088) and 0·878 (SD 0·056) for diets C, B and I (including inulin) respectively. Hemicellulose and cellulose fermentability was significantly higher for diet B than for diets C and I, whereas uronic acid fermentability was not significantly different between diets B and C (Table 4). However, hemicellulose, cellulose and uronic acid fermentability was significantly lower for diet I than for the other two diets. Variability of cellulose fermentability was high because of very low values for the same two or three volunteers on each of the three diets, resulting in heavier stools and higher stool cellulose content.

Increases in faecal DM excretion with diets B and I were accompanied by significant increases in faecal energy excretion and resulted in significant reductions of apparent digestibility of energy (Table 4). Starch apparent digestibility was over 0·990. Decreases in lipid ($P < 0·01$) and fatty acid (NS) digestibilities were similar with diet B but negligible with diet I. Similarly, the 16% increase in faecal N excretion ($P < 0·05$) resulted in a 1·8% reduction of apparent N digestibility with both experimental diets ($P < 0·01$). However, after deduction of microbial N excretion, the corrected N digestibilities obtained were not significantly different between diets C, B and I. Thus, sugarbeet fibre and inulin intake did not significantly alter digestive utilization of dietary proteins and N balance (Table 4).

**Table 4.** Daily intake, excretion and apparent digestibility values for dietary constituents and fermentability of NSP in subjects consuming a control diet and diets containing sugarbeet fibre or inulin†

(Values are adjusted least square means for nine subjects during an 8 d period, with their pooled standard errors)

| Diet... | Control | Sugarbeet | Inulin | SEM |
|---|---|---|---|---|
| Energy | | | | |
| intake (kJ/d) | 11467[a] | 11441[a] | 11677[a] | 92 |
| faecal excretion (kJ/d) | 585[a] | 734[b***] | 707[b**] | 22 |
| apparent digestibility | 0·949[a] | 0·936[b**] | 0·939[b**] | 0·002 |
| urinary excretion (kJ/d) | 562[a] | 543[a] | 562[a] | 12 |
| Metabolizability | 0·900[a] | 0·889[b***] | 0·891[b**] | 0·002 |
| Nitrogen | | | | |
| intake (g/d) | 18·1[a] | 17·7[a] | 17·6[a] | 0·2 |
| faecal excretion (g/d) | 1·72[a] | 1·99[b*] | 1·98[b*] | 0·07 |
| apparent digestibility | 0·906[a] | 0·888[b**] | 0·888[b**] | 0·004 |
| corrected digestibility‡ | 0·927[a] | 0·922[a] | 0·931[a] | 0·003 |
| urinary excretion (g/d) | 15·8[a] | 15·3[a] | 15·3[a] | 0·25 |
| balance (g/d) | 0·52[a] | 0·29[a] | 0·29[a] | 0·27 |
| Fat | | | | |
| intake (g/d) | 69·2[a] | 67·4[ab] | 63·3[b*] | 1·7 |
| faecal excretion (g/d) | 3·72[a] | 4·39[b**] | 3·52[a] | 0·13 |
| apparent digestibility | 0·945[a] | 0·932[b**] | 0·942[a] | 0·002 |
| Total fatty acids | | | | |
| intake (g/d) | 54·8[a] | 53·4[ab] | 50·3[b*] | 1·3 |
| faecal excretion (g/d) | 1·59[a] | 2·00[a] | 1·48[a] | 0·17 |
| apparent digestibility | 0·970[a] | 0·959[a] | 0·969[a] | 0·004 |
| Hemicellulose | | | | |
| intake (g/d) | 10·46[a] | 21·50[b***] | 10·19[a] | 0·37 |
| faecal excretion (g/d) | 1·89[a] | 2·41[a] | 3·16[b**] | 0·23 |
| fermentability | 0·822[a] | 0·881[b]§ | 0·697[c***] | 0·018 |
| Cellulose | | | | |
| intake (g/d) | 4·13[a] | 13·86[b**] | 3·81[a] | 0·34 |
| faecal excretion (g/d) | 2·09[a] | 4·30[b]§ | 2·85[a] | 0·71 |
| fermentability | 0·511[a] | 0·687[b]§ | 0·330[c]§ | 0·065 |
| Uronic acids | | | | |
| intake (g/d) | 5·61[a] | 12·77[b***] | 5·37[a] | 0·23 |
| faecal excretion (g/d) | 0·51[a] | 0·77[ab] | 1·08[b*] | 0·14 |
| fermentability | 0·919[a] | 0·939[a] | 0·814[b*] | 0·026 |

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
Mean values were significantly different from control: $^{*}P < 0·05$, $^{**}P < 0·01$, $^{***}P < 0·001$, §$P < 0·1$.
† For details of diets and procedures, see Tables 1 and 2 and pp. 344–346.
‡ Digestibility corrected by subtraction of faecal bacterial N.

*Fermentability, digestible energy value and metabolizable energy value of NSP isolates*

Fermentability of sugarbeet fibre, calculated from the results obtained with diets B and C, averaged 0·943 (SD 0·066), 0·815 (SD 0·288) and 0·965 (SD 0·035) for hemicellulose, cellulose and uronic acids respectively. As a whole, NSP fermentability averaged 0·886 (SD 0·117) for the nine volunteers and 0·935 (SD 0·056) for seven of them. Only traces of inulin were detected in faeces (from 0 to 3 g/kg DM and less than 1 g/kg, on average) with diet I, so that inulin fermentability was close to 1·00.

Sugarbeet and inulin digestible energy values averaged 11·1 (SD 1·0) and 13·5 (SD 2·1) kJ/g DM respectively. Apparent energy digestibilities of sugarbeet fibre and inulin calculated as the ratio digestible energy value : gross energy (0·731 (SD 0·070) and 0·814 (SD 0·127)) were lower than NSP fermentability.

Urinary energy losses averaged 4·85 (SD 0·43) % of gross energy intake and were not significantly different between the three diets. Ignoring $H_2$ and $CH_4$ energy losses, ME amounted to 0·900 (SD 0·015) energy intake with diet C, 0·888 (SD 0·015) with diet B ($P<0·001$) and 0·891 (SD 0·013) with diet I ($P<0·01$). ME values of NSP isolates were calculated assuming that decreases in dietary ME contents resulted only from experimental NSP-isolate-intake. Sugarbeet fibre and commercial inulin ME values averaged 10·7 (SD 1·2) and 13·0 (SD 2·3) kJ/g DM respectively, and were not significantly different.

*Energy expenditure and maintenance energy requirements of the volunteers*

EE of volunteers given the control diet averaged 9·84 (SD 0·83) MJ/d. This high SD may partly result from differences in lean body mass, which was not determined in the present study. However, repeatability of EE measurement was good since the difference between the two consecutive days averaged 1·3 (SD 1·1) %. Daily ingestion of 50 g sugarbeet fibre induced significant increases in EE during the

**Table 5.** Daily energy expenditure (expressed in kJ/d and as a percentage of the control mean) of subjects consuming a control diet and diets containing sugarbeet fibre or inulin†

(Values for 24 h expenditure are adjusted least square means for nine subjects with their pooled standard error)

| Diet... | Control | Sugarbeet | Inulin | SEM |
|---|---|---|---|---|
| Energy expenditure (kJ/d) | 9842[a] | 10042[b]* | 9897[ab] | 47 |
| Energy expenditure (% control mean) | | | | |
| daily | 100·0 | 102·0* | 100·5 | |
| sleeping | 100·0 | 102·6* | 101·6 | |
| postprandial: | | | | |
| after lunch (3 h) | 100·0 | 102·0 | 100·0 | |
| after dinner (5 h) | 100·0 | 106·3* | 102·0 | |
| exercise | 100·0 | 104·1* | 102·0 | |

[a,b] Mean values within the row not sharing a common superscript letter were significantly different, $P < 0·05$.
Mean values were significantly different from control, *$P < 0·05$.
† For details of diets and procedures, see Tables 1 and 2 and pp. 344–346.

various activities and over 24 h. Inulin ingestion caused slight but non-significant increases in EE, especially after dinner and during sleep (Table 5). Retained energy was slightly positive with diet B and a little bit more with diets C and I. MEm values of the volunteers were 2·3% higher ($P < 0·05$) and slightly but not significantly higher with diet I than with diet C (Table 6).

### *Net energy content of NSP isolates for maintenance*

The maintenance NE contents of NSP isolates calculated from their ME content and differences in MEm (see p. 346 and Table 6) averaged 5·0 (SD 5·0) and 11·9 (SD 3·3) kJ/g DM for sugarbeet fibre and inulin respectively (Table 7).

## Discussion

### *Digestive utilization of NSP isolates*

The two types of NSP studied (sugarbeet fibre and commercial inulin) were selected because of their technological and nutritional assets and their differences in physico-chemical properties. Inulin, a fructose polymer, is characterized by high water-solubility, gel-forming capacity and water-binding capacity (Dysseler & Hoffem, 1995). Although inulin cannot be hydrolysed by the endogenous secretions of the human digestive system, due to the specific structure of the D-fructofuranosyl $\beta$(1-2) link, in rats 18–26% of inulin was digested enzymically in the small intestine and the remainder was almost totally fermented in the distal colon by bacterial microflora (Nilsson & Björk, 1988), especially bifidobacteria and bacteroides (Roberfroid, 1993). By contrast, sugarbeet fibre is mainly composed of insoluble compounds (50·5% cellulose + hemicellulose) which are fermented in the distal colon. It also contains 17·6% uronic acids, water-soluble compounds fermented in the proximal colon.

**Table 6.** Daily metabolizable energy (ME) intake, retained energy (RE) and maintenance ME requirement (MEm) of subjects consuming a control diet and diets containing sugarbeet fibre or inulin*

(Values are adjusted least square means for nine subjects with their pooled standard errors)

| Diet... | Control | Sugarbeet | Inulin | SEM |
|---|---|---|---|---|
| ME (kJ/d) | 10320[a] | 10163[a] | 10408[a] | 78 |
| RE (kJ/d) | 478[a] | 121[b] | 510[a] | 102 |
| MEm (kJ/d) | 9776[a] | 10010[b] | 9831[ab] | 54 |

[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Tables 1 and 2 and pp. 344–346.

The average 57% increase in wet stool weight obtained with sugarbeet fibre and inulin in the present study agreed with the results previously obtained with various NSP (Kelsay *et al.* 1981). It resulted mainly from an increase in water excretion due to the high water-binding capacity of the products (Cherbut, 1989). Furthermore, as inulin incorporated into bread was hydrolysed during cooking and true inulin intake was only 22 g/d, it can be concluded that inulin had a much higher water-binding capacity than sugarbeet fibre. This assumption is supported by results obtained with rats, since gut content was similar in rats fed on diets containing 80 g inulin/kg or 150 g sugarbeet or carrot NSP/kg (Cubizolles & Vermorel, unpublished results).

Interindividual variability in faecal output was high: the CV were 28, 31 and 22% for energy, lipids and N respectively. Furthermore, the highest and the lowest values were observed for the same volunteers for the three diets, which stresses the advantage of using a Latin-square design in human studies. The variability of fermentability was much greater for cellulose than for hemicellulose, in agreement with the results of Southgate & Durnin (1970). The lowest values for hemicellulose and cellulose fermentability (close to nil) were obtained for two or three subjects exhibiting the lowest energy digestibility, the highest faecal starch excretion and the greatest bacterial mass excretion. These results might indicate a greater flow of undigested starch into the large intestine, which may have altered fermentation processes to the detriment of the cellulolytic activity of the caecal flora. In addition, the lower fermentability values might result from differences in the composition of the intestinal microflora as suggested by Southgate & Durnin (1970).

Daily ingestion of 50 g sugarbeet fibre was followed by significant increases in dietary cellulose and hemicellulose fermentability. These may have resulted from both the high

**Table 7.** Determination of net energy (NE) content of sugarbeet fibre and inulin

(Values are means and standard deviations for nine (sugarbeet) or eight (inulin) measurements)

| | Sugarbeet fibre | | Inulin | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Fibre DM intake (g/d) | 43·85 | | 48·45 | |
| Fibre ME intake (kJ/d) | 471·7 | 52·4 | 627·5 | 113·2 |
| Difference in MEm (kJ/d)* | 249·4 | 228·8 | 49·2 | 233·7 |
| NE (kJ/g DM) | 5·0 | 5·0 | 11·9 | 3·3 |

ME, metabolizable energy; MEm, maintenance ME requirement.
* Difference of MEm between control and fibre diet.

fermentability of the present sugarbeet fibre (processing may have altered its structure and physical properties, Ellis *et al.* 1996) and improved microbial degradation of dietary NSP in the large intestine. In fact, ingestion of the same sugarbeet fibre by growing rats caused a drop in caecal pH and increases in caecal VFA content and percentages of acetate and butyrate in the VFA mixture, which could reflect increased cellulolytic activity of the caecal flora (Cubizolles & Vermorel, unpublished results). By contrast, in the present study, daily ingestion of 50 g inulin resulted in significant decreases in dietary hemicellulose, cellulose and uronic acid fermentability. These results could be partly explained by the development of a specific microflora (Roberfroid, 1993) and alteration of fermentation in the large intestine, as shown in growing rats given inulin: a 54 % increase in caecal VFA concentration and a decrease in the percentage of acetate in the VFA mixture (Cubizolles & Vermorel, unpublished results).

Decreases in apparent digestibility of energy, protein and lipids resulting from sugarbeet fibre or inulin intake in the present study were less than those obtained in other studies with similar quantities of cereal, vegetable or fruit NSP (0·01 *v.* 0·03–0·06) (Göranzon *et al.* 1983; Wisker *et al.* 1988; Miles, 1992). These differences could be partly explained by the higher fermentability of processed sugarbeet fibre (and inulin) in the present study compared with that of undamaged NSP supplied by cereals, vegetables and fruit (Southgate & Durnin, 1970): 0·943 *v.* 0·684 for hemicellulose and 0·815 *v.* 0·259 for cellulose.

The average 50 % increase in faecal excretion of bacterial biomass probably resulted from the increase in carbohydrates fermented in the large intestine. It is noteworthy that dry bacterial mass contributed 50 % or more of the dry stool weight. About 35 % of the fermented sugarbeet NSP energy appeared as faecal bacterial energy, which agrees with the accepted figure of 30 % (range 20–40 %; Livesey, 1992). In other respects the increased excretion of faecal microbial N accounted for the decreased apparent N digestibility of NSP-enriched diets. It may have resulted mainly from urea utilization by the bacterial flora and maybe from desquamation of the enlarged intestinal mucosa. It was compensated for by a slight, non-significant, reduction of urinary N excretion and consequently N balances tended to be reduced in subjects on diets B and I, but were not significantly different between treatments. These results agree with those obtained in human subjects consuming high-NSP diets containing fruit and vegetables (Kelsay *et al.* 1978) and in growing rats fed on diets supplemented with purified NSP sources with a wide range of fermentability (Tetens *et al.* 1996). Furthermore, N retention was significantly increased for the same ME intake in growing rats given sugarbeet fibre or inulin supplements (Cubizolles & Vermorel, unpublished results).

### *Digestible and metabolizable energy content of diets and NSP*

$CH_4$ and $H_2$ energy losses could not be determined reliably due to the very low concentrations of these gases in air leaving the calorimetric chambers, so they were not taken into account to calculate the dietary ME content. In adult volunteers given 50 g lactitol monohydrate/d in place of 49 g sucrose/d, $H_2$ production increased by 1·9 litres/d on average, corresponding to 25 kJ/d, i.e. 0·25 % of daily gross energy intake or 3 % of lactitol gross energy (Van Es *et al.* 1986). Consequently, ignoring gas energy losses probably resulted in errors similar to those for gross energy intake.

The average measured ME contents of the control and NSP diets were compared with ME contents predicted from dietary chemical composition using several published predictive equations (Fig. 2). Agreement was satisfactory for the three diets (average differences lower than 2 %) with the Miller & Payne (1959) and Livesey (1991) predictive equations which assume that the ME content of NSP is close to 8·4 kJ/g. In fact, in the present study the ME contents of sugarbeet fibre and inulin were 10·7 and 13·0 kJ/g respectively. However, the Southgate & Durnin (1970) and Miller & Judd (1984) predictive equations assume a strong depressive effect of NSP on food digestibility and resulted in 3·2 % and 9·6 % underestimation of ME content for diets B and I respectively, whereas agreement was satisfactory for diet C with the Southgate & Durnin (1970) equation (Fig. 2).

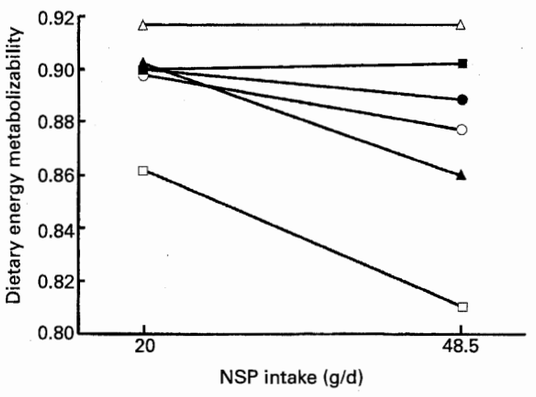


**Fig. 2.** Variation of dietary energy metabolizability with NSP intake. Comparison of the measured values (●) obtained with control and sugarbeet diets in the present study and those calculated using different predictive equations: (△) Atwater & Bryant (1900); (■) Miller & Payne (1959); (▲) Southgate & Durnin (1970); (□) Miller & Judd (1984); (○) Livesey (1991).

Calculations of digestible energy and ME contents were based on the assumption that increases in faecal energy losses obtained with diets B and I resulted entirely from NSP intake (see p. 346). Faecal energy losses increased on average 3·40 kJ and 2·52 kJ per g sugarbeet fibre or inulin ingested respectively. These results were much lower than those obtained in human subjects with cereal NSP (23·8 kJ/g NSP; Wisker *et al.* 1988).

In the present study the ME content of sugarbeet fibre was similar to that obtained for cereal NSP (Göranzon & Forsum, 1987) and in between those obtained by these authors and Wisker & Feldheim (1990) in human subjects. They agreed with the results obtained in growing rats given the same NSP types at higher feeding levels (Cubizolles & Vermorel, unpublished results). The high fermentability and ME content of inulin were in accordance with the results obtained in rats (Nilsson *et al.* 1988; Cubizolles & Vermorel, unpublished results). However, its ME content was much higher than that (4·18 kJ/g) assumed by Bastiaens *et al.* (1989) and accepted in Belgium and Switzerland (Dysseler & Hoffem, 1995).

### *Net energy value of NSP isolates*

The maintenance NE values of NSP isolates could be calculated from their ME content and the differences in MEm of the volunteers between the experimental and control diets. Such an approach required a high accuracy in the measurement of daily EE, preferably during two consecutive days after adaptation to the facilities, and a Latin-square design to overcome the great interindividual variability of MEm.

The maintenance NE content of inulin in human subjects (11·9 kJ/g DM on average) was close to that obtained in growing rats (10·0 kJ/g DM) using the comparative slaughter method (Cubizolles & Vermorel, unpublished results). It showed a 91·5 % efficiency of ME utilization for maintenance in agreement with the weighted efficiency of VFA (85 %, Krebs, 1960; Armstrong & Blaxter, 1957; Livesey, 1992) and glucose or fructose (95 %, Van Es *et al.* 1986) probably resulting from inulin hydrolysis during bread baking.

The maintenance NE content of sugarbeet fibre (5·0 kJ/g DM on average) was much lower than that of inulin. It resulted from both a lower ME content of sugarbeet fibre and higher MEm of the volunteers. EE was increased during all the considered circadian periods and especially during the postprandial phase. This phenomenon may result from (1) enlargement and thickening of caecal and colonic tissues (Cubizolles & Vermorel, unpublished results) known to have a high metabolic rate, (2) increased motility of the gastrointestinal tract (Cherbut *et al.* 1994) and (3) the lower efficiency of VFA utilization compared with glucose. The great SD of the NE values resulted from the variability of differences in MEm of the volunteers between diets C and B (2·65 (SD 2·53) %). If the two extreme values were excluded, the average NE value of sugarbeet fibre would be 4·4 (SD 3·2) kJ/g DM.

The maintenance NE values of sugarbeet fibre and inulin determined in the present study were compared with those predicted from fibre fermentability, estimated energy lost as microbial mass, $H_2$, $CH_4$ and fermentation heat (0·30, 0·02 and 0·05 kJ/kJ carbohydrate fermented respectively) and efficiency of VFA utilization (Livesey, 1992). The predicted NE value of sugarbeet fibre was higher than the measured maintenance NE value (7·6 *v.* 5·0 kJ/g DM) probably because the increased metabolic rate of the digestive tract was not taken into account in Livesey's approach. However, the predicted NE value of inulin agreed with the measured value (12·0 *v.* 11·8 kJ/g DM) probably because the increased metabolic rate of the digestive tract was compensated for by a better efficiency of utilization of fructose, deriving from inulin hydrolysis during bread baking, compared with VFA.

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# Prebiotic effects of inulin and oligofructose

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Prebiotics are non-digestible food ingredients that target certain components within the microbiota of the human large intestine. Efficient prebiotics need to have a specific fermentation therein and thereby have the ability to alter the faecal microflora composition towards a more 'beneficial' community structure. This should occur by the stimulation of benign or potentially health promoting genera but not the harmful groups. Because of their positive attributes bifidobacteria and lactobacilli are the most frequent target organisms. Both inulin and oligofructose have been demonstrated to be effective prebiotics. This has been shown through both *in vitro* and *in vivo* assessments in different laboratories. Because of their recognised prebiotic properties, principally the selective stimulation of colonic bifidobacteria, both inulin and oligofructose are increasingly used in new food product developments. Examples include drinks, yoghurts, biscuits and table spreads. Because of the recognised inhibitory effects that bifidobacteria can exert against gut pathogens, one of the most important aspects of prebiotic ingestion is fortification of the gut flora to resist acute infections.

**Human gut microflora: Prebiotics: Inulin**

## Introduction

Oligosaccharides are major components of various dietary products (e.g. plant cells, milk) and since 1980 their use in functional foods has been increasingly researched (Roberfroid, 2002). Certain non-digestible (in the upper gastrointestinal tract) oligosaccharides are prebiotics. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that can improve the host health (Gibson & Roberfroid, 1995). For a food ingredient to be classified as a prebiotic it must fulfil the following criteria:

- Neither be hydrolyzed, nor absorbed in the upper part of the gastrointestinal tract.
- Be selectively fermented by one or a limited number of potentially beneficial bacteria commensal to the colon, e.g. bifidobacteria and lactobacilli, which are stimulated to grow and/or become metabolically activated.
- Prebiotics must be able to alter the colonic microflora towards a healthier composition, for example by increasing numbers of saccharolytic species while reducing putrefactive microorganisms.

Thus, prebiotic fermentation should be directed towards bacteria seen as health promoting, with indigenous lactobacilli and bifidobacteria currently being the preferred targets (Gibson, 1998).

The ultimate aim of supplementation of the human diet with prebiotics is beneficial management of the gut microbiota. The resident bacterial microflora of the human colon comprises approximately 95 % of the total cells of the body and plays a key role in the host nutrition, health and disease. More than 500 different bacterial species have been cultured from human faeces belonging to fifty different genera (Blaut *et al.* 2002; Tannock, 2002). Of these, there are indications that bifidobacteria are the main health promoting group. Bifidobacteria are thought to play an important role in the improved health and development of breast-fed infants as compared to those which are formula-fed. *Bifidobacterium* sp. dominate the gut microflora of breast-fed infants. Among the beneficial effects of bifidobacteria are thought to be:

- Protection from enteric infection.
- Lowering of intestinal pH by formation of acids after assimilation of carbohydrates.
- Suppression of putrefactive and pathogenic bacteria.
- Production of vitamins.
- Activation of intestinal function, assistance of digestion and absorption.
- Stimulation of the immune response.

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**Abbreviations:** GOS, galacto-oligosaccharide; SCFA, short-chain fatty acid; TOS, transgalactosylated oligosaccharide.
**Note:** For the definition of the terms inulin and oligofructose please refer to the introductory paper (p. S139) and its footnote.
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Because bifidobacteria are susceptible to oxygen and heat, their application in foods, as probiotics, has been limited in comparison to the lactobacilli. Therefore, there has been much interest in food-grade bifidogenic factors, which endure normal processing and show effectiveness in the human body after ingestion

The prebiotic market is expanding rapidly and the demand for novel compounds may not be limited to oligosaccharides although these are the current market leaders (Crittenden & Playne, 1996). A variety of products containing inulin and/or oligofructose formulations, claiming to have beneficial effects on gut health and general well-being, are starting to become prevalent in the European market. Table 1 gives examples of foodstuffs containing the ingredients, whilst numerous other products existing in supplement form are available in many health food stores.

Inulin and oligofructose are legally classified as food or food ingredients in all countries in which they are used. They are well accepted for food use without limitations (Coussement, 1999).

Inulin and oligofructose are amongst the most studied and well-established prebiotics. Fig. 1 summarises their behaviour and effects in the gastrointestinal tract.

As previously mentioned inulin and oligofrustose escape digestion in the upper gastrointestinal tract and reach the large intestine virtually intact. This attribute constitutes them as being ideal for fermentation in the colon by the saccharolytic resident microbiota. Whilst variable data have ensued on the different application of inulin and oligofructose, it is incontestable that they act as prebiotics. The effects of inulin and oligofructose on the human gut microbiota has been extensively studied both *in vivo* and *in vitro* and the majority of the studies report selective fermentation by the beneficial flora, namely bifidobacteria and to lesser extent lactobacilli (Table 2).

Gibson & Wang (1994) confirmed the prebiotic effects of inulin and oligofructose in an *in vitro* study. The fermentability was compared to a range of reference carbohydrates in batch culture. Bacterial growth data showed preferential fermentation by bifidobacteria while populations of *Escherichia coli* and *Clostridium perfringens* were maintained at relatively low levels. Further pure culture studies confirmed increased ability of bifidobacteria populations to ferment these substrates when compared to glucose. In a later study, Gibson & Wang (1994) determined the bifidogenic effect of oligofructose in single stage continuous culture systems containing human faecal bacteria. Oligofructose preferentially enriched for bifidobacteria when compared to inulin and sucrose. Experiments with a three-stage continuous culture model of the human colon further confirmed the bifidogenic effect of oligofructose. Karppinen *et al.* (2000) compared the *in vitro* fermentability of inulin by human faecal bacteria to that of rye, wheat and oat bran. Inulin was the most rapidly fermented of the test substrates giving most butyrate production and the largest decrease in pH, but also the highest and fastest gas production. Again, the butyrate generating capacity, as well as increased gas formation, does not agree with metabolic profiles exhibited by bifidobacteria.

Kaplan & Hutkins (2000) screened a selection of twenty-eight lactic acid bacteria and bifidobacteria for their ability to ferment inulin and oligofructose on MRS agar. Twelve of sixteen *Lactobacillus* strains and seven of eight *Bifidobacterium* strains tested were able to ferment the substrates. Hopkins *et al.* (1998) also documented the ability of seven *Bifidobacterium* isolates to utilise a selection of fifteen different carbohydrate sources in 48 h batch culture experiments. In a continuous culture study Sghir *et al.* (1998) demonstrated, through molecular techniques, that inulin and oligofructose were selectively fermented not only by bifidobacteria but also by lactobacilli. Oligofructose and galacto-oligosaccharides preferentially supported growth of the test bacteria.

The effect of inulin on faecal bifidobacteria in eight healthy free living humans was investigated by Kruse *et al.* (1999). Subjects consumed a typical Western diet (45 % energy as fat, 40 % energy as carbohydrate) followed by a reduced fat diet (30 % energy as fat) using inulin as fat replacement (maximum inulin consumed 34 g per day). Controls consumed identical diets but without inulin supplementation. The effect on faecal flora was monitored using fluorescent probes targeting diagnostic regions of the 16S rRNA molecule. A significant increase in bifidobacterial populations was observed, while short-chain fatty acids (SCFA), blood lipids and gas production remained unaffected. Bouhnik *et al.* (1999) assessed the tolerance and threshold dose of oligofructose (from sucrose) that significantly increased faecal bifidobacteria counts in a 7-day study of forty healthy human volunteers. They reported that the optimal dose for increased bifidogenesis without significant side effects, such as flatulence, was 10 g per day. Gibson *et al.* (1995) studied the selective stimulation of bifidobacteria by inulin and oligofructose in

**Table 1.** Examples of fructo-oligosaccharide containing foodstuffs in the European market (after Young, 1998)

| Product | Company | Active Ingredients |
|---|---|---|
| Symbalance (yogurt) | Tonilait (Switzerland) | Three *Lactobacillus* strains plus inulin |
| Jour apres Jour (milk) | Lactel (France) | Vitamins plus oligofructose (from sucrose) |
| Probiotic plus Oligofructose (yogurt) | Bauer (Germany) | Two *Lactobacillus* strains plus oligofructose |
| Actiline (spread) | Vamdermoortele (Belgium) | inulin |
| Ligne Bifide dietetic range (biscuits, ready meals) | Vivis (France) | Oligofructose (from sucrose) |
| Aviva (biscuits and chocolate drink) | Novartis (Switzerland) | Oligofructose (from sucrose) |
| Low-sugar sorbet | Thiriet (France) | Oligofructose (from sucrose) |
| Actimel (Cholesterol control yogurt) | Danone (Belgium) | *L. acidophilus* plus oligofructose (from sucrose) |
| Fysiq (dairy drink) | Mona (Holland) | *L. acidophilus* plus inulin |

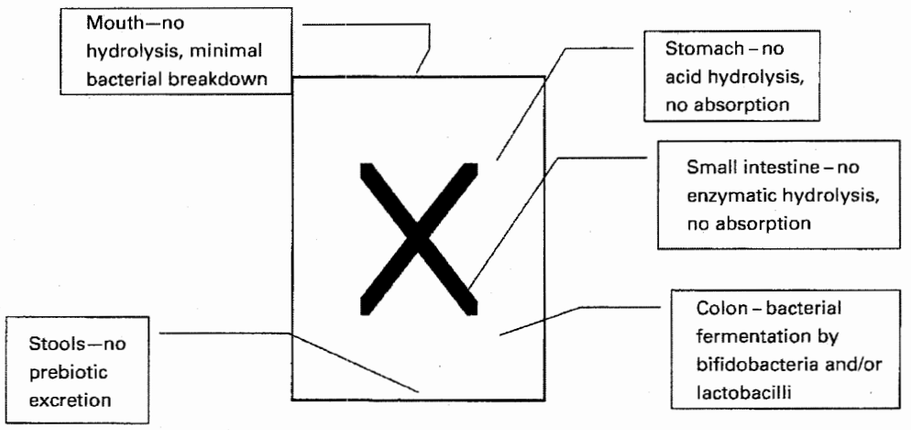


**Fig. 1.** Behaviour of inulin and oligofructose, as prebiotics, in the human gut.

a 45-day study of eight healthy male human subjects. Volunteers were fed controlled diets of 15 g/d sucrose for the first 15 days followed by 15 g/d oligofructose for a further 15 days. Four volunteers went on to consume 15 g/d inulin for the final 15 days of the study. Both oligofructose and inulin caused significant increases in faecal bifidobacteria. Bacteroides, clostridia and fusobacteria all decreased during oligofructose supplementation and Gram-positive cocci were reduced during inulin supplementation. Total bacterial levels remained unaffected, while little change was observed in faecal SCFA and breath $CH_4$. Wet and dry matter nitrogen and energy excretion was increased with both inulin and oligofructose. Kleessen *et al.* (1997) studied the effect of dietary supplementation on faecal flora, microbial activity and bowel habit in thirty-five elderly constipated patients. Groups of fifteen and ten patients received lactose and inulin supplements, respectively for 19 days. They were initially administered a 20 g/d dose for days 1–8 which was gradually increased to 40 g/d during days 9–11 and was maintained at these levels until the end of the study. A significant increase was observed in bifidobacterial levels in the inulin group while a decrease in enterococci numbers and enterobacteria occurred. Lactose had no effect on bifidobacteria while it increased enterococci counts and decreased lactobacilli levels. A better laxative effect was reported with inulin. Den Hond *et al.* (2000) investigated the effect of high performance inulin on constipation in six healthy humans with a low stool frequency in a double-blind placebo control crossover study. Subjects consumed an active diet of 15 g/d inulin and a placebo of 15 g/d sucrose. A significant increase in stool frequency and faecal bulk was observed with inulin administration. Hunter *et al.* (1993) carried out a double-blind crossover trial using oligofructose against sucrose, at a dose of 6 g/d, for the management of irritable bowel syndrome, but no gastrointestinal effects were evident. In a double-blind placebo controlled crossover study of thirty-one healthy human volunteers, the prebiotic effects of biscuits containing a blend of partially hydrolysed guar gum and oligofructose were confirmed using fluorescently labelled molecular probes targeting 16S rRNA for the bacteriology. A significant increase in bifidobacterial numbers occurred while bacteroides, lactobacilli, clostridia and total bacteria remained unaffected throughout the study (Tuohy *et al.* 2001). Bouhnik *et al.* (1996) studied the effect of a fermented milk product containing *Bifidobacterium* sp. with or without inulin on faecal bacteriology of twelve healthy human volunteers. The authors observed that addition of the *Bifidobacterium* fermented milk substantially increased bifidobacterial levels after 12 days, but addition of 18 g/d inulin to this formulation did not enhance the effect. Buddington *et al.* (1996) studied the influence of oligofructose (from sucrose) supplementation on the faecal flora of twelve healthy adult humans. Subjects were fed a controlled diet for 42 days which was supplemented with 4 g/d oligofructose (from sucrose) between days 7 and 32. The controlled diet increased bifidobacterial levels but highest increases were observed during oligofructose (from sucrose) supplementation. In a similar study on the effects of 4 g/d oligofructose (from sucrose) on ten healthy adult humans, Williams *et al.* (1994) reported a significant increase in bifidobacteria levels and an increase in lactobacilli in six volunteers. Le Blay *et al.* (1999) studied the effect of the prolonged intake of oligofructose (from sucrose) in rats. Subjects were fed either a low fibre diet (basal) or the basal diet supplemented with 9 g/100 g body weight daily for 2, 8 or 27-week periods. Supplementation with oligofructose (from sucrose) led to an increase in lactic acid bacteria after 2 weeks without changing total anaerobic bacterial levels. The majority of the effects were however abolished by weeks 8 and 27 of oligofructose (from sucrose) consumption. Djouzi & Andrieux (1997) performed a trial on germ-free rats inoculated with human faecal flora fed either control or active diets with 40 g/kg of oligofructose, galacto-oligosaccharide (GOS), or transgalactosylated oligosaccharide (TOS). A significant increase in bifidobacterial levels was observed with oligofructose and TOS as well as increases in $H_2$ and $CH_4$ excretion. In two studies, the effect of inulin on dextran sulphate sodium (DSS) induced colitis rats was reported (Videla *et al.* 1998; Videla, 1999). It was established that dietary inulin promoted growth of lactobacilli in the rat colon, reduced the severity of DSS induced colitis and reduced the luminal pH in a wide area extending from left to right colon.

**Table 2.** Summary of studies designed to determine the prebiotic effect of fructo-oligosaccharides

| Oligosaccharide | Mode of study | Evidence of prebiotic effect | Reference |
|---|---|---|---|
| Oligofructose, GOS, TOS | *In vivo* gnotobiotic rats | High increases in bifidobacteria numbers with FOS and TOS | Djouzi & Anrdieux, 1997 |
| Oligofructose (from sucrose) | *In vivo* rats | Increase in lactic acid bacteria after 2 weeks, but in the long-term any effect was lost | Le Blay *et al.* 1999 |
| Inulin | *In vivo* DSS induced colitis rats | Decrease in luminal pH between left and right colon | Videla, 1999 |
| Inulin | *In vivo* DSS induced colitis rats | Increase in lactobacilli | Videla *et al.* 1998 |
| Inulin | *In vivo* eight healthy humans | Significant increase in bifidobacteria established by FISH | Kruse *et al.* 1999 |
| Oligofructose | *In vivo* double-blind placebo controlled IBS patients | No therapeutic effect at 6 g/d | Hunter *et al.* 1993 |
| Oligofructose (from sucrose) | *In vivo* forty healthy humans | Significant increase in bifidobacteria levels without excessive gas production at 10 g/d | Bouhnik *et al.* 1999 |
| Inulin and oligofructose | *In vivo* eight healthy humans | 15 g/d inulin or oligofructose led to bifidobacteria becoming predominant in faeces | Gibson *et al.*1995 |
| Inulin and lactose | *In vivo* twenty-five elderly constipated humans | Significant increase in bifidobacteria, decreases in enterococci and fusobacteria. Better laxative effect than lactose | Kleessen *et al.* 1997 |
| Oligofructose | *In vivo* double blind placebo controlled crossover study of thirty healthy humans | Significant increase in bifidobacteria established via FISH at 7 g/d, no change in total bacterial levels | Tuohy *et al.* 2001, in press |
| Oligofructose (from sucrose) | *In vivo* twelve healthy adult humans | Significant increase in bifidobacteria, no change in total bacteria levels | Buddington *et al.* 1996 |
| Oligofructose (from sucrose) | *In vivo* ten healthy adult humans | Significant increase in bifidobacteria, some increase in lactobacilli | Williams *et al.* 1994 |
| BFM and BFM plus inulin | *In vivo* twelve healthy humans | Increase in bifidobacteria with BFM but addition of inulin did not enhance effect | Bouhnik *et al.* 1996 |
| Inulin | *In vivo* six healthy humans (low stool frequency) double blind placebo controlled crossover study | Significant increase in stool frequency and faecal bulk | Den Hond *et al.* 2000 |
| Inulin and oligofructose | *In vitro* human faecal flora batch cultures | Significant increase in bifidobacteria, suppression of *E. coli* and clostridia | Wang & Gibson, 1993 |
| Oligofructose and fourteen other carbohydrates | *In vitro* batch cultures of seven *Bifidobacterium* isolates | Best supported growth of test bacteria on oligofructose and GOS | Hopkins *et al.* 1998 |
| Inulin, rye, wheat and oat brans | *In vitro* human fecal batch cultures | Highest decrease in pH with inulin and highest increase in butyrate, very fast fermentation and high gas production | Karppinen *et al.* 2000 |
| Oligofructose | *In vitro* human faecal flora continuous culture and *in vitro* 3-stage gut model | Significant bifidogenic effect compared to sucrose and inulin | Gibson & Wang, 1994 |
| Oligofructose | Sixteen strains lactobacilli eight bifidobacteria | Twelve of sixteen lactobacilli and seven of eight bifidobacteria strains fermented FOS | Kaplan & Hutkins, 2000 |
| Oligofructose | *In vitro* human faecal flora continuous culture | Increases in bifidobacteria. Lactobacilli outcompeted bifidobacteria at pH 5·2–5·4 | Sghir *et al.* 1998 |

**Abbreviations:** GOS (galacto-oligosaccharides), TOS (transgalactosylated oligosaccharides), BFM (*Bifidobacterium* sp. Fermented Milk), FISH (fluorescent *in situ* hybridisation).
Unless otherwise stated, observations on the bacterial flora listed in the Table 2 were obtained via microbial culture techniques.

## Conclusion

The prebiotic effects of inulin and oligofructose have been confirmed in numerous laboratory and human trials. New developments in molecular procedures for diagnostic bacteriology will help determine health applications, and explain mechanisms of effect. A further desirable attribute for prebiotics is the ability to persist towards the distal region of the colon. This is the site of origin of several chronic disease states including colon cancer and ulcerative colitis. It is thought that the microflora in this region of the gut may play an important role in the onset or maintenance of such disorders. Dietary carbohydrate is the main fermentable substrate in the proximal colon and as this is degraded during bacterial fermentation, protein takes over as the dominant fermentable substrate towards distal areas. The products of bacterial protein metabolism include toxic and potentially carcinogenic compounds such as amines, ammonia and phenolic compounds. There is currently much scientific interest in developing prebiotics, which target this region of the colon. This may include FOS of differing molecular sizes, such that the lower degree of polymerisation oligomers are proximally fermented thereby leaving the longer chain prebiotic for more distal colonic activity.

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# Inulin, oligofructose and mineral metabolism — experimental data and mechanism

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Numerous investigations performed in animal models in the past 10 years have shown repeatedly that non-digestible oligosaccharides (NDO), such as inulin, oligofructose or transgalacto-oligosaccharides (TOS), stimulate mineral absorption, mainly calcium and magnesium. Long-term beneficial effects on bone health have been indicated by accumulation of bone mineral content in growing rats or prevention of bone loss in ovariectomized rats. However, bone mineral content or density are not necessarily associated with bone quality. In recent studies both oligofructose and calcium prevented loss of trabecular bone area induced by oestrogen deficiency, this, however, occurred at different trabecular shapes. The effects of NDO on mineral metabolism may be based on the enhancement of passive and active mineral transport across the intestinal epithelium, mediated by an increase in certain metabolites of the intestinal flora and a reduction of pH. The possible impact of short-chain fatty acids, butyrate in particular, and of polyamines on the stimulation of mineral absorption capacity, and the interaction of oligofructose and antibiotics is discussed.

**Prebiotics: Oligofructose: Mineral metabolism: Bone quality**

## Introduction

Dietary habits have changed in the past 50 years in westernized societies (Ernährungsbericht, 2000) including higher consumption of proteins, fats, sucrose, and sodium chloride, and lower intake of cereals and complex carbohydrates including nondigestible, bulking dietary fibres and non-digestible but fermentable carbohydrates (NDC) like inulin and oligofructose. Since wheat provides 70 % of inulin and oligofructose in American diets (Moshfegh *et al.* 1999), it is assumed that the decreased intake of cereals lowered intake of NDC. Some 'modern' diseases, such as coronary heart disease, some cancers or osteoporosis are thought to be associated at least in part with these changes in dietary patterns (Fraser, 1999).

Nondigestible oligosaccharides (NDO), such as inulin, oligofructose, galacto-oligosaccharides (GOS), transgalacto-oligosaccharides (TOS), and soybean-oligosaccharides, or lactulose, but also certain resistant starches, sugar alcohols, fermentable fibre, and gums like guar gum hydrolysate (GGH), attracted attention in the past decade for their physiological and health promoting properties and thus for their potential to represent candidates for functional food ingredients (Fooks *et al.* 1999). It was observed that NDO had specific effects on gut physiology. Changes herein were reported to be associated with benefits for the host, such as improved gastrointestinal well-being associated with changes in the intestinal flora (van Loo *et al.* 1999). Although fibre substances like pectin or cellulose also pass through the small intestine undigested, there are several differences in physical properties like dispersibility, viscosity, and adsorbing capacity compared to inulin and oligofructose (Schneeman, 1999). These physical differences presumably explain the variation of physiological effects and why different NDO, fibre substances, and sugar alcohols affect mineral metabolism differently. Lactulose for example, increased calcium absorption from 26 % to 37 % in rats as did other sugars whose digestion was limited, like L- and D-arabinose, raffinose or sugar alcohols like xylitol (Brommage *et al.* 1993). In animal experiments it was shown that inulin and oligofructose improved mineral absorption (for review see Scholz-Ahrens *et al.* 2001) and that this was associated with the production of short-chain fatty acids (SCFA) and a lower pH in the

**Abbreviations:** $1,25[OH]_2D_3$, calcitriol; GGH, guar gum hydrolysate; GOS, galacto-oligosaccharides; NDC, nondigestible but fermentable carbohydrates; NDO, nondigestible oligosaccharides; ODC, ornithine decarboxylase; OVX, ovariectomy; SCFA, short-chain fatty acids; T.Ar, tissue area; Tb.Ar, trabecular area; Tb.Ar/T.Ar, trabecular area as percentage of tissue area; TBPf, trabecular bone pattern factor; Tb.Pm, trabecular perimeter; TOS, transgalacto-oligosaccharides.

**Note:** For the definition of the terms inulin and oligofructose please refer to the introductory paper (p. S139) and its footnote.

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**Table 1.** Documented effects of nondigestible carbohydrates and related compounds on bioavailability of different minerals in rats

| *Substance* | % of Diet | Mineral | Animal model — rat | Method | Effect | Authors |
|---|---|---|---|---|---|---|
| Oligofructose | 2·5–20 % | Ca | ● Growing | ● Isolated caecum, colon | +++++ | Brommage *et al.* 1993; Bouhnik *et al.* 1997; |
| TOS | | | ● Mg-deficient | ● Balances | +++ | Chonan *et al.* 1995; Delzenne *et al.* 1995; |
| Lactulose | | | ● Fe-deficient | ● DEXA | + | Goda *et al.* 1995; Hämäläinen 1994; |
| Resistant starch | | | ● Gastrectomized | ● Femur Ca | +++ | Hara *et al.* 1996; Heijnen *et al.* 1993; |
| Resistant sugar | | | ● Ovariectomized | ● Bone structure | +++++ | Lopez *et al.* 2000; Mattila *et al.* 1998; |
| Xylitol, maltitol | | | | | | Ohta *et al.* 1994, 1995*a*, 1995*b*, 1998*b*, 1998*c*; |
| GGH | | | | | | Sako *et al.* 1999; Scholz-Ahrens *et al.* 1998; |
| | | | | | | Scholz-Ahrens *et al.* 2001; |
| | | | | | | Schulz *et al.* 1993; Takahara *et al.* 2000 |
| Oligofructose | 5 – 10 % | P | ● Growing | ● Balances | +, 0, – | Hämäläinen, 1994; Heijnen *et al.* 1993; |
| Lactulose | | | ● Mg-deficient | ● Femur P, Humerus P | + | Mattila *et al.* 1998; Ohta *et al.* 1994, 1998*a*; |
| Xylitol | | | ● Gastrectomized | | | Scholz-Ahrens *et al.* 2002 |
| | | | ● Ovariectomized | | | |
| Oligofructose | 5 – 10 % | Mg | ● Growing | ● Balances | +++++ | Baba *et al.* 1996; Delzenne *et al.* 1995; |
| TOS | | | ● Mg-deficient | ● Isolated caecum, isolated colon | | Heijnen *et al.* 1993; Lopez *et al.* 2000; |
| Lactulose | | | ● Fe-deficient | | | Ohta *et al.* 1994, 1995*b*; Schulz *et al.* 1993 |
| Resistant starch | | | | | | |
| Oligofructose | 10 % | Cu | ● Growing | ● Balances | +++ | Delzenne *et al.* 1995; Lopez *et al.* 2000; |
| | | Fe | ● Fe-deficient | ● Haematology | +++ | Ohta *et al.* 1995*b*, 1998*b* |
| | | Zn | ● Gastrectomized | | ++ | |

Negative (–), no (0), or positive effects with increasing degree of markedness (+, +++, +++++) are indicated. TOS = Transgalacto-oligosaccharides, GGH = Guar gum hydrolysates.

intestinal lumen. Compared to inulin, oligofructose stimulated calcium absorption slightly more effectively, while the effect on magnesium was similar (Delzenne *et al.* 1995). The production of total SCFA was not different but lactate was significantly higher following xylo-oligosaccharides and butyrate and was highest in oligofructose (derived both from inulin and sucrose) fed rats (Campbell *et al.* 1997). Moreover, the dose of NDO and the background dietary calcium used in different studies have their own impact on mineral absorption (Scholz-Ahrens *et al.* 2002).

To what extent observations on mineral balance allow assumptions on bone mineralization or bone quality requires information on the persistence of the stimulating effect of inulin and oligofrucose on mineral absorption, and on the relevance of improved calcium absorption with respect to bone mineralization, bone density and bone structure. These aspects are discussed in the following review.

## Mineral balance

Most experiments on NDO lasted 3–4 weeks and were done in young growing rats (Table 1). In some cases animals were gastrectomized or they were magnesium or iron deficient. In some experiments mineral disappearance from the isolated or ligated caecum or colon was studied or the positive effect on mineral availability was demonstrated by prevention of anaemia, i.e. haematological parameters or by prevention of mineral deficiency. The doses used varied mostly between 2·5 % and 10 % in the diet. Minerals that were analysed included calcium, phosphorous, magnesium, iron, copper, and zinc. In young growing rats NDO exerted their most prominent effects on calcium and magnesium availability (Table 1). In aged ovariectomized rats 5 % oligofructose added to a diet high in calcium (1 %) persistently reduced faecal calcium loss after 4 and 8 weeks. This effect became significant after 16 weeks (Fig. 1). Calcium retention was slightly but persistently higher after 4, 8, and 16 weeks. This effect failed significance because increased absorption was associated with increased urinary calcium. Higher urinary calcium however, reflected metabolic reaction on higher calcium influx from the gut and not calcium mobilization from the skeleton because bone mineral content was higher on oligofructose (Scholz-Ahrens *et al.* 2002). Iron absorption was less effectively stimulated compared to calcium or magnesium but more effectively than zinc (Table 1, Delzenne *et al.* 1995). Phosphorous absorption was not affected (Ohta *et al.* 1994) or was significantly lower on a diet with 1 % calcium on oligofructose with 76·3 ± 2·82 [mean (pooled SEM)] compared to the group on maize starch with 87·8 (2·82) after 4 weeks (Scholz-Ahrens *et al.* 2002). Since phosphorous excretion via urine was significantly lower as well, phosphorous retention was not affected after 4 and 8 weeks (not shown), and after 16 weeks (Fig. 2).

## Bone mineralization

Few experiments were reported, in which the effect of

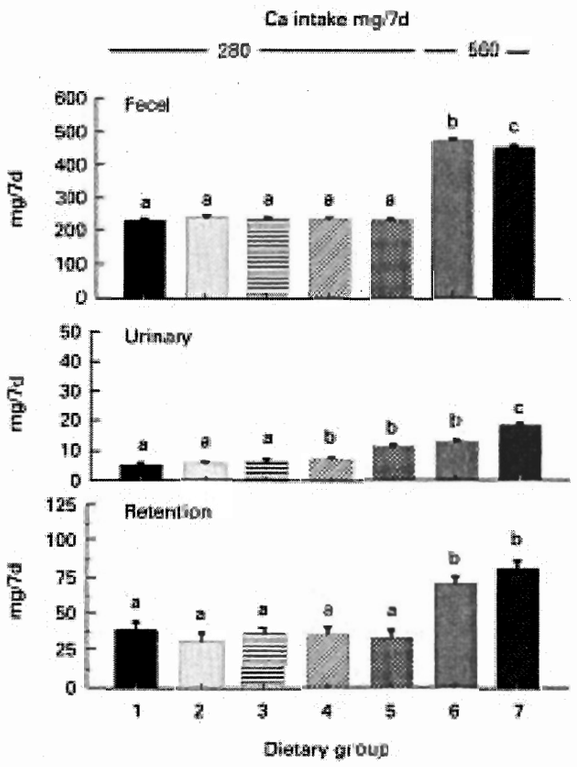


**Fig. 1.** Effect of oligofructose on 7-day calcium (Ca) balance in ovariectomized rats after 16 weeks on diets containing (g calcium/kg diet) / (g oligofructose/kg diet) as follows: ■, 5/0; □, 5/0; ▤, 5/25; ▨, 5/50; ▩, 5/100; ■, 10/0; ■, 10/50. Animals in group 1 were sham operated, animals in groups 2–7 were ovariectomized. Least square means and pooled SEM of MANOVA. a, b, c indicates significant differences between groups with $P$ of at least $<0{\cdot}05$.

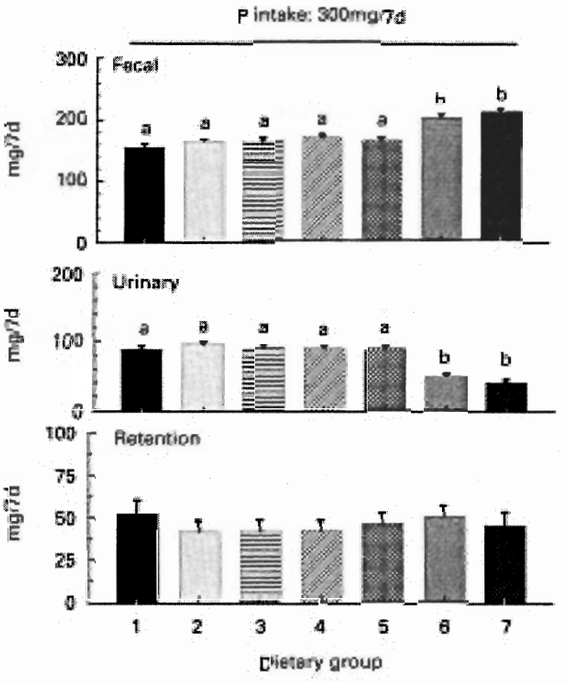


**Fig. 2.** Effect of oligofructose on 7-day phosphorous (P) balance in ovariectomized rats after 16 weeks on diets containing (g calcium/kg diet) / (g oligofructose/kg diet) as follows: ■, 5/0; □, 5/0; ▤, 5/25; ▨, 5/50; ▩, 5/100; ■, 10/0; ■, 10/50. Animals in group 1 were sham operated, animals in groups 2–7 were ovariectomized. Least square means and pooled SEM of MANOVA. a, b, indicates significant differences between groups with $P$ of at least $<0{\cdot}05$.

NDO on bone mineral content was investigated (Chonan *et al.* 1995; Ohta *et al.* 1998*a*; Scholz-Ahrens *et al.* 1998). Ovariectomy (OVX) in the adult or aged rat is an accepted method to simulate human postmenopausal state (Kimmel, 1996). In young (Chonan *et al.* 1995) and adult (Scholz-Ahrens *et al.* 2002) ovariectomized rats galacto-oligosaccharides and oligofructose effectively prevented loss of bone mineral content or density. The preventive effect in the femur was more prominent after 8 weeks than after 16 weeks while in the lumbar vertebra it occurred persistently over 16 weeks but with less magnitude. The addition of 5 % oligofructose prevented bone loss significantly in the femur and lumbar vertebra in the presence of high dietary calcium (1 %) but not at 0·5 % (Fig. 3). At 0·5 % calcium 10 % oligofructose was needed to significantly increase bone mineralization, and this was only observed in the femur (Scholz-Ahrens *et al.* 2002).

### Bone trabecular structure — bone quality

In an experiment by our group, oligofructose was studied for its effect on bone trabecular structure in a long-term study in adult ovariectomized rats. Trabecular structure was assessed by computer-supported image analyses of contact microradiographs, a method described in detail elsewhere (Hein, 1997) and applied before to assess bioavailability of calcium from different sources (Scholz-Ahrens *et al.* 1997). Ovariectomy induced loss of tibia trabecular bone, particularly trabecular area (Tb.Ar/T.Ar), and trabecular perimeter (Tb.Pm) was reduced while tissue area (T.Ar) was not affected (Fig. 4a). To prevent ovariectomy-induced bone loss, rats were treated with higher dietary calcium (1·0 % *v.* 0·5 %,) or by including 5 % oligofructose in the diet or by combining both treatments for 8 and 16 weeks. Trabecular structure was affected differently by these treatments: Higher dietary calcium as well as including oligofructose preserved the trabecular area almost to the same extent (Fig. 4b). Higher dietary calcium, however, conserved fewer but thicker trabecules, while oligofructose conserved more trabecules compared to dietary calcium. This was also indicated by the higher values for trabecular perimeter after oligofructose, compared to the control group, 2 (Fig. 4b). The combined intervention, i.e. including 5 % oligofructose into a diet containing 1 % calcium, preserved a larger Tb.Ar/T.Ar at almost the same number but larger trabecular perimeter (Tb.Pm), thus indicating longer trabecules. Moreover, cortical thickness (C.Th) was significantly higher (Fig. 4c). Based on the bone structure analysis we demonstrated different bone quality following varying diets

(Fig. 4). It may be speculated that a certain bone structure represents bone with more or less elasticity and break force resistance. From civil engineering it is known that a construction made of many thin beams is more stable than one made of a few thick ones. Therefore a certain trabecular architecture may reflect a surrogate for a lower fracture risk. Further data, however, are needed to define an architecture that is advantageous for bone health.

## Mechanism

In the first place an enhancement of the passive calcium transport by NDO was favoured and postulated by several authors (Ohta *et al.* 1993; Levrat *et al.* 1991; Chonan *et al.* 1995), because the higher calcium solubility in the large intestine and its absorption was associated with a lower luminal pH, and a selective stimulation of bacterial growth *in vitro* and in the intestinal lumen, mainly bifidobacteria and lactobacilli (Wang & Gibson,1993; Rowland & Tanaka, 1993; Gibson & Roberfroid, 1995). At fermentation of inulin and oligofructose by the microflora the reduction of pH occurs as the result of stimulated production of lactic acid and short-chain fatty acids, mainly propionate and acetate, and at a lower level but at a higher rate, butyrate (Levrat *et al.* 1991). At lower pH more mineral is soluble in the gut lumen and thus is more readily absorbed from the gut mucosa cell (Ohta *et al.* 1995*a*). Apart from stimulating the passive calcium absorption indirectly by increasing its solubility via lowering the pH (Table 2), SCFA directly stimulated calcium disappearance across the colon in humans more effectively than a solution containing calcium + NaCl, although the pH of the latter was lower (Trinidad *et al.* 1996). Calcium propionate stimulated calcium uptake more effectively than calcium acetate, although the pH of the infusion solutions were not different. It was discussed that a more rapid uptake of propionate compared to acetate was due to its greater lipid-solubility, which is associated with chain length (Trinidad *et al.* 1996).

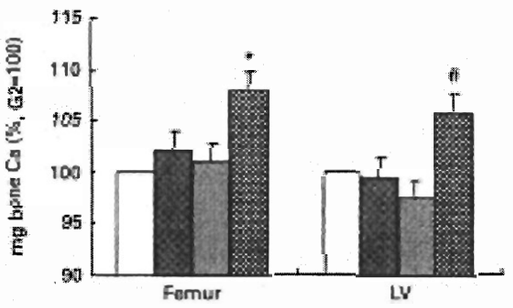


**Fig. 3.** Effect of oligofructose on calcium content (mg) of femur (left) and 1.+4. lumbar vertebra (LV, right) in ovariectomized rats after 16 wks on diets containing (g calcium/kg diet) / (g oligofructose/kg diet) as follows: □, 5/0; ▨, 5/50; ■, 10/0; ◪, 10/50. Least square means and pooled SEM of MANOVA. Values given are percentages of the mean of 5/0. Significantly different ($P$ of at least $<0{\cdot}05$) as follows: *different from 5/0 and 10/0, #different from 5/0, 5/50 and 10/0.

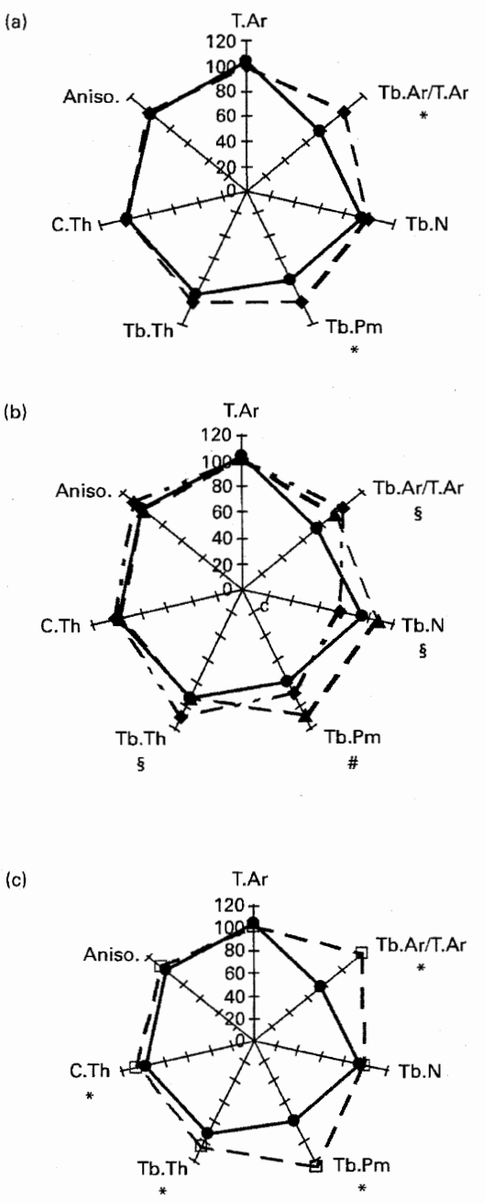


**Fig. 4.** Parameters of tibia trabecular structure following microradiography and computer-supported image analysis of the proximal tibia of adult ovariectomized rats on different diets. The figures indicate different bone quality depending on whether ovariectomy-induced bone loss (a) was prevented by oligofructose or calcium (b) or a combination of both (c). The experimental groups were fed semipurified diets with 5 g/kg phosphorous and 5 g/kg calcium without oligofructose ((a)—◆—; group 1 and —●—; group 2), 5 g/kg calcium plus 50 g oligofructose /kg (—▲—; group 4) and 10 g/kg calcium either without ((b) —◆—; group 6) or with (—□—; group 7) 50 g oligofructose /kg. Group 1 was sham operated, groups 2, 4, 6, and 7 were ovariectomized. Least-squares means from MANOVA were given. Significant differences with $P$ at least $< 0{\cdot}05$ as follows: in (a) *1 *v.* 2; in (b) #4 different from 2, §6 different from 2; in (c) * 7 *v.* 2.

**Table 2.** Aspects probably involved in the oligofructose-mediated stimulation of mineral absorption and bone mineralization

| Effect of oligofructose on | | Mediated via | Effect |
|---|---|---|---|
| Passive transport | ↑ | ● SCFA production (acetate > propionate > butyrate) > lactate | ↓ pH, ↑ mineral solubility |
| | | ● Propionate > acetate | ↑ exchange of cellular proton (H+) for luminal cation (Ca++) |
| Resorptive surface | ↑ | ● Polyamines | Growth and proliferation factor |
| | | ● SCFA, mainly butyrate | Growth substrate for colonocytes |
| Active transport | ↑ | ● Butyrate | ↑ synthesis of CaBP |
| | | ● Polyamines ? | ↑ synthesis of $1,25(OH)_2D_3$ receptor ? |

Butyrate is given prominence to be a potent candidate for enhancing mineral absorption. Butyrate is a substrate for cell growth and proliferation (Lupton & Kurtz, 1993). By increasing the floral butyrate production, fructo-oligosaccharides (FOS) might indirectly induce cell growth and growth of the gut's absorptive area, another way to contribute to the enhanced mineral absorption (Table 2). Moreover, FOS might be stimulative on the active calcium transport via butyrate production. Sodium butyrate increased the concentration of calbindin and of $1,25[OH]_2D_3$ receptor activity in chick primary culture cells (Anita & Anthony, 1992). In rats that were fed a diet containing oligofructose (from sucrose), Ohta *et al.* (1998*c*) observed a higher expression of calcium binding protein (calbindin-D9k) in the large intestine, while that in the small intestine was decreased. Results reported by Le Blay *et al.* (1999) support the hypothesis that butyrate may have a marked impact on the effect of oligofructose. They observed that oligofructose reduced the pH in the caecal lumen after 2 weeks and this was mainly due to stimulated synthesis of lactate (63·5 μmol/g wet contents at oligofructose and 3·6 μmol/g at basal) > acetate > butyrate. However, after 8 weeks, lactate production came down to concentration of basal diet, while acetate and butyrate were persistently stimulated over 27 weeks. Acetate was 69·5 μmol/g wet contents at oligofructose and 47·6 μmol/g at basal. Butyrate was 15·3 μmol/g wet contents at oligofructose and 4·7 μmol/g at basal. One might ask whether the low concentration of butyrate in relation to acetate, or lactate could have such a strong effect. Butyrate is very quickly taken up by the enterocytes and makes its detection difficult (Trinidad *et al.* 1996). This circumstance may explain the lack of coincidence of high luminal butyrate concentration and increased mineral uptake across the apical membrane. Inulin and oligofructose mainly stimulate growth of bifidobacteria, but these microbes produce only a little butyrate compared to other strains (Djouzi & Andrieux, 1997). Therefore, data presently available do not support a causal relationship between stimulated mineral absorption by bifidobacteria via butyrate.

Polyamines, e.g. spermine, spermidine, and putrescine, are metabolites generated by several strains of microbes (Noack *et al.* 2000) as well as by higher organisms (Straub *et al.* 1995). Hence they occur almost ubiquitously. Polyamines are potent agents to stimulate proliferation of several organs including intestine (Buts *et al.* 1993; Lupton & Kurtz, 1993; Löser *et al.* 1999) or cells in culture like osteoblasts (Klein & Carlos, 1995). Thus they might at least in part be responsible for cell growth and enlargement of the resorptive surface and by this are further candidates for mineral absorption enhancers. Moreover it was proposed that polyamines have a metabolic effect. The low bone mass in subjects who consume excessive amounts of alcohol was explained by its inhibitory effect on osteoblast proliferation or on the activity of ornithine

**Table 3.** Effects of exogenous polyamines, oligofructose (as potential initiator of microbial polyamines synthesis), or antibiotics (for eradication of microbial polyamine production) on bone mineralization and intestinal weight and pH in adult ovariectomized rats

| | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| Femur weight | 494$^{b}$ | 448$^{a}$ | 457$^{ab}$ | 445$^{a}$ | 479$^{ab}$ | 500$^{b}$ | 486$^{ab}$ | 476$^{ab}$ | 489$^{ab}$ | 0·000 |
| (mg) | (12) | (12) | (10) | (12) | (9) | (10) | (7) | (10) | (9) | |
| Femur ash | 240bc | 235ab | 233ab | 232ab | 242bc | 248c | 228ab | 223a | 237bc | 0·001 |
| (mg) | 3·3 | 4·0 | 3·2 | 4·5 | 3·6 | 2·8 | 3·0 | 2·1 | 3·3 | |
| Caecum weight | 0·57 $^{a}$ | 0·55 $^{a}$ | 0·55 $^{a}$ | 0·55 $^{a}$ | 0·85 $^{b}$ | 1·31 $^{c}$ | 0·86 $^{b}$ | 0·83 $^{b}$ | 1·33 $^{c}$ | 0·001 |
| (g) | (0·02) | (0·01) | (0·01) | (0·01) | (0·03) | (0·06) | (0·05) | (0·05) | (0·09) | |
| Caecal contents | 2·37 $^{a}$ | 2·56 $^{a}$ | 2·50 $^{a}$ | 2·31 $^{a}$ | 5·58 $^{b}$ | 8·85 $^{c}$ | 4·97 $^{b}$ | 5·17 $^{b}$ | 9·95 $^{c}$ | 0·001 |
| (g) | (0·11) | (0·01) | (0·01) | (0·11) | (0·37) | (0·57) | (0·42) | (0·53) | (0·99) | |
| Caecum pH | 7·95 $^{b}$ | 7·98 $^{bc}$ | 8·15 $^{cd}$ | 8·05 $^{bc}$ | 7·63 $^{a}$ | 7·62 $^{a}$ | 8·26 $^{d}$ | 8·29 $^{d}$ | 7·91 $^{b}$ | 0·001 |
| | (0·03) | (0·08) | (0·03) | (0·05) | (0·04) | (0·04) | (0·04) | (0·06) | (0·10) | |
| Jejunum Ph | 7·97$^{ab}$ | 7·75$^{a}$ | 7·97$^{ab}$ | 8·13$^{b}$ | 7·75$^{a}$ | 7·73$^{a}$ | 8·02$^{ab}$ | 8·20$^{b}$ | 7·97$^{ab}$ | 0·001 |
| | (0·07) | (0·17) | (0·08) | (0·12) | (0·03) | (0·06) | (0·08) | (0·06) | (0·04) | |

ANOVA, mean and (SEM), $N = 12–14$, values within a row not sharing a superscript letter are significantly different with $P$ as indicated. The rats were fed semisynthetic diets for 16 weeks that contained 7 g calcium /kg diet and either no additives (G1, G2; G1 = sham operated), or spermidine, spermine and putrescine in physiologic amounts (G3), high amounts (G4, equivalent to 10 × polyamine content of G3), oligofructose (50 g/kg diet, G5), oligofructose + antibiotics (neomycin/metronidazol) (G6), antibiotics alone (G7), antibiotics + high amounts of polyamines (G8) or oligofructose + antibiotics + high amounts of polyamines (G9).

decarboxylase (ODC), the rate-limiting enzyme for polyamine synthesis (Klein & Carlos, 1995). Dietary inulin stimulated ODC activity (Rémésy *et al.* 1993), indicating increased polyamine synthesis by the intestinal microflora. Stimulated intestinal microbial polyamine production was also observed after feeding of guar gum or pectin, two highly fermentable dietary fibres (Noack *et al.* 1998). Since exogenous or microbial polyamines are readily taken up by enterocytes or can pass into the circulation (Bardócz *et al.* 1993), polyamines may also stimulate gene expression of calcium binding proteins in the gut.

When rats were fed oligofructose for 4 weeks, putrescine concentration was higher and spermidine concentration lower, while total polyamine concentration was unchanged in the caecal contents compared to a standard diet. In the caecal tissue putrescine, spermidine and total polyamine concentrations were increased with diets containing oligofructose (Delzenne *et al.* 2000). Higher polyamine concentrations were also observed in rats after feeding of pectin or guar gum (Noack *et al.* 1998).

*In vitro* studies have demonstrated that fusobacterium synthesised mainly putrescine and bacteroides mainly spermidine, while bifidobacterium did not synthesise any of the polyamines (Noack *et al.* 1998). This observation does not support a causal relation between oligofructose-stimulated mineral absorption and increased polyamine synthesis due to selective stimulation of growth of intestinal bifidobacteria.

To test whether the oligofructose-induced stimulation of mineral absorption is mediated by polyamines, a study with adult ovariectomized Fisher-344 rats was done by our group. The animals were fed semisynthetic diets for 16 weeks that contained 7 g calcium/kg diet and either no additives (G1, G2; G1 = sham operated), or spermidine, spermine and putrescine in physiological amounts (G3), high amounts (G4, equivalent to 10 × polyamine content of G3), oligofructose (50 g/kg diet, G5), oligofructose + antibiotics (neomycin/metronidazol) in order to eradicate the intestinal flora (G6), antibiotics alone (G7), antibiotics + high amounts of polyamines (G8) or oligofructose + antibiotics + high amounts of polyamines (G9). The hypothesis was that oligofructose would increase mineral availability to a more or less comparable degree than exogenous polyamines and that this effect may be diminished in the presence of antibiotics, which eradicate most polyamine producers. We observed a significant loss of femur weight following ovariectomy (Table 3). The highest femur ash was gained in the groups containing oligofructose, either alone (G5) or in combination with antibiotics (G6) or in combination with antibiotics + polyamines (G9). Exogenous polyamines slightly increased the pH of cecal contents and significantly of jejunal contents at the high dose. In contrast to diets containing oligofructose, especially in combination with antibiotics (G6), exogenous polyamines alone did not prevent loss of femur weight and femur ash (Table 3). Antibiotics alone almost prevented loss of femur weight, but not loss of femur ash, which was prevented when antibiotics were combined with oligofructose (G6, Fig. 5). Antibiotics increased caecum weight and weight of caecal contents in a comparable magnitude as oligofructose. However, antibiotics did not reduce the pH in the caecal contents, in contrast to oligofructose, but rather increased it. Data of this experiment revealed that the femur ash, in contrast to femur weight, was highest correlated with pH of the caecal content and to a much less degree to the weight of the caecum or of the caecal contents (Table 4). Femur weight was highest correlated with caecal weight.

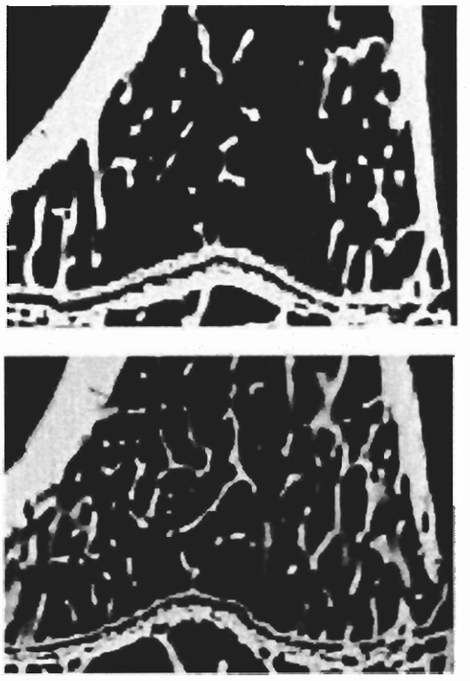


**Fig. 5.** Microradiograph of the proximal tibia of a 9-month old ovariectomized Fisher-344 rat after treatment with antibiotics (1) or after treatment with antibiotics in the presence of oligofructose (2).

We conclude that the potential of oligofructose to prevent ovariectomy-induced loss of bone weight and bone mineral may not be mediated via polyamines, since exogenous polyamines were not protective, possibly because endogenous polyamine synthesis compensated for it. The bone-protecting effect of oligofructose in combination with a reduction of fecal flora was associated with a significant drop of caecal pH. This indicates that oligofructose can prevent not only loss of bone mineral

**Table 4.** Pearson correlation coefficient (*r*) of means of bone mineral and of caecal pH, caecal weight and weight of caecal content in ovariectomized rats fed semisynthetic diets that contained physiological amounts of polyamines, high amounts of polyamines, oligofructose, anitibiotics, oligofructose + antibiotics, polyamines + antibiotics or oligofructose + antibiotics + polyamines to induce different femur ash

| | | Caecum | |
|---|---|---|---|
| Femur | pH | Content (g) | Organ weight (g) |
| | | *r* | |
| Bone weight (g) | −0·35 | 0·67 | 0·71 |
| Ash weight (g) | −0·94 | 0·37 | 0·39 |
| Calcium (mg) | −0·88 | 0·37 | 0·40 |

content but also the undesirable side effect of raising alkalinity in the large bowel following treatment with antibiotics.

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Nutritional and Health Benefits of Inulin and Oligofructose



# Dietary Modulation of the Human Gut Microflora Using the Prebiotics Oligofructose and Inulin[1]

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ABSTRACT Although largely unproven in humans, better resistance to pathogens, reduction in blood lipids, antitumor properties, hormonal regulation and immune stimulation may all be possible through gut microflora manipulation. One approach advocates the oral intake of live microorganisms (probiotics). Although the probiotic approach has been extensively used and advocated, survivability/viability after ingestion is difficult to guarantee and almost impossible to prove. The prebiotic concept dictates that non viable dietary components fortify certain components of the intestinal flora (e.g., bifidobacteria, lactobacilli). This concept has the advantage that survival of the ingested ingredient through the upper gastrointestinal tract is not a prerequisite because it is indigenous bacterial genera that are targeted. The feeding of oligofructose and inulin to human volunteers alters the gut flora composition in favor of bifidobacteria, a purportedly beneficial genus. Future human studies that exploit the use of modern molecular-based detection methods for bacteria will determine the efficacy of prebiotics. It may be possible to address prophylactically certain gastrointestinal complaints through the selective targeting of gut bacteria. J. Nutr. 129: 1438S–1441S, 1999.

KEY WORDS: • *prebiotics* • *gut microbiota modulation* • *inulin* • *oligofructose*



### *Bacterial fermentation in the human colon*

Functions of the human gut include absorption of water, certain minerals, and the storage and excretion of waste materials. However, because of the resident microbiota, it is clear that the colon has an important role in human nutrition and possibly health (Gibson and Macfarlane 1995). It is known that many disease states involve bacterial metabolism. However, the gut microflora may also be considered relevant to host welfare. Gut bacteria carry out a multidisciplinary process known as fermentation in which dietary and endogenously produced residues are metabolized in a process that involves a large amount of cross-feeding by the microflora. Large intestinal microorganisms have a strictly anaerobic metabolism; the numbers of obligate anaerobes are many orders of magnitude higher than those of facultative anaerobes. Numerically predominant anaerobes are gram-negative rods belonging to the genus *Bacteroides*. These bacteria can represent up to 30% of the total microbial flora. Other groups that have been identified to date as present in high numbers include bifidobacteria, clostridia, eubacteria, lactobacilli, gram-positive cocci, coliforms, methanogens and dissimilatory sulfate-reducing bacteria. It is thought that between 400 and 500 different bacterial species are present in the human large intestine.

The principal substrates for gut bacterial growth are dietary carbohydrates that have escaped digestion in the upper gastrointestinal tract. In addition, amino acids can also be effective as growth substrates for colonic bacteria; bacterial secretions, lysis products, sloughed epithelial cells and mucins may also make a contribution.

A number of different microbial metabolic niches, bacterial habitats and interrelationships occur in the large gut and respond mainly to substrate availability, the physicochemical environment of the gut and the metabolic capabilities of the microflora (Freter 1992). Gut bacteria are able to metabolize substrates for increased energy and growth. The major end-products of metabolism are short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate, but a variety of other metabolites are also produced, including electron sink products such as lactate, pyruvate, ethanol, hydrogen and succinate (**Table 1**). These substances are formed to maintain the redox balance during fermentation. Electron sink products act as fermentation intermediates because they are further metabolized to SCFA by other species. Although the products of gut proteolysis may be generally thought of as toxic toward host health, those of carbohydrate digestion may be considered benign and in some cases can contribute positively (Table 1).

Although the gut microflora contain certain bacteria that are pathogenic, there may also be a positive aspect to gut microbiology. In this context, the intestinal flora are considered to be key in influencing human well-being. Under normal homeostatic conditions, the intestinal microflora are of central importance in preventing coloniza-

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**TABLE 1**

*Predominant products of carbohydrate metabolism in the human colon*

| End product | Bacterial group involved | Metabolic fate |
|---|---|---|
| Acetate | bacteroides, bifidobacteria, eubacteria, lactobacilli, clostridia, ruminococci, peptococci, veillonella, peptostreptococci, propionibacteria, fusobacteria, butyrivibrio | Metabolized in muscle, kidney, heart and brain |
| Propionate | bacteroides, propionibacteria, veillonella | Cleared by the liver, possible gluceogenic precursor, suppresses cholesterol synthesis |
| Butyrate | clostridia, fusobacteria, butyrivibrio, eubacteria, peptostreptococci | Metabolised by the colonic epithelium, regulator of cell growth and differentiation |
| Ethanol, succinate, lactate, pyruvate | bacteroides, bifidobacteria, lactobacilli, eubacteria, peptostreptococci, clostridia, ruminococci, actinomycetes, enterococci, fusobacteria, | Absorbed, electron sink products, further fermented to short-chain fatty acids |
| Hydrogen | clostridia, ruminococci, fusobacteria | Partially excreted in breath, metabolized by hydrogenotrophic bacteria |



tion by pathogens;they are also thought to have many beneficial local and systemic roles such as improved lactose tolerance, supply of SCFA as energy substrates for the host, antitumor properties, neutralization of certain toxins, stimulation of the intestinal immune system and possibly reduction of blood lipid levels (Fuller 1989,1992 and 1997, Gorbach et al. 1988, Isolauri et al. 1991, Kohwi et al. 1978, Lin et al. 1989, Sanders 1994).

For obvious reasons, there is much interest in increasing numbers and activities of beneficial bacteria in the large gut, preferably at the expense of more harmful species. A way in which this can be achieved is through dietary supplementation.

### *Probiotics*

Fuller (1992) defined a probiotic as *A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance*. The probiotic organism(s) used for human consumption are usually lactic acid excretors.

The history of probiotics dates back as far as the first intake of fermented milks, over 2000 years ago. However, it is probably from the work of Metchnikoff (1907) in the early years of this century that the first scientific assessments of probiotics were made. Common probiotics include the following: *1*) Lactobacilli such as *Lactobacillus acidophilus, L. casei, L. delbrueckii* subsp. *bulgaricus, L. reuteri, L. brevis, L. cellobiosus, L. curvatus, L. fermentum, L. plantarum*; *2*) Gram-positive cocci such as *Lactococcus lactis* subsp. *cremoris, Streptococcus salivarius* subsp. *thermophilus, Enterococcus faecium, S. diaacetylactis, S. intermedius*; and *3*) Bifidobacteria such as *Bifidobacterium bifidum, B. adolescentis, B. animalis, B. infantis, B. longum, B. thermophilum*.

Selection criteria for probiotics is an area of much debate and should be taken into account when defining appropriate strains (Huis In't Veld and Havennar 1991). One important characteristic is survival (and establishment) of the fed microorganism after ingestion. Some studies with feces rely on phenotypic traits of the probiotics, such as different morphologies or biochemical tests. However, these are probably unreliable because the bacteria may exhibit metabolic variation. Future developments in molecular techniques directed toward gut microbiology will more clearly define the survival characteristics of probiotics (McCartney and Gibson 1997).

### *Prebiotics*

Because the viability of live bacteria in food products and during transit through the gastrointestinal tract may be variable, the prebiotic concept has been developed. Here the selective growth of indigenous gut bacteria is required. *A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that can improve the host health* (Gibson and Roberfroid 1995). Thus, the prebiotic approach advocates the administration of nonviable entities and therefore overcomes survival problems in the upper gastrointestinal tract. Certain oligosaccharides that cannot be digested, except through bacterial activity, are prebiotics. Those that contain fructose can alter the composition of the human gut flora, by a specific fermentation, towards a community predominated by bifidobacteria.

### *Oligofructose and inulin as prebiotics*

Criteria that allow the classification of a food ingredient as a prebiotic, include the following: *1*) It must be neither hydrolyzed, nor absorbed in the upper part of the gastrointestinal tract. *2*) It must be selectively fermented by one or a limited number of potentially beneficial bacteria in the colon. *3*) It must alter the composition of the colonic microbiota towards a healthier composition. *4*) It must preferably induce effects that are beneficial to the host health.

Any food that reaches the colon such as nondigestible carbohydrates, some peptides and proteins, as well as certain lipids, is a prebiotic candidate. Nondigestible carbohydrates, in particular fructose oligosaccharides, are authentic prebiotics. Fructooligosaccharides are β-D-fructans with degrees of polymerization (DP) varying between 2 and 60 (inulin) and 2 and 20 (oligofructose).

In vitro studies have indicated that inulin and oligofructose have a specific fermentation (Hidaka et al. 1986, Wang and Gibson 1993). This has also been confirmed in human volunteer trials that assessed the bifidogenic effects of both

**TABLE 2**

*Differences in fecal microbial counts of volunteers fed a controlled diet supplemented with 15 g/d sucrose, oligofructose or inulin*[1,2]

| Bacterial group | Sucrose | Oligofructose | Inulin |
|---|---|---|---|
| Total aerobes | 6.5 ± 1.0 | 6.2 ± 1.0 | 6.7 ± 1.0 |
| Coliforms | 6.0 ± 1.2 | 5.9 ± 0.7 | 6.2 ± 1.4 |
| Gram positive cocci | 5.8 ± 0.7 | 5.8 ± 0.9 | 5.5 ± 0.27 |
| Total anaerobes | 10.3 ± 0.8 | 10.2 ± 0.9 | 10.7 ± 0.25 |
| Bifidobacteria | 8.9 ± 0.6 | 9.5 ± 0.7 | 10.1 ± 0.44 |
| Bacteroides | 9.3 ± 0.7 | 8.8 ± 1.1 | 9.8 ± 0.5 |
| Fusobacteria | 8.5 ± 0.6 | 7.7 ± 0.9 | 8.9 ± 0.62 |
| Clostridia | 8.0 ± 0.8 | 7.5 ± 0.9 | 8.1 ± 0.72 |
| Lactobacilli | 6.6 ± 1.1 | 7.0 ± 1.4 | 6.3 ± 0.76 |

[1] Counts are $\log_{10}$/g wet weight of feces and are given as mean values ± SD.
[2] See Gibson et al. (1995) for study details.

inulin and oligofructose in vivo. The influence of oligofructose (Raftilose, P95) on the fecal bacterial composition in healthy persons was evaluated during a 45-d feeding period in which the volunteers were given a strictly controlled diet (Gibson et al. 1995). Eight volunteers participated in the experiment. They had never suffered from any form of gastrointestinal disorder and had not taken antibiotics for at least 3 mo before the start of the study. During the first 5 d, subjects were given a noncontrolled diet; at that time, a stool sample was collected for bacteriological analysis. Subsequently, the volunteers were given the controlled diet supplemented with 15 g of sucrose for a 15-d period. This was then replaced by 15 g of oligofructose for a further 15 d, followed by another period with sucrose. Stool samples were taken periodically for bacterial enumeration. In summary, the use of oligofructose as a replacement for sucrose in diet caused a marked increase in bifidobacteria, whereas bacteroides, fusobacteria and clostridia all decreased. Other bacteria tested (total aerobes, total anaerobes, lactobacilli, coliforms and gram-positive cocci) remained more or less unchanged. Bacteroides was the numerically predominant genus with sucrose consumption, whereas bifidobaceria dominated with oligofructose. Similar results were detected during the feeding of inulin (Raftiline, ST) (**Table 2**). Other investigators have since confirmed the prebiotic effect of inulin and oligofructose in vivo (Buddington et al. 1996, Kleesen et al. 1997).

### *Health aspects of prebiotics*

Although prebiotics offer one rational approach to the probiotic concept, the health consequences have not yet been defined. In theory, a number of potential benefits may arise. However, it may be that improved resistance to pathogens offers the most feasibility. The lactic microflora of the human gastrointestinal tract are thought to play a significant role in improved colonization resistance. Increased bifidobacterial numbers in the breast-fed infant may be one factor that contributes towards improved competitive exclusion of pathogens seen in this group compared with those who are formula fed (Gibson et al. 1997).

In terms of the mechanism of inhibition, metabolic end products, such as acids excreted by these microorganisms, may lower the gut pH, in a microniche, to levels below those at which pathogens are able to effectively compete. Another factor that could be considered is a competitive effect by occupation of normal colonization sites (by anti-infective prebiotics or probiotic microrganisms) and competition for available nutrients. Enhanced immune function would also be a further important factor.

Many lactobacilli and bifidobacterial species are able to excrete natural antibiotics, which can have a broad spectrum of activity (e.g., lactocins, helveticins, lactacins, curvacins, nisin or bifidocin). For the bifidobacteria, our studies have indicated that some species are able to exert antimicrobial effects on various gram-positive and gram-negative intestinal pathogens including salmonellae, campylobacters and *Escherichia coli* (Gibson and Wang 1994).

The outbreak of *E. coli* 0157 in Lanarkshire, Scotland at the end of 1996 resulted in 20 fatalities. The deaths have highlighted the continuing concern about bacterial gastroenteritis to consumers, the food industry, researchers and the medical profession. In recent laboratory tests, we have also shown that some bifidobacteria exert powerful antagonistic effects towards *E. coli* 0157. The inhibition was variable in species of bifidobacteria, with *Bifidobacterium infantis* and *B. longum* exerting the greatest effect on *E. coli* 0157. The possibility exists therefore that increased levels of bifidobacteria (and consideration of the species type) in the large gut, together with other factors such as immune status, may offer improved protection.

In humans older than ~55 y, fecal bifidobacterial counts are known to show a marked decrease in comparison to those of younger persons (Kleessen et al. 1997, Mitsuoka 1990). It may be of some relevance that the UK fatalities during the *E. coli* outbreak all involved the elderly, whereas hundreds of people in different age groups reported the infection. A potential analogy exists with reduced pathogen resistance, decreased numbers of bifidobacteria in the elderly and the production of natural resistance factors. In essence, the natural gut flora may have been compromised through reduced bifidobacterial numbers and possibly a diminished ability to deal with the pathogen. The design of prebiotic-based health foods for selected populations such as the elderly may therefore have much virtue.

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# Calcium intake, calcium bioavailability and bone health

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Calcium accounts for 1–2 % of adult human body weight. Over 99 % of total body Ca is found in the teeth and bones. Therefore, in addition to the obvious structural role of the skeleton, it also serves as a reservoir for Ca. Dietary Ca intake has an important impact on bone metabolism and bone health. Chronic Ca deficiency resulting from inadequate intake or poor intestinal absorption is one of several important causes of reduced bone mass and osteoporosis. It is vital, therefore, that adequate dietary Ca is consumed at all stages of life – in early life so that the genetically programmed peak bone mass can be reached and in later adulthood so that the skeletal mass can be maintained and age-related bone loss minimised. Unfortunately, there is wide variation in the estimates of daily Ca requirements made by different expert authorities. Furthermore, there is evidence that many individuals are not consuming these recommended levels. The consequence of this for bone health will be discussed in the present review. Besides the amount of Ca in the diet, the absorption of dietary Ca in foods is also a critical factor in determining the availability of Ca for bone development and maintenance. Thus, there is a need to identify food components and/or functional food ingredients that may positively influence Ca absorption in order to ensure that Ca bioavailability from foods can be optimised. This approach may be of particular value in individuals who fail to achieve the dietary recommended level of Ca.

**Calcium intake: Calcium absorption**

## Introduction

Osteoporosis is a global health problem that will take on increasing significance as people live longer and the world's population continues to increase in number. Dietary composition is an important determinant of the bone mineral density in the growth period, and of the magnitude of the age-related bone mineral loss, in particular among postmenopausal women (Michaelsen *et al.* 1994). Calcium, in particular, plays an important role in skeletal health (European Commission, 1998). A sufficient intake of Ca and vitamin D can reduce the risk of fractures in postmenopausal women, and it is likely that a low Ca intake may affect peak bone mass negatively (Michaelsen *et al.* 1994). Nonetheless, dietary Ca intakes are below recommended levels in many EU member states (European Commission, 1998), with consequences for bone health and risk of osteoporosis in these populations. The present review will define the principal disease of bone mass (i.e. osteoporosis) as well as considering its epidemiology and risk factors. The review will then focus on the importance of dietary Ca in bone health, with particular emphasis on the role of Ca intake and Ca bioavailability in maintaining optimal skeletal health.

## Definition of osteoporosis and osteopenia

Osteoporosis is defined as a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture (Consensus Development Conference, 1993). Osteopenia is sometimes referred to as borderline low density because there is a loss of bone density, but less than is seen with osteoporosis. For the purposes of clinical diagnosis, a Working Party of the World Health Organisation has redefined osteoporosis and osteopenia according to bone mass, at least for women. Their diagnostic criteria for osteoporosis and osteopenia, based on bone mineral content (BMC) or bone mineral density (BMD) include: normal, within 1 standard deviation (SD) of young adult reference mean for the population; osteopenia, between −1 and −2·5 SD of the young adult mean; osteoporosis, more than −2·5 SD below the young adult mean, and established osteoporo-

**Abbreviations:** CPP, casein phosphopeptides; NDO, non-digestible oligosaccharide; PBM, peak bone mass; PTH, parathyroid hormone.
**Note:** For the definition of the terms inulin and oligofructose please refer to the introductory paper (p. S139) and its footnote.
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sis as the same mass definition but associated with a fragility fracture (World Health Organisation, 1994). Fragility fractures are the hallmark of osteoporosis and are particularly common in the spine, hip and distal forearm, although they can occur throughout the skeleton.

## Epidemiology of osteoporosis

Osteoporotic fractures constitute a major public health problem. Currently, in the US alone, 10 million individuals already have osteoporosis, and a further 18 million more have low bone mass, placing them at increased risk for this disorder (National Institutes of Health, 2000). One in eight EU citizens over the age of 50 years will fracture their spine this year (European Commission, 1998). The estimated remaining lifetime risk of fractures in Caucasian women at the age of 50 years, based on incidence rates in North America is 17·5 %, 15·6 % and 16 % for hip, spine and forearm respectively; the remaining lifetime risk for any fragility fracture approaches 40 % in women and 13 % in men (Melton *et al.* 1992). Similar rates have been reported from parts of Europe, although there is a marked variation in the incidence of fractures between countries and regions (Johnell *et al.* 1992) and even within countries (Elffors *et al.* 1994). Hip fractures in particular are associated with significant morbidity, necessitating hospital admission for an average of 20–30 days (Johnell *et al.* 1992). Osteoporosis patients currently occupy 500 000 hospital bed nights per year in the European Community (European Commission, 1998). Moreover, they have an overall mortality of 15–30 % (Browner *et al.* 1996), the majority of excess deaths occurring within the first 6 months after the fracture. Vertebral fractures are also associated with reduced survival (Copper *et al.* 1993), probably due to clustering of comorbidity which predisposes independently to osteoporosis and premature death. Fractures can also have a profound impact on quality of life, as evidenced by the finding that 80 % of women older than 75 years preferred death to a bad hip fracture resulting in nursing home placement (National Institutes of Health, 2000). Fear, anxiety, and depression are frequently reported in women with established osteoporosis and are likely to be under-addressed when considering the overall impact of this condition (National Institutes of Health, 2000).

The incidence of vertebral and hip fractures increases exponentially with advancing age while that of wrist fractures levels off after the age of 60 years (Compston, 1993). This is of particular concern as it is projected that the number of elderly (80 years and older, in whom the incidence of osteoporotic fracture is greatest) in the EU population will grow from 8·9 million and 4·5 million women and men, respectively, in 1995 to 26·4 million and 17·4 million women and men, respectively, in the year 2050 (European Commission, 1998). Because of the increase in incidence rates of osteoporotic fractures with age, the above demographic changes and increasing life expectancy will have a huge impact on the number of fractures that can be expected to occur. For example, the number of hip fractures occurring each year in the EU alone is estimated to rise from current figures of 414 000 to 972 000 by the year 2050, representing an increase of 135 % (European Commission, 1998). The increase in the number of vertebral fractures occurring each year is not expected to be of the same magnitude as for hip fractures; thus the estimated increase is from current figures of 237 000 to 373 000 by the year 2050, representing a rise of 57 % (European Commission, 1998).

From an economic perspective, the expenses of hospital care and rehabilitation associated with osteoporotic fractures are a considerable fiscal drain for the health care system, exceeding those of other highly prevalent pathologies of the elderly, such as myocardial infarction (Schurch *et al.* 1996). Osteoporosis costs national treasuries over 3500 million ECU annually in hospital health care alone (European Commission, 1998).

## Risk factors for osteoporosis

Low bone mineral mass is the main factor underlying osteoporotic fracture (Prentice, 1997). Bone mass in later life depends on the peak bone mass (PBM) achieved during growth and the rate of subsequent age-related bone loss. Bone mineral is laid down throughout childhood, with the most rapid increase occurring during puberty. The deposition continues, at a slower rate, after growth in height has stopped (British Nutrition Foundation, 1989). PBM is achieved in early life (20–35 years), although the exact timing is not certain and may vary between different regions of the skeleton (Teegarden *et al.* 1995; Institute of Medicine, 1997). From the age of 20 years until approximately 40 years, bone mass is stable in both sexes (Reid & New, 1997). At older ages, bone is gradually lost from the skeleton in both men and women (Prentice, 1997). For women, there is also a period of about 10–15 years when bone loss (especially at trabecular-rich sites such as the spine or wrist) is accelerated due to oestrogen withdrawal at the menopause, when more than one-third of bone is lost from the skeleton (Compston, 1993). This accelerated rate of loss seen in women, when associated with a low attainment of PBM, leads to excessive risk of future fracture (Reid & New, 1997).

Bone is a living, dynamic tissue, and is constantly undergoing breakdown and formation as part of the natural process of renewal and repair (Prentice, 1997). Development of maximal bone mass during growth and reduction of loss of bone later in life are the two main strategies of preventing osteoporosis (Weaver, 2000). Consequently, any factor that influences the development of PBM or the loss of bone in middle age will affect later fracture risk. Several factors are thought to influence bone mass. These can be broadly grouped into factors that cannot be modified, such as gender, age, body (frame) size, genetics and ethnicity, and those factors that can be modified, such as hormonal status (especially sex and calciotropic hormone status), lifestyle factors including physical activity levels, smoking and alcohol consumption patterns, and diet. The interaction of these genetic, hormonal, environmental and nutritional factors influences both the development of bone to PBM at maturity and its subsequent loss. It has been suggested that genetic factors probably account for

up to 80 % of the bone mass variation in the population (Morrison *et al.* 1994). While diet and lifestyle factors, such as physical activity, may have a smaller influence than genetics on bone mass, these factors are nonetheless important since they are modulators for the achievement of maximum genetic potential peak bone mass as well as the subsequent rate of bone loss and, unlike genotype, they can be modified (Cashman & Flynn, 1998).

## Calcium and bone health

A large number of macro- and micronutrients have been proposed as possible determinants of bone health and osteoporosis risk. Of the bone-building nutrients, Ca is the most likely to be inadequate in terms of dietary intake (Weaver, 2000). Therefore, the remainder of the present review focuses only on the impact of Ca (in particular, Ca intake and Ca bioavailability) on bone health.

The adult human body contains about 1200 g of Ca, which amounts to about 1–2 % of body weight. Of this, 99 % is found in mineralised tissues, such as bones and teeth, where it is present as Ca phosphate (together with a small component of Ca carbonate), providing rigidity and structure (Nordin, 1997). The remaining 1 %, found in blood, extracellular fluid (ECF), muscle, and other tissues, plays a role in mediating vascular contraction and vasodilation, muscle contraction, nerve transmission and glandular secretion (Institute of Medicine, 1997).

Calcium is under close homeostatic control with processes such as absorption, excretion and secretion and storage in bone being involved in maintaining the concentration of ionised Ca in the plasma within a tightly regulated range (1·1–1·3 mmol/l; British Nutrition Foundation, 1989). This tight regulation of plasma Ca concentration is achieved through a complex physiological system comprising the interaction of the calcitropic hormones, such as parathyroid hormone (PTH), 1,25 dihydroxycholecalciferol (1,25 $(OH)_2D_3$) and calcitonin, with specific target tissues (kidney, bone and intestine) which serve to increase or to decrease the entry of Ca into the extracellular space. Only in extreme circumstances, such as severe malnutrition or hyperparathyroidism, is the serum ionised Ca concentration below or above the normal range. The secretion of these hormones is governed wholly, or in part, by the plasma concentration of ionised Ca, thus forming a negative feedback system. PTH and 1,25 $(OH)_2D_3$ are secreted when plasma Ca is low, while calcitonin is secreted when plasma Ca is high (British Nutrition Foundation, 1989).

Calcium is required for normal growth and development of the skeleton (National Research Council, 1989*a*; Nordin, 1997). During skeletal growth and maturation, i.e. until the age of the early twenties in humans, Ca accumulates in the skeleton at an average rate of 150 mg per day. During maturity, the body – and therefore the skeleton – is more or less in Ca equilibrium. From the age of about 50 in men and from the menopause in women, bone balance becomes negative and bone is lost from all skeletal sites. This bone loss is associated with a marked rise in fracture rates in both sexes, but particularly in women. Adequate Ca intake is critical to achieving optimal peak bone mass and modifies the rate of bone loss associated with ageing (National Institutes of Health, 1994).

In recent years, convincing evidence has emerged with respect to effects of dietary Ca on bone health in all age groups (European Commission, 1998). Intervention and cross-sectional studies have reported a positive effect of Ca on bone mass in children and adolescents (Kanders *et al.* 1988; Johnston *et al.* 1992; Dawson-Hughes, 1996). Välimäki *et al.* (1994) reported that dietary Ca intake in childhood and adolescence was positively related to bone mineral density in young women. A meta-analysis of thirty-three studies concluded that there was an overall association between Ca intake and bone mass in premenopausal women (Welten *et al.* 1995). There is considerable evidence that increasing Ca intake above that usually consumed in the diet may have benefits for the development and maintenance of bone, and may reduce the risk of osteoporosis in later life (Flynn & Cashman, 1999). The findings of many of these controlled Ca intervention trials have been reviewed (Dawson-Hughes, 1991; Institute of Medicine, 1997; Prentice, 1997; Department of Health, 1998).

A number of studies of Ca supplementation in children and adolescents, typically of one to two years duration, have shown that increased Ca intake is associated with a higher rate of accrual of bone mass (as measured by BMC or BMD) of approximately 1–5 %, depending on the skeletal site (Johnston *et al.* 1992; Lloyd *et al.* 1993; Andon *et al.* 1994; Lee *et al.* 1994; 1996; Bonjour *et al.* 1997; Cadogan *et al.* 1997; Dibba *et al.* 1998; 1999). There is strong consistency in the results of these studies despite the differences in ages of subjects, forms of Ca used (e.g. as supplements, dairy products or Ca enriched foods) and in habitual Ca intake. There is still considerable debate on the meaning of these effects of Ca on bone. For example, some researchers argue that the increase in bone mass is due to a decrease in bone turnover and is transient and reversible (Department of Health, 1998). In the absence of longitudinal studies of sufficient duration it is not clear whether additional Ca consumed throughout early life results in increased PBM in adulthood. This question is of great significance since PBM in adulthood is predictive of bone mass, and therefore osteoporosis risk, in later life (Hansen *et al.* 1991).

Studies of Ca supplementation in postmenopausal women, typically of one to two years duration, have shown that Ca cannot prevent bone loss but can reduce the rate of bone loss to some extent. These studies reveal that the effectiveness of Ca varies by skeletal site, by menopausal age, and with usual Ca intakes of the study subjects (Institute of Medicine, 1997). For example, supplementation studies indicate that an increase in Ca intake for women during the first 5 years of menopause (the period of most rapid bone loss) is not effective in retarding bone loss from trabecular regions of the skeleton, including those most vulnerable to osteoporotic fracture (Riis *et al.* 1987; Dawson-Hughes *et al.* 1990; Elders *et al.* 1994). However, reductions in cortical bone loss due to Ca supplementation are observed during this period (Polley *et al.* 1987; Riis *et al.* 1987; Smith *et al.* 1989; Dawson-Hughes *et al.* 1990; Elders *et al.* 1994).

Women who are more than 5 years past menopause tend to be more responsive to supplemental Ca (Nelson *et al.* 1991; Reid *et al.* 1993; Chevalley *et al.* 1994; Prince *et al.* 1995), and those with very low Ca intakes generally gain more from Ca supplementation than do women with higher usual Ca intakes (Dawson-Hughes *et al.* 1990; Elders *et al.* 1994). Trials in women with the highest usual Ca intakes demonstrate that increasing Ca intake above 750 mg (Reid *et al.* 1995), 800 mg (Prince *et al.* 1995), or 1000 mg (Riis *et al.* 1987) reduces loss of bone mineral from cortical-rich sites, such as the proximal radius, femoral neck, and total body. Increases in Ca intake have little effect on spinal-bone mineral in older women (Nelson *et al.* 1991; Chevalley *et al.* 1994; Prince *et al.* 1995).

There is still considerable debate on the significance of the reduction in the rate of bone loss observed in these Ca supplementation studies. A meta-analysis of Ca supplementation trials (Mackerras & Lumley, 1997) confirmed that Ca supplementation reduces bone loss, but the effects were only significant in the first year of supplementation. Although osteoporosis is usually defined in terms of reduced bone mass, it is the end result, i.e. the greater tendency to sustain fractures, which is of major concern. There have been only a few studies on the effect of Ca supplementation on fracture rates in postmenopausal women. A reduction in vertebral fractures with Ca supplementation was observed in two studies in which habitual Ca intakes were low (450–620 mg, Chevalley *et al.* 1994).

Studies of combined supplementation with Ca and vitamin D for 1·5–3 years have shown impressive reductions in hip-fracture incidence in elderly women (mean age 84 years) (Chapuy *et al.* 1992; 1994). More recently, Dawson-Hughes *et al.* (1997) showed that combined supplementation with Ca and vitamin D for 3 years significantly reduced non-vertebral fracture rates in men and women (mean age 71 years). Correction of poor vitamin D status and reduction in serum PTH levels appear to be central to the mechanism of this effect (Prentice, 1997).

The effect of Ca supplementation on bone turnover in the aforementioned studies is due to the increased Ca intake increasing plasma Ca, leading to a suppression of plasma PTH and, consequently, the renal production 1,25 $(OH)_2D_3$. Reduced serum levels of PTH and 1,25 $(OH)_2D_3$ reduce the stimulus for osteoclastic bone resorption (Rubinacci *et al.*1996).

### *Consequences of inadequate calcium intakes*

Because of the small metabolic pool of Ca (less than 0·1 % in the ECF compartment) relative to the large skeletal reserve, for all practical purposes metabolic Ca deficiency probably never exists, at least not as a nutritional disorder. An inadequate intake or poor intestinal absorption of Ca causes the circulating ionised Ca concentration to decline acutely, which triggers an increase in PTH synthesis and release. PTH acts on three target organs to restore the circulating Ca concentration to normal. At the kidney, PTH promotes the reabsorption of Ca in the distal tubule. PTH affects the intestine indirectly by stimulating the production of 1,25 $(OH)_2D_3$, which in turn leads to increased Ca absorption. PTH also induces bone resorption, thereby releasing Ca into blood. Due to the action of PTH and 1,25 $(OH)_2D_3$ on the target tissues, plasma Ca levels are restored within minutes to hours (Cashman & Flynn, 1998; Flynn & Cashman, 1999).

If, on the other hand, there is a chronic Ca deficiency resulting from a continual inadequate intake or poor intestinal absorption of Ca, circulating Ca concentration is maintained largely at the expense of skeletal mass, i.e. from an increased rate of bone resorption. This PTH-mediated increase in bone resorption is one of several important causes of reduced bone mass and osteoporosis (National Research Council, 1989*b*; National Institutes of Health, 1994; Institute of Medicine, 1997). The cumulative effect of Ca depletion on the skeleton over many years contributes to the increasing frequency of osteoporotic fractures with age (Flynn & Cashman, 1999).

### *Calcium requirements and recommendations, and prevalence of calcium deficiency*

Given the high proportion of body Ca which is present in bone and the importance of bone as the major reservoir for Ca, development and maintenance of bone is the major determinant of Ca needs. Thus, unlike other nutrients, the requirement for Ca relates not to the maintenance of the metabolic function of the nutrient but to the maintenance of an optimal reserve and the support of the reserve's function (i.e. providing internal structural rigidity needed for locomotion and gravity resisting activity, Heaney, 1997).

Calcium is stored in skeletal tissue as Ca phosphate crystals embedded in a protein matrix. This composite is laid down as a result of cell-based activity, which, in turn, is determined by the combined effects of genetics and mechanical usage, as well as Ca availability. Calcium is a threshold nutrient, i.e. at sub-optimal intakes the ability of the organism to store Ca as bone tissue is limited by the intake of Ca, but increasing Ca intake above that required as optimal for genetic or mechanical purposes further increases are not stored (Heaney, 1997). Thus, Ca can only be stored as bone and increasing Ca intake beyond that which produces optimal bone mass will not result in more bone.

Calcium requirements vary throughout an individual's life, with greater needs during the periods of rapid growth in childhood and adolescence, during pregnancy and lactation, and in later life. There are important genetic and environmental influences of Ca requirements. Genetic influences include such factors as bone architecture and geometry and responsiveness of bone to hormones which mediate the function of bone as the body's Ca reserve (Heaney, 1997). Environmental influences include factors such as dietary constituents and the degree of mechanical loading imposed on the skeleton in everyday life. Because of their effects on urinary Ca losses, high intakes of both sodium and protein increase dietary Ca requirements (Shortt & Flynn, 1990; Massey & Whiting, 1996; Heaney, 1997).

There is considerable disagreement on human Ca

requirements, and this is reflected in the wide variation in estimates of daily Ca requirements made by different expert authorities. For example, expert committees in the US, UK and EU have established very different recommendations for Ca intake (European Commission, 1993; Institute of Medicine, 1997; Department of Health, 1998), see Table 1. Much of this divergence arises due to different interpretations of available human Ca balance data. The higher recommendations in the US derive from defining Ca requirements based on desirable Ca retention estimated from human Ca balance studies, i.e. that which results in the maximum skeletal Ca reserve (Institute of Medicine, 1997).

Low Ca status as reflected in reduced bone mass appears to be common in western countries. According to recent estimates obtained using WHO diagnostic criteria (based on bone mineral content), approximately 4–6 million older women and 1–2 million older men have osteoporosis in the US (Looker *et al.* 1997). Because life expectancy in western countries is increasing (it will soon average more than 80 years in the US and the EU), it is anticipated that this disease will affect an even larger proportion of the population in future (Melton *et al.* 1992). However, while low bone mass may be taken as an estimate of low Ca status, it should be noted that there are a number of contributory factors to this besides dietary Ca deficiency (e.g. altered hormonal status associated with amenorrhoea or menopause, physical inactivity).

In the absence of reliable indicators of nutritional adequacy for Ca, estimates of Ca deficiency are based largely on adequacy of dietary intake relative to recommendations. However, this approach is complicated by the lack of agreement between expert groups on recommended Ca intakes (Table 1). In practice, estimates of the proportion of the population in different countries with inadequate Ca intake are based on recommended intakes for the individual countries. Using this approach, it has been reported that a significant proportion of some population groups fails to achieve the recommended Ca intakes in a number of western countries.

For example, about half of adult women and one third of adult men in Germany are consuming Ca intakes lower than recommended (Heseker *et al.* 1992; van Dokkum, 1995). Similarly, in Switzerland, a large proportion of adult women fail to achieve the recommended Ca intake (van Dokkum, 1995). In Ireland, over 50 % of females aged 12 to 18 years fail to achieve the recommended Ca intake (Irish Nutrition and Dietetic Institute, 1990). In Italy, 50 % of elderly subjects (> 60 years) do not meet the recommended allowance for Ca (van Dokkum, 1995). In the Netherlands, a significant proportion (8–25 %) of adult males and females fail to achieve even 80 % of the recommended allowance for Ca (van Dokkum, 1995). In the US, most females aged 9 to 18 years and 31 years onwards fail to achieve the recommended Ca intake (Cleveland *et al.* 1996). Even in countries where recommended intakes are relatively low inadequate intakes have been reported in some population subgroups. For example, for females in the UK 13–18 % of 14–34-year-olds and 8–15 % of those over 65 years have habitual Ca intakes less than the lower reference nutrient intake, a level below which intake is almost certainly deficient (Department of Health, 1998).

It should be noted that estimates of Ca intakes from foods might provide an underestimate of Ca intake due to under-reporting of food intakes in self-reported food consumption surveys. Furthermore, many surveys do not include the contribution of supplements, medicines or drinking water to total Ca intakes. Such contributions may be significant for some people.

**Table 1.** Recommended Ca intakes in the EU, UK and US

| EU PRI (1993)* | | UK RNI (1998)† | | US AI (1997)‡ | |
|---|---|---|---|---|---|
| Age group (years) | mg/d | Age group (years) | mg/d | Age group (years) | mg/d |
| 0·5–1 | 400 | 0–1 | 525 | 0–0·5 | 210 |
| 1–3 | 400 | 1–3 | 350 | 0·5–1 | 270 |
| 4–6 | 450 | 4–6 | 450 | 1–3 | 500 |
| 7–10 | 550 | 7–10 | 550 | 4–8 | 800 |
| 11–14 M | 1000 | 11–14 M | 1000 | 9–13 | 1300 |
| 15–17 M | 1000 | 15–18 M | 1000 | 14–18 | 1300 |
| >18 M | 700 | 11–14 F | 800 | 19–30 | 1000 |
| 11–14 F | 800 | 15–18 F | 800 | 31–50 | 1000 |
| 15–17 F | 800 | 19–50 | 700 | 51–70 | 1200 |
| >18 F | 700 | >50 | 700 | >70 | 1200 |
| | | | | Pregnancy | |
| Pregnancy | 700 | Pregnancy | NI | ≤ 18 | 1300 |
| | | | | 19–50 | 1000 |
| Lactation | 1200 | Lactation | + 550 | Lactation | |
| | | | | ≤ 18 | 1300 |
| | | | | 19–50 | 1000 |

* Population Reference Intakes (PRI) (European Commission, 1993).
† Reference nutrient intake (RNI) (Department of Health, 1998).
‡ Adequate intake (AI) (Institute of Medicine, 1997).
Estimates of Ca requirements refer to males and females unless stated otherwise.
M = requirements for males; F = requirements for females. NI = No increment.

*Improving calcium intakes and calcium bioavailability in the population*

The dietary deficiency of Ca identified in some population groups may be addressed in a number of ways. This includes changing eating behaviour at the population level by increasing the consumption of foods which are naturally rich in Ca (e.g. milk and milk products), Ca fortification of foods consumed by target groups, or increasing Ca intakes from Ca supplements. These may be seen as complementary rather than alternative strategies and each has advantages and disadvantages (Flynn & Cashman, 1999). For example, it is notoriously difficult to achieve changes in the diet of entire populations, and thus persuading individuals to consume more dairy produce represents a considerable challenge. The use of Ca supplements can be effective in increasing Ca intake in individuals who consume them regularly, but it has limited effectiveness at the population level due to the poor compliance with supplement use (Flynn & Cashman, 1999). Calcium-fortified food products could provide additional choices for meeting Ca requirements; however, attention should be paid to the selection of products so that they reach the target groups (i.e. those population groups who have the greatest difficulty in meeting Ca requirements).

Besides the amount of Ca in the diet, the absorption of dietary Ca in foods is also a critical factor in determining the availability of Ca for bone development and maintenance. Thus, there is a need to identify food components and/or functional food ingredients that may positively influence Ca absorption in order to ensure that Ca bioavailability from foods can be optimised (Kennefick & Cashman, 2000).

A number of food constituents have been suggested as potential enhancers of Ca absorption. Individual milk components, such as lactose, lactulose and casein phosphopeptides have attracted considerable attention. Phosphopeptides derived from the intestinal digestion of casein (casein phosphopeptides, CPP) have been proposed as potential enhancers of Ca absorption (Mellander, 1950; Kitts & Yuan, 1973). Berrocal *et al.* (1989) demonstrated that such phosphopeptides have the capacity to chelate Ca and to prevent the precipitation of Ca phosphate salts and suggested that they may help to maintain a high concentration of soluble Ca in the intestinal lumen. There is some evidence that CPP increase Ca absorption in rats, minipigs, and chicks (West, 1991). However, there have been only a few studies in humans examining the effect of CPP on Ca absorption. Hansen *et al.* (1997*a*) found that Ca absorption from a high-phytate-containing bread meal was not significantly influenced by the addition 250 mg of CPP in healthy adults but was significantly reduced by the addition of 1000 mg. The same group also reported that fractional Ca absorption was not affected by CPP addition (1000 or 2000 mg) to a rice-based cereal or from whole-grain cereal, in healthy adults (Hansen *et al.* 1997*b*). Furthermore, Heaney *et al.* (1994) reported that CPP administration was associated with better absorption of co-ingested Ca by postmenopausal women with low basal absorptive performance. Therefore, the significance of these phosphopeptides for Ca absorption in humans remains unclear.

Ziegler & Fomon (1983) showed that Ca absorption in human infants was significantly higher from a soy-based infant formula containing lactose than from a similar formula in which the carbohydrate source was a mixture of starch hydrolysate and sucrose. Enhancement of Ca absorption by lactose has also been shown in rats (Lengemann, 1959; Armbrecht & Wasserman, 1976; Brommage *et al.*, 1993; Suzuki *et al.* 1985). However, studies on the effect of lactose on Ca absorption in human adults generally have failed to demonstrate this effect. Miller, in a review of this area, concluded that it is likely that lactose enhances Ca absorption in human infants and in rats, while, at levels normally present in milk, lactose does not have a significant effect on Ca absorption by healthy adults consuming normal diets (Miller, 1989). Recently, Van den Heuvel *et al.* (1999) reported that a 9-day consumption of lactulose (5 or 10 g/day) increased Ca absorption in postmenopausal women in a dose-responsive manner.

Emerging evidence has shown that certain non-digestible oligosaccharides (NDOs) can improve Ca absorption in adolescents and adults. For example, Coudray *et al.* (1997) fed nine healthy young men a control diet or the same diet supplemented with 40 g/day of either inulin or sugar beet fibre for a period of 26 days (2 days of control diet followed by 14 days of progressive increase in inulin amount and then 12 days at the maximum inulin consumption) and determined apparent Ca absorption. They found that upon inulin ingestion, apparent Ca absorption increased significantly ($P<0{\cdot}01$) from 21·3 % to 33·7 % (an increase of 58 %); ingestion of sugar beet fibre had no effect. In a randomised, double blind, cross-over design study, Van den Heuvel *et al.* (1999) fed twelve healthy male adolescents (aged 14–16 years) either orange juice supplemented with 5 g oligofructose or sucrose (control treatment) three times daily for 9 days, after which time, they measured true fractional Ca absorption by a dual stable isotope technique. An increase of 26 % in true fractional Ca absorption (47·8 % with placebo to 60·1 % with oligofructose) was observed upon ingestion of the daily 15 g supplement of oligofructose. In an earlier study by the same group, a daily supplement of 15 g of oligofructose had no effect on Ca absorption in healthy adult men (Van den Heuvel *et al.* 1998). However, in that study, unlike the latter one, the colonic component of Ca absorption (a putative target for enhancement by NDO) was not included because the urine collection was limited to 24 h after isotope administration. In a recent, randomised, double-blind, crossover design study, twenty-nine young adolescent girls (11–14 years, consuming a relatively high Ca intake, 1500 mg/day) received either 8 g servings of a mixture of inulin + oligofructose or placebo (sucrose) in a Ca-fortified orange juice daily for 3 weeks. True Ca absorption was measured using a dual stable isotope method at the end of each 3-week period (Abrams & Griffin, 2001). A 48 h urine collection was carried out after isotope administration so as to detect any modulatory effect of the mixture of inulin + oligofructose on the colonic component of Ca absorption. Consumption of the mixture of inulin + oligofructose resulted in an 18 %

increase in true fractional Ca absorption and in an absolute increase in Ca absorption of 90 mg/day (Abrams & Griffin, 2001).

The findings of these studies strongly suggest that addition of some NDO to food represent an opportunity for increasing the uptake of Ca present in the diet. However, studies are necessary to prove that the benefits of these ingredients to Ca absorption persist in the longer term and, importantly, that they can be translated into benefits to bone health.

## Conclusion

Adequate and appropriate nutrition is important for all individuals, but not all follow a diet that is optimal for bone health. Calcium is the specific nutrient most important for attaining PBM and for preventing and treating osteoporosis. However, significant proportions of some population groups fail to achieve the recommended Ca intakes in a number of western countries. The challenge remaining for interested groups (including nutritionists, health professionals, and the food industry) is to encourage such individuals to meet their Ca requirements. This task is not an easy one when so few high-Ca foods, except for dairy products, are readily available. Calcium supplements or Ca-fortified foods may be needed by individuals who do not or will not consume Ca-rich foods as recommended in the dietary guidelines of many western countries. In addition, consumption of a functional food, which contains an ingredient (such as lactulose, inulin, oligofructose or both) that may positively influence Ca absorption, will ensure that the Ca bioavailability from foods can be optimised.

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AMERICAN ACADEMY OF PEDIATRICS

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# Calcium Requirements of Infants, Children, and Adolescents

**ABSTRACT. This statement is intended to provide pediatric caregivers with advice about the nutritional needs of calcium of infants, children, and adolescents. It will review the physiology of calcium metabolism and provide a review of the data about the relationship between calcium intake and bone growth and metabolism. In particular, it will focus on the large number of recent studies that have identified a relationship between childhood calcium intake and bone mineralization and the potential relationship of these data to fractures in adolescents and the development of osteoporosis in adulthood. The specific needs of children and adolescents with eating disorders are not considered.**

Approximately 99% of total body calcium is found in the skeleton, with only small amounts found in the plasma and extravascular fluid. Serum calcium exists in 3 fractions: ionized calcium (approximately 50%), protein-bound calcium (approximately 40%), and a small amount of calcium that is complexed, primarily to citrate and phosphate ions. Serum calcium is maintained at a constant level by the actions of several hormones, most notably parathyroid hormone and calcitonin. Calcium absorption is by the passive vitamin D-independent route or by the active vitamin D-dependent route.[1]

Understanding calcium needs for different age groups requires a consideration of the variable physiologic requirements for calcium during development. For example, during the first month of life, the regulatory mechanisms that maintain serum calcium levels may not be entirely adequate in some otherwise healthy infants, and symptomatic hypocalcemia can occur. However, in general, hypocalcemia is uncommon in healthy children and adolescents, and the primary need for dietary calcium is to enhance bone mineral deposition.

Calcium requirements also are affected substantially by genetic variability and other dietary constituents. The interactions of these factors make identification of a single unique number for the calcium "requirement" for all children impossible.[2–4] However, several recent dietary guidelines have considered the data about calcium requirements and recommended calcium intake levels that are calculated to benefit most children (Table 1).[2,3]

In addition to calcium intake, exercise is an important aspect of achieving maximal peak bone mass. There is evidence that childhood and adolescence may represent an important period for achieving long-lasting skeletal benefits from regular exercise.[5] For example, Welten et al[6] showed in a large Dutch cohort of children that regular weight-bearing activity had a greater influence on peak bone mass than dietary calcium.

The recommendations in this statement do not indicate an exclusive course of treatment or serve as a standard of medical care. Variations, taking into account individual circumstances, may be appropriate.



## IDENTIFICATION OF MINERAL REQUIREMENTS DURING CHILDHOOD

### Overview

It is recognized that a very low calcium intake can contribute to the development of rickets in infants and children, especially those consuming very restrictive diets (eg, a macrobiotic diet).[7] There are no reliable data on the lowest calcium intake needed to prevent rickets or on the relationship among ethnicity, vitamin D status, physical activity, and diet in the causation of rickets in children fed low-calcium diets.[8,9]

Recent data support the possibility that a low bone mass may be a contributing factor to some fractures in children. A relationship between the adolescent growth spurt and the risk of fractures has been shown.[10,11] Goulding et al[12] reported lower bone mass at multiple sites in a group of 100 girls aged 3 to 15 years with distal forearm fractures compared with age-matched girls. For girls aged 11 to 15 years in the study by Goulding et al[12] a lower calcium intake was reported for those with fractures compared with the control subjects. Wyshak and Frisch[13] similarly reported that high calcium intakes seem to exert a protective effect against fractures in adolescent boys and girls. They also reported a positive relationship between cola beverage intake and bone fracture. Whether this is attributable to a potential effect of excessive phosphorus in the colas impairing bone mineral status or to the lack of calcium intake related to the substitution of colas for dairy products is uncertain. However, a direct harmful effect of a high phosphorus intake affecting the bone mineral status is unlikely in older children and adults.[2] Further data on the relationship between calcium intake and fractures are needed before the magnitude of increased fracture risk at different calcium intake levels can be assessed. However, it is reasonable to conclude that low calcium intakes may be an important risk factor for fractures in adolescents. This risk may be an issue that adolescents can more readily relate to than a long-term risk of osteoporosis.

Maintaining adequate calcium intake during childhood is necessary for the development of a maximal peak bone mass. Increasing peak bone mass may be

**TABLE 1.** Dietary Calcium Intake (mg/d) Recommendations in the United States[2,3]*

| Age | 1997 NAS[3] | 1994 NIH[2] |
|---|---|---|
| 0 to 6 mo† | 210 | 400 |
| 6 mo to 1 y† | 270 | 600 |
| 1 through 3 y | 500 | 800 |
| 4 through 8 y | 800 | 800 (4–5 y)<br>800–1200 (6–8 y) |
| 9 through 18 y | 1300 | 800–1200 (9–10 y)<br>1200–1500 (11–18 y) |

* Recommended intakes were provided in different forms by each source cited. The Food and Nutrition Board of the National Academy of Sciences (NAS) released Recommended Dietary Allowances until 1997. In 1997, it chose to use the term *adequate intake* for the recommendations for calcium intake but indicated that these values were to be used as Recommended Dietary Allowances. The NIH Consensus Conference did not specify a specific term but indicated that these values were the "optimal" intake levels. Dietary recommendations by the NAS are set to meet the needs of 95% of the identified population of healthy subjects. The NAS guideline should be the primary guideline utilized.
† For infant values, the 1994 NIH Consensus Conference indicated values for formula-fed infants, whereas the 1997 NAS report used the infant fed human milk as the standard.

an important way to reduce the risk of osteoporosis in later adulthood.[2,14] This is a more difficult end point to identify than the development of rickets or fractures. Therefore, surrogate markers of mineral status are used to assess the consequences of differing levels of calcium intake. The primary surrogates used are optimization of calcium balance or achievement of greater bone mass in children with increased calcium intake.[3,14,15]

In children with chronic illnesses, fractures may occur during childhood secondary to mineral deficiency associated with the disease process or the effects of therapeutic interventions (ie, corticosteroids) on calcium metabolism.[16] However, minimal data generally are not available on the risks and benefits of increasing calcium intake in children with chronic illnesses above current dietary recommendations. Supplementation of vitamin D along with calcium may be necessary for a maximal response.[17]

### Methods

Multiple approaches are used to assess mineral requirements in children. They include the following: 1) measurement of calcium balance in persons with various levels of calcium intake; 2) measurement of bone mineral content, by dual-energy radiograph absorptiometry or other techniques, in groups of children before and after calcium supplementation; and 3) epidemiologic studies relating bone mass or fracture risk in adults with childhood calcium intake.

The calcium balance technique consists of measuring the effects of any given calcium intake on the net retention of calcium by the body. This approach has been the most commonly used to estimate requirement for minerals. Its usefulness is based on the rationale that virtually all retained calcium must be used, especially by children, to enhance bone mineralization. It therefore is reasonable to expect that the dietary intake that leads to the greatest level of calcium retention is the intake that will lead to the greatest benefit for promoting skeletal mineralization and decreasing the ultimate risk of osteoporosis.[18,19]

The substantial limitations involved in obtaining and interpreting data about calcium balance are well known. These include substantial technical problems with measuring calcium excretion and the difficulty obtaining dietary intake control in children. Both of these are necessary for adequate balance studies. These problems have been partly overcome by the development of stable isotopic methods to assess calcium absorption and excretion.[20] Nevertheless, more data are needed to establish the "optimal" level of calcium retention at different ages and the effects of development on calcium balance.[6]

A major advance in the field during the last 25 years has been the development and improvement of methods to measure total body and regional bone mineral content by using various bone density techniques. Currently, the technique used in many studies is dual-energy radiograph absorptiometry. This technique can rapidly measure the bone mineral content and bone mineral density of the entire skeleton or of regional sites with a virtually negligible level of radiation exposure. Furthermore, recent enhancements in the precision of the technique have made it particularly suitable for assessing the effects of calcium supplementation on bone mass in children of all ages.[21]

Several groups have directly assessed the effects of calcium supplementation on bone mass by using dual-energy radiograph absorptiometry or similar techniques.[22–25] These studies, however, also have limitations. First, most supplementation studies done in children involved relatively short-term supplementation of 1 to 2 years. This period may be inadequate to fully assess the long-term benefits of calcium supplements on bone mineral density. The second is that these studies generally have been done using only 1 level of supplementation, which frequently has been given in pill form. This limited dosing approach makes it difficult to identify an optimal intake level or determine the relative benefits of dietary calcium versus supplements as a method of increasing calcium intake in children.

Several investigators have performed population-based epidemiologic studies relating childhood or adult bone mass or fracture risk to calcium intake in childhood. Although many of these studies are limited by their retrospective design, they have generally shown a positive association between calcium intake in childhood and childhood and adult bone mass. Not all studies have shown a benefit, however, and further data about this relationship are needed.[3,26–28]

## RECOMMENDATIONS BY AGE GROUP

### Overview

The specific requirements for calcium intake by infants, children, and adolescents have been extensively reviewed by 2 panels in North America since 1994.[2,3] A summary of their recommendations is shown in Table 1.

### Infants

The optimal primary nutritional source during the first year of life is human milk. No available evidence shows that exceeding the amount of calcium retained by the exclusively breastfed term infant during the first 6 months of life or the amount retained by the human milk–fed infant supplemented with solid foods during the second 6 months of life is beneficial to achieving long-term increases in bone mineralization. Available data demonstrate that the bioavailability of calcium from human milk is greater than that from infant formulas or cow's milk, although this comparison has not generally been made at comparable intake concentrations, ie, such as found in human milk.[29] Nevertheless, it has been deemed prudent to increase the concentration of calcium in all infant formulas relative to human milk to ensure at least comparable levels of calcium retention. Relatively greater calcium concentrations are found in specialized formulas, such as soy formulas and casein hydrolysates, to account for the potential lower bioavailability of the calcium from these formulas relative to cow's milk-based formula. Specific concentration requirements cannot be set readily, but all formulas marketed should have demonstrated a net calcium retention at least comparable to that of human milk. Research data are not available to justify the use of very high levels of calcium in infant formula for full-term infants.

Premature infants have higher calcium requirements than full-term infants while in the nursery. These may be met by using human milk fortified with additional minerals or with specially designed formulas for premature infants.[30] After hospitalization, there may be benefits to providing formula-fed premature infants formulas with higher calcium concentrations than those of routine cow's milk–based formulas.[31] The optimal concentrations and length of time needed for such formulas are unknown.

### Children

Few data are available about the calcium requirements of children before puberty. Calcium retention is relatively low in toddlers and slowly increases as puberty approaches. Most available data indicate that calcium intake levels of about 800 mg/d are associated with adequate bone mineral accumulation in prepubertal children. The benefits of greater levels of intake in this age group have been studied inadequately.[20,32] One study found a benefit of calcium supplements to children as young as 6 years of age.[16] However, further supporting data are needed for this finding. Perhaps of most importance in this age group is the development of eating patterns that will be associated with adequate calcium intake later in life.

### Preadolescents and Adolescents

The majority of research in children about calcium requirements has been directed toward 9- to 18-year-olds. The efficiency of calcium absorption is increased during puberty, and the majority of bone formation occurs during this period.[15,20,21,32,33] Data from balance studies suggest that for most healthy children in this age range, the maximal net calcium balance (plateau) is achieved with intakes between 1200 and 1500 mg/d. That is, at intake levels above this, almost all of the additional calcium is excreted and not used. At intakes below that level, the skeleton may not receive as much calcium as it can use, and peak bone mass may not be achieved.[2,3,9,15,18–20] Virtually all the data used to establish this intake level are from white children; minimal data are available for other ethnic groups. The exact level that is best for a given person depends on other nutrients in the diet, genetics, exercise, and other factors.

Several controlled trials have found an increase in the bone mineral content in children in this age group who have received calcium supplementation.[22–25] However, the available data suggest that if calcium is supplemented only for relatively short periods (ie, 1 to 2 years), there may not be long-term benefits to establishing and maintaining a maximum peak bone mass.[34,35] This emphasizes the importance of diet in achieving adequate calcium intake and in establishing dietary patterns consistent with a calcium intake near recommended levels throughout childhood and adolescence. Unfortunately, long-term studies evaluating the consequences of maintaining currently recommended calcium intakes beginning in childhood or early adolescence are not available. Most available epidemiologic data, recently reviewed by the National Academy of Sciences and the National Institutes of Health, support the view that maintaining such a diet will increase peak bone mass and lower the incidence of fractures.[2,3]

Recent data obtained in African American adolescents suggest a link between lower diastolic blood pressure and increased calcium intake. Further studies are necessary to evaluate this relationship in children of multiple ethnicities and age groups.[36]

## ACHIEVING RECOMMENDED INTAKES

The gap between the recommended calcium intakes and the typical intakes of children, especially those 9 to 18 years of age, is substantial (Table 1). Mean intakes in this age group are between approximately 700 and 1000 mg/d, with values at the higher side of this range occurring in males.[3] Preoccupation with being thin is common in this age group, especially among females, as is the misconception that all dairy foods are fattening. Many children and adolescents are unaware that low-fat milk contains at least as much calcium as whole milk.

Knowledge of dietary calcium sources is a first step toward increasing the intake of calcium-rich foods. Table 2 gives typical amounts of calcium for some common food sources. The largest source of dietary calcium for most persons is milk and other dairy products.[37] Other sources of calcium are, however, important, especially for achieving calcium intakes of 1200 to 1500 mg/d. Most vegetables contain calcium, although at low density. Therefore, relatively large servings are needed to equal the total intake achieved with typical servings of dairy products. The bioavailability of calcium from vegetables

**TABLE 2.** Approximate Calcium Contents of 1 Serving of Some Common Foods*

| Food | Serving Size | | Calcium Content |
|---|---|---|---|
| Milk† | 1 cup | 240 mL | 300 mg |
| White beans | ½ cup | 110 g | 113 mg |
| Broccoli cooked | ½ cup | 71 g | 35 mg |
| Broccoli raw | 1 cup | 71 g | 35 mg |
| Cheddar cheese | 1.5 oz | 42 g | 300 mg |
| Low-fat yogurt | 8 oz | 240 g | 300–415 mg |
| Spinach cooked‡ | ½ cup | 90 g | 120 mg |
| Spinach raw‡ | 1½ cup | 90 g | 120 mg |
| Calcium-fortified orange juice | 1 cup | 240 mL | 300 mg |
| Orange | 1 medium | 1 medium | 50 mg |
| Sardines or salmon with bones | 20 sardines | 240 g | 50 mg |
| Sweet potatoes | ½ cup mashed | 160 | 44 |

* Adapted from Raper et al,[37] Weaver,[38,39] and Weaver and Plawecki.[40]
† Low-fat milk has comparable or greater calcium levels than whole milk.
‡ The calcium from spinach is essentially nonbioavailable.

is generally high. An exception is spinach, which is high in oxalate, making the calcium virtually nonbioavailable. Some high-phytate foods, such as whole bran cereals, also may have poorly bioavailable calcium.[38–40]

Several products have been introduced that are fortified with calcium. These products, most notably orange juice, are fortified to achieve a calcium concentration similar to that of milk. Limited studies of the bioavailability of the calcium in these products suggest that it is at least comparable to that of milk.[41] It is likely that more such products will soon become available. Breakfast foods also are frequently fortified with minerals, including calcium. Calcium intakes on food labels are indicated as a percentage of the "daily value" in each serving. This daily value is currently set as 1000 mg/d. Therefore, it is important to instruct families about reading and interpreting food labels.

Several alternatives exist for children with lactose intolerance. Lactose intolerance is more common in African American, Mexican Americans, and Asian Pacific Islanders than in whites.[42] Many children with lactose intolerance can drink small amounts of milk without discomfort. Other alternatives include the use of other dairy products, such as solid cheeses and yogurt, that may be better tolerated than milk. Lactose-free and low-lactose milks are available. Increasing the intake of nondairy products, such as vegetables, may be helpful, as may the use of calcium-supplemented foods.

For children and adolescents who cannot or will not consume adequate amounts of calcium from any dietary sources, the use of mineral supplements should be considered. Although supplements vary in their bioavailability, they may have bioavailability comparable to or greater than that of dairy products.[43] Decisions about their use must be made on an individual basis, keeping in mind the usual dietary habits of the person, any individual risk factors for osteoporosis, and the likelihood that the use of the supplement will be maintained.

## CONCLUSION

Recent studies and dietary recommendations have emphasized the importance of adequate calcium nutriture in children, especially those undergoing the rapid growth and bone mineralization associated with pubertal development. The current dietary intake of calcium by children and adolescents is well below the recommended optimal levels. The available data support recent recommendations for calcium intakes of 1200 to 1500 mg/d beginning during the preteen years and continuing throughout adolescence as recommended by the National Institutes of Health Consensus Conference[2] and the National Academy of Sciences.[3] Currently, evidence is inadequate to alter the dietary recommendations for children with chronic illnesses or those taking medications, such as corticosteroids, that alter bone metabolism. However, an effort should be made to achieve at least the recommended intake levels. The provision of adequate vitamin D also may be important for children with chronic illnesses.

## RECOMMENDATIONS

1. Pediatricians should actively support the goal of achieving calcium intakes in children and adolescents comparable to those in recently recommended guidelines.[2,3] The prevention of future osteoporosis, as well as the possibility of a decreased risk of childhood and adolescent fractures, should be discussed as potential benefits to achieving these goals. Currently, relatively few children and adolescents achieve dietary calcium intake goals.
2. To emphasize the importance of calcium nutriture, pediatricians should consider including the following questions about dietary calcium intake.
   - What do you drink, either white or chocolate milk, with your meals?
   - Do you drink milk with meals, snacks, or cereal or any other time during the day?
   - Do you eat cheese, yogurt, or other dairy products such as cottage cheese?
   - Do you drink calcium-fortified juices or eat any calcium-fortified foods?
   - Do you eat any of the following: broccoli, tofu, oranges, or legumes (dried beans and peas)?
   - Do you take any mineral or vitamin supplements?
3. For children and adolescents whose calcium intake seems deficient, specific information about the sources of dietary calcium should be pro-

vided. Adolescents may need to be reminded that low-fat dairy products, including skim milk and low-fat yogurts, are good sources of calcium that are not high in fat.



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# Appendix 6

## Petition to add to the National List the substance "Oligofructose enriched with Inulin Documented for Calcium Absorption"

**January 12, 2007**

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ORAFTI



**BENEO® Synergy 1**

Material Safety Data Sheet Date Prepared: 10/11/01

## CHEMICAL PRODUCT AND COMPANY DESCRIPTION

ORAFTI
Food Ingredients
101 Lindenwood Drive
Malvern, PA 19355

**For Information Call:**
(610) 889-9828

**Product Status:**
FDA regulated use only.

**Chemical Name or Synonym:**
Inulin Powder

## COMPOSITION/ INFORMATION ON INGREDIENTS

| *Component* | *CAS Reg Number* | *OSHA Hazard* | *Percentage* |
|---|---|---|---|
| Fructose Chains | 9005-80-5 | N | 90 – 94 |
| Sucrose | 57-50-1 | Y | 2 - 4 |
| Glucose | 50-99-7 | N | 0 – 6 |
| Fructose | 57-48-7 | N | 0 – 6 |

## HAZARDS IDENTIFICATION

*A. Emergency Overview:*

Physical Appearance and odor: White fine powder solid, odorless.

Warning Statements: Dusts in high concentration may cause skin, eye and respiratory tract irritation.

B. *Potential Health Effects:*

Acute Eye: Non-irritating
Acute Skin: Non-irritating
Acute Inhalation: Low acute inhalation toxicity. Dusts may cause upper respiratory tract irritation.
Acute Ingestion: Low acute oral toxicity.
Chronic Effects: This product does not contain any ingredient designated by IARC, NTP, ACGIH or OSHA as probable or suspected human carcinogens.

## FIRST AID MEASURES

*First Aid measures for accidental:*

| | |
|---|---|
| Eye exposure: | Hold eyelids open and flush with a steady, gentle stream of water for at least 15 minutes. Seek medical attention if irritation develops or persists or if visual changes occur. |
| Skin exposure: | In case of contact, wash with plenty of soap and water. Seek medical attention if irritation develops or persists. |
| Inhalations: | If respiratory irritations or distress occurs, remove victim to fresh air. Seek medical attention if respiratory irritation or distress continues. |
| Ingestion: | No harmful affects expected. If appreciable quantities are swallowed, call a physician or poison control center. Do not leave victim unattended. |

*Medical conditions possibly aggravated by exposure:*
No specific information found.

Notes to Physician:
All treatments should be based on observed signs and symptoms of distress in the patient. Consideration should be given to the possibility that overexposure to materials other than this product may have occurred. Treat symptomatically. No special antidote available.

## FIRE FIGHTING MEASURES

| | | |
|---|---|---|
| Flash Point: | 93°C (200°F). Flammability Class: will burn | |
| Method used: | Closed cup | |
| Flammability limits(vol/vol%) | Lower: | Upper: |
| | No data | No data |
| Extinguishing Media: | Recommended: water fog, carbon dioxide. | |
| Special fire fighting procedures: | Firefighters should wear NIOSH/MSHA approved self-contained breathing apparatus and full protective clothing. Cool containers exposed to fire with water. | |
| Unusual fire and explosion hazards: | Product will burn under fire conditions. | |
| Hazardous Decomposition materials (Under fire conditions): | Oxides of carbon. | |

## ACCIDENTAL RELEASE MEASURES

| | |
|---|---|
| Evacuation procedures and safety: | Ventilate closed spaces before entering. |
| Containment of Spill: | Follow procedure described below under cleanup and disposal of spill. |
| Cleanup and Disposal of Spill: | Absorb with vermiculite or other inert absorbent. Scrape up and place in appropriate closed container (see **Handling and Storage**). Pump any free liquid into an appropriate closed container. (See |

**Handling and Storage**). Clean up residual material by washing area with water. Collect washings for disposal.

Environmental and regulatory reporting:

Do not flush to drain. If spilled on the ground, the affected area should be scraped clean and placed in an appropriate container for disposal.

## HANDLING AND STORAGE

Min/ Max. storage temperatures: Not Available.

Handling: Keep containers closed when not being used.

THIS PRODUCT MAY PRESENT A DUST - EXPLOSION HAZARD.
It is recommended that all dust control equipment and material transport systems involved in handling of this product contain explosion relief vents or explosion suppression system or an oxygen deficient environment. In addition, all conductive elements of the system that contact this material should be electrically bonded and grounded. This powder should not be flowed through non-conductive ducts or pipes. Use only appropriately classed electrical equipment.

Storage: Store in an area that is dry, sanitary, cool. Store in tightly closed containers.

## EXPOSURE CONTROL/PERSONAL PROTECTION

Introductory remarks:

These recommendations provide general guidance for handling this product. Because specific work environments and material handling practices vary, safety procedures should be developed for each intended application. While developing safe handling procedures, do no overlook the need to clean equipment and piping systems for maintenance and repairs. Waste resulting from these procedures should be handled in accordance with **DISPOSAL CONSIDERATIONS.**

Assistance with selection, use and maintenance or worker protection equipment is generally available from equipment manufacturers.

Exposure Guidelines: Exposure limits represent regulated or recommended worker breathing zone concentrations measured by validated sampling and analytical methods, meeting the regulatory requirements. The following limits apply to this material, where, if indicated, S = skin and C = ceiling limit:

Sucrose

| | Notes | TWA | STEL |
|---|---|---|---|
| ACGIH | | 10 mg/cu m | |
| OSHA | | 5 mg/cu m | |
| OSHA | | 15 mg/cu m | |

| | |
|---|---|
| Engineering controls: | Where engineering controls are indicated by use conditions or a potential for excessive exposure exists, the following traditional exposure control techniques may be used to effectively minimize employee exposures. |
| Respiratory Protection: | When respirators are required, select NIOSH/MSHA approved equipment based on actual or potential airborne concentrations and in accordance with the appropriate regulatory standards an/or industrial recommendations. Under normal conditions, in the absence of other airborne contaminants, the following devices should provide protection from this material up to the conditions specified by the appropriate OSHA, WHMIS or ANSI standard(s): dust/mist filtering respirator. |
| Eye/Face Protection: | Eye and face protection requirements will vary dependent upon work environment conditions and material handling practices. Appropriate ANSI Z87 approved equipment should be selected for the particular use intended for this material. It is generally regarded as good practice to wear a minimum of safety glasses with side shields when working in industrial environments. |
| Skin protection: | Skin contact should be minimized through use of gloves and suitable long-sleeved clothing (i.e., shirts and pants). Consideration must be given both to durability as well as permeation resistance. |
| Work Practice Controls: | Personal hygiene is an important work practice exposure control measure and the following general measures should be taken when working with or handling this material: |

1. Do not store, use, and/or consume foods, beverage, tobacco products, or cosmetics in areas where this material is stored.
2. Wash hands and face carefully before eating, drinking, using tobacco, applying cosmetics, or using the toilet.
3. Wash exposed skin promptly to remove accidental splashes of contact with this material.

## PHYSICAL AND CHEMICAL PROPERTIES

Physical and chemical properties here represent typical properties of this product.

| | |
|---|---|
| Physical Appearance: | white fine powder solid |
| Odor: | odorless |
| pH: | 5.0 to 7.0 at 10 wt/wt% |
| Specific gravity: | not available |

| | |
|---|---|
| Water solubility: | soluble |
| Melting point range: | not available |
| Boiling point range: | not available |
| Vapor pressure: | not available |
| Vapor density: | not available |

## STABILITY AND REACTIVITY

| | |
|---|---|
| Chemical stability: | This material is stable under normal handling and storage conditions described in section **HANDLING AND STORAGE**. |

| | |
|---|---|
| Conditions to be avoided: | none known |
| Materials/Chemicals to be avoided: | strong oxidizing agents |

The following hazardous decomposition products might be expected:
Decomposition: thermal
none known
Hazardous polymerization with not occur. Avoid the following to inhibit hazardous polymerization: not applicable

## TOXICOLOGICAL INFORMATION

| | |
|---|---|
| Acute Eye Irritation: | no test data found for product |
| Acute Skin Irritation: | no test data found for product |
| Acute dermal toxicity: | no test data found for product |
| Acute oral toxicity: | no test data found for product |
| Chronic toxicity: | This product does not contain any substances that are considered by OSHA, NTP, IARC or ACHIG to be "probable" or suspected: human carcinogens. |

No additional test data found for product.

## ECOLOGICAL INFORMATION

| | |
|---|---|
| Ecotoxicological information: | no data found for product |
| Chemical fate information: | no data found for product |

## DISPOSAL CONSIDERATIONS

| | |
|---|---|
| Waste disposal method: | Chemical additions, processing or otherwise altering this material may make the waste management information presented in this MSDS incomplete, inaccurate or otherwise inappropriate. Please be advised that state and local requirements for waste disposal may be more restrictive or otherwise different from federal laws and regulations. Consult state and local regulations regarding the proper disposal of this material. |
| Chemical handling and disposal: | DO NOT REUSE CONTAINERS. Rinse containers before disposal. |

EPA Hazardous Waste – NO



## TRANSPORTATION INFORMATION

| | |
|---|---|
| Transportation status: | The listed Transportation classification does not address regulatory variations due to changes in package size, mode of shipment or other regulatory descriptors. |

US Department of Transportation Shipping name:
Not regulated

## REGULATORY INFORMATION

Federal regulations:

TSCA Inventory Status: This product is excluded from TSCA

## TRANSPORTATION INFORMATION

Transportation status: The listed Transportation classification does not address regulatory variations due to changes in package size, mode of shipment or other regulatory descriptors.

US Department of Transportation Shipping name:
Not regulated

## REGULATORY INFORMATION

Federal regulations:

TSCA Inventory Status: This product is excluded from TSCA because it is solely for FDA regulated use.

SARA Title III Hazard Classes:

| | |
|---|---|
| Fire Hazard: | No |
| Reactive Hazard: | No |
| Release of pressure: | No |
| Acute health hazard: | No |
| Chronic health hazard: | No |

State regulations: This product does not contain any components that are regulated under California Proposition 65.

## OTHER INFORMATION

National Fire Protection Association Hazard Ratings – NFPA (R):
0 Health hazard rating – minimal
1 Flammability rating – slight
0 Reactivity rating – minimal

National Paint & Coating Hazardous Materials Identification System – HMIS (R):
0 Health hazard rating – minimal
1 Flammability rating – slight
0 Reactivity rating – minimal

Key legend information:
ACGIH – American Conference. of Governmental Industrial Hygienists
OSHA – Occupational Safety and Health Administration
TLV – Threshold Limit value
PEL – Permissible exposure limit
TWA – Time weighted average
STEL – Short term exposure limit
NTP – National Toxicology program
IARC – International Agency for Research on Cancer
ND – not determined

Disclaimer: The information herein is given in good faith but no warranty, expressed or implied, is made.

# Appendix 7

## Petition to add to the National List the substance "Oligofructose enriched with Inulin Documented for Calcium Absorption"

**January 12, 2007**

# Organic/ Biological/ Eco Statement

**DOC A8-06*06/05**

Inulin and Oligofructose cannot be produced 'organically' economically on an industrial scale.

ORAFTI does not produce these ingredients 'organically'.

European Regulation CEE2092/91 allows to add non-organic ingredients to foods up to 5%, and still call these foods 'organic'. The only requirement is, that the ingredients are listed in annex VI.C. of this regulation.

Neither inulin nor oligofructose are part of Annex VI.C. of European Regulation CEE2092/91.

A request for this was sent to the Belgian Authorities in 1997. In this request, the CEFI (European Confederation of Inulin Producers) confirms that none of its members can produce organic inulin (Annex 1).

It might take quite some time before this request will be treated or accepted.

In the mean time, customers can obtain a temporary permission from their local authorities. This is based on EC Regulation 207/93 as adapted by Regulation 345/97.

Tienen, June 2005

Paul Coussement
CEO

Annex : CEFI request



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Annex1

C
E
F
I

# Comité Européen des Fabricants d'Inuline

MINISTERE DE L'AGRICULTURE
DG4/Qualité et protection des Végéteux
A l'attention de Mr Christian PAPRIANS

WTC 3 Bd Simon Bolivar 30

1000 BRUXELLES



Warcoing, December 22th, 1997 CEFI9738

Dear Mr. PAPRIANS,

CONCERNING : use of inulin and oligofructose in "Biological" products

Hereby we would like to ask to

**to add "inulin" and "oligofructose" in list C of annex VI of Regulation CEE2092/91.**

The reasons and background information for this request are summarised in the following points :

1. **Inulin and Oligofructose**

Inulin and oligofructose are obtained from chicory roots (cichorium intybus) by extraction and eventually partial enzymatic hydrolysis. Their properties and applications are summarised in the brochures in Annex 1 and 2.
Both products are authorised for food use in Europe. They are classified as "food ingredients", not "food additives". They can be labelled as "dietary fibre". They are analysed with AOAC Method n° 997.08 (Annex 3).

.../...

Association internationale à but scientifique (Loi 25 mai 1919)
Siège social Avenue de Tervueren 182, 1150 Bruxelles, Belgique
Présidence rue de la Sucrerie 1, 7740 Warcoing, Belgique - Tél 069/55.32.22
Fax 069/55.81.37

## 2. Chicory Roots and Production

The roots that are used for the production of inulin and oligofructose are from the same type as that used for the production of coffee chicory. They are planted mainly in Belgium and the Netherlands. No GMO-derived plants are used for the moment, or will be in the near future. Although the chicory root can be cultivated in a "Biological" way, the quantities that would be needed for an economically feasible production are too high to allow the production of "biological" inulin or oligofructose. This is explained in point 3.



The production processes used for inulin and oligofructose are based on :
- hot water extraction
  (using only anti-foaming agents commonly used and allowed in the sugar industry)
- purification by precipitation processes used in the sugar industry, ion exchange, absorption, ultrafiltration
- spray-drying
- enzymatic hydrolysis for oligofructose ; using an inulinase enzyme derived from A. niger this enzyme is food grade.

During these processes, some slight pH control may be done using regular acids and bases, with the main aim to prohibit the chemical breakdown of inulin or oligofructose.
See also : Annex 4.

## 3. Production Site and Capacities

At the moment, there are only three production sites for inulin : one in the Netherlands (operated by SENSUS in Roosendaal) and two in Belgium (operated by WARCOING INDUSTRIE in Warcoing and by ORAFTI in Oreye).
These three facilities operate at a high production capacity : a few thousand of tons of chicory roots are extracted every day in each unit.
In total, a yearly amount of 700.000 tons of chicory roots will be extracted in 1997.
A large part of this chicory volume is transformed into fructose syrups. The use of inulin and oligofructose in Europe has reached several thousands of tons.

.../...



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For the economical production of biological inulin or oligofructose, it would be necessary to stop the production, clean the systems, run on biological roots for a few days and separate the production. This would be almost impossible due to :
- too high turnover costs because the factories need to stop activities during at least one day;
- too high volumes of chicory roots that would be required to operate the factory for a few days (approx. 10.000 tons for each run).



**4. Need for inulin and oligofructose in "Biological" products**

Both inulin and oligofructose have some exceptional technical and nutritional properties (See Annex I and 2), such as
- both are dietary fibres with excellent taste and technical advantages such as : high solubility and good effect on texture. Oligofructose is the most soluble dietary fibre in the world.



- both are unique products because of their "bifidogenic" properties; no other products have this property for the moment.
- inulin is a powerful fat mimic for which there is no alternative.
- oligofructose has excellent sugar-replacing properties and combines well with fruits.

These properties appear typically at dosages below 5 % in foods products.
Several customers in Europe have declared their wish to use inulin or oligofructose in "Biological Products" (up to a maximum of 5 %).
Hence the need to add these products to list C of annex VI of Regulation CEE2092/91.
The applications for inulin and oligofructose are in a very wide range of sectors : a.o.
- dairy products
- bakery products
- chocolate products
- meat products

This request is sent to you by the CEFI, the "Confederation des Fabricants d'Inuline", which represents all the active producers of inulin. The request is signed by its president.

.../...



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