

Clinical Validation of

CALCIUM

Includes Scientific Papers, Research Papers,
University Studies & Articles

Clinical Documentation for Calcium

The World's First L (+) Lactic Acid Probiotic Activated Liquid Calcium
w/Puracal[®] & Greens

Puracal[®]

Puracal[®] is the world's first L (+) Lactic Acid Probiotic Activated Calcium Lactate. It is unique in that it is soluble in both an acid or neutral environment and has an extremely high bioavailability of 45% - (That's approximately 17% - 20% greater bioavailability than the competitive calcium supplement like: Calcium Citrate & Calcium Carbonate).

- High Bioavailability – 45% Absorption Rate
- 100% Vegetarian
- 100% Dairy & Lactose Free

Puracal[®] Manufacturing Process

Puracal[®] is created through a Proprietary Process in which 350 Million Year Old Devonian Age Purified Lime Stone is Liquefied and combined with L (+) Lactic Acid derived from Proprietary Strains of Probiotic Bacteria which yields the Purest, Most Soluble & Most Bioavailable form of Calcium in the Industry. It is interesting to note that the L (+) Lactic Acid Bacteria used in the Proprietary Manufacturing Process is not derived from Animal, Dairy Sources or even "Human Strains" and is completely 100% Vegetarian.

Clinical Studies

PURACAL[®]

Study #1

Availability for absorption of calcium from four calcium supplements during passage through an in vitro gastrointestinal model (TIM-1)

Research by: TNO Nutrition and Food Research Institute, Zeist, The Netherlands, 2003

Objective

- Determine the availability for absorption of calcium from four different supplements (calcium L-lactate, calcium lactate gluconate, calcium carbonate, calcium citrate) during passage through the dynamic, computer controlled model of the stomach and small intestine.
- Simulating two GI conditions (fast and slow pH gastric curve), using calcium supplement intake with either a glass of water or a meal, and determination of its effect on availability of calcium for absorption.
- Determine the effect of addition level of calcium on the bioaccessibility of calcium from calcium L-lactate and calcium citrate.

Design

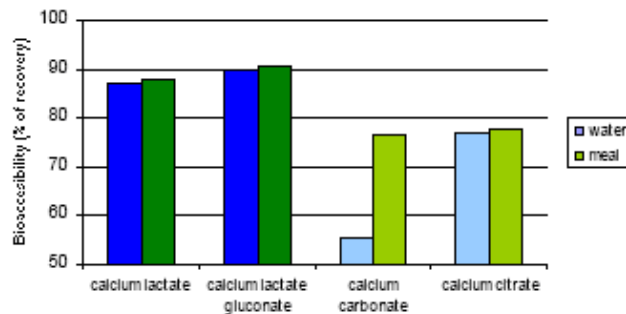
- Four supplements were being tested: calcium L-lactate, calcium lactate gluconate, calcium carbonate and calcium citrate

- The study was performed in the TNO dynamic, multi-compartmental model system of the stomach and small intestine (TIM-1). This model simulates very closely the successive dynamic conditions in the gastric-small-intestinal tract, such as body temperature, pH curves, electrolyte concentrations, and the enzyme activity in the stomach and small intestine.

Results

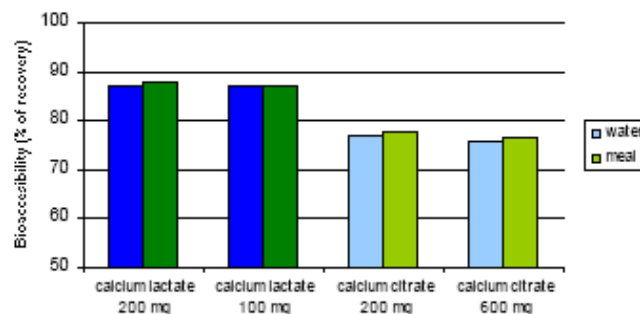
- Order of decreasing calcium availability for absorption from different calcium supplements was: **ca lactate gluconate** > **ca lactate** > **ca citrate** > **ca carbonate**

Figure 1: Availability for absorption of calcium for the four tested calcium sources with two different conditions (intake with a glass of water or a meal) in TIM-1



- Availability of calcium was not influenced by GI conditions (calcium supplement intake with a glass of water or a meal), with the exception of calcium carbonate. Calcium from the calcium carbonate supplement was significantly less available for absorption when consumed with a glass of water as compared to intake with a meal.

Figure 2: Availability for absorption of calcium for calcium L-lactate (addition level 200 mg calcium and 100 mg calcium) and calcium citrate (addition level 200 mg calcium and 600 mg calcium) with two different conditions (intake with a glass of water or a meal) in TIM-1



- Calcium bioaccessibility is independent from the calcium level (within the limits tested in these experiments).

Study #2

Calcium absorption from calcium L-lactate and calcium lactate gluconate and other calcium sources in postmenopausal women.

Research by: TNO Nutrition and Food Research Institute, Zeist, The Netherlands, 1991

Objective

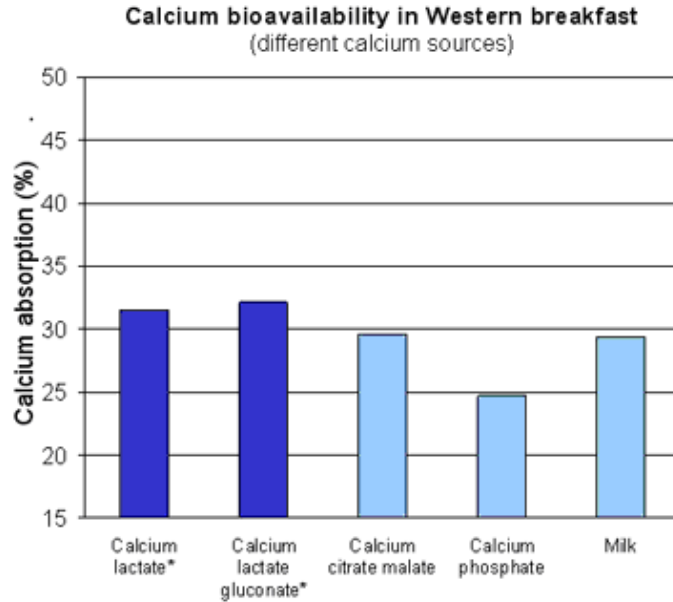
- Compare the absorption of calcium from calcium L-lactate (PURACAL[®] PP) and calcium lactate gluconate (PURACAL[®] XPro) with absorption from milk, calcium carbonate, calcium citrate malate and calcium phosphate.
- Determine the effect of dietary background composition, by means of a Western or Asian breakfast, on calcium absorption from PURACAL[®] PP.

Design

- Ten postmenopausal women participated in the study.
- Each calcium source was administered with a Western breakfast.
- In addition, calcium L-lactate was given with an Asian breakfast as well as without any breakfast.
- Calcium sources provided 200 mg of calcium. The Asian breakfast provided 50.2 mg and the Western breakfast provided 60.2 mg.

Results

- PURACAL[®] calcium L-lactate and calcium lactate gluconate have shown to be highly bioavailable calcium sources from which calcium is significantly better absorbed when compared to tri-calcium-di-phosphate.
- Calcium from PURACAL[®] calcium L-lactate and calcium lactate gluconate is as bioavailable as calcium from milk.
- Calcium from PURACAL[®] PP is well absorbed when consumed with a Western or an Asian breakfast and is even better absorbed when taken without food.



* calcium sources supplied by PURAC

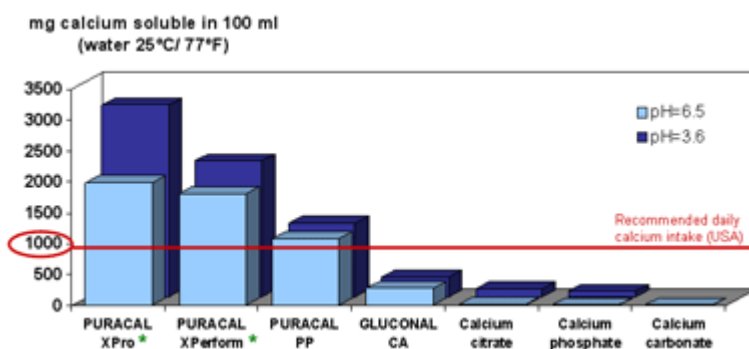
Bioavailability

Note: By definition, bioavailability is the fraction of the ingested nutrient that is absorbed and utilized for normal physiological functions or storage in the human body. The determination of bioavailability is very difficult as there is no specific marker for utilization. A critical step is the absorption into the blood. Once absorbed, the origin of the calcium source does not matter any more, differences only show up because of individual differences (genetic, hormonal etc.) and impact of diet. The food matrix and type of calcium have significant influence on the actual amount that is absorbed. Fortification of food and mineral supplementation has become a very popular method to compensate for inadequate diets. When developing a calcium-fortified food product or mineral supplement, bioavailability is an important parameter to determine the right calcium sources.

Solubility

Calcium sources can be divided into soluble and insoluble calcium. The soluble calcium sources are Puracal[®] (calcium L-lactate and calcium lactate gluconate), Gluconalcalcium (calcium gluconate) and calcium chloride. Insoluble calcium sources like calcium carbonate, calcium citrate and calcium phosphate. Puracal[®] calcium sources are very soluble at both low and neutral pH.

PURACAL[®] calcium sources are very soluble at both low and neutral pH. This solubility is best presented by showing the amount of calcium that can be solubilized in water (see calcium solubility graph). PURACAL[®] calcium sources have a solubility of more than 100% of the RDI (Recommended Daily Intake) per 100 ml water. PURACAL[®] XPro and PURACAL[®] XPerform provide extremely high solubility, making these products very suitable for syrups, fruit concentrates, in-line blending and clear beverages with a high calcium content.



* For PURACAL XP range solubility is shown for 48-hours stable solutions. Initial solubility in some formulations up to 4000 mg Ca/100ml

Study # 3

Current Topics in Nutraceutical Research Volume 1, Number 1, pp. 161-168 (2003)

Comparison of Six Different Calcium Sources and Meal Type on True Fractional Calcium Absorption in Postmenopausal Women

ELIZABETH J. BRINK, ELLEN G.H.M. VAN DEN HEUVEL, AND THEO MUIJS

ABSTRACT: The present study was designed to compare true calcium absorption from six different calcium sources, being milk, carbonate, citrate/malate, phosphate, L-lactate and lactate/gluconate and to determine the effect of congestion of a Western or Asian breakfast on calcium absorption from calcium L-lactate. The study was conducted according to a randomized, 8-way crossover design. Ten postmenopausal women participated. Each calcium source was given once with a Western breakfast. In addition, calcium L-lactate was given with an Asian breakfast and without a breakfast. Calcium sources were labeled extrinsically or intrinsically with ⁴⁴calcium. True calcium absorption was determined with the dual label stable isotope technique. True calcium absorption from the six calcium sources consumed with a Western breakfast ranged from 24.7% for tri-calcium-di-phosphate to 32.1% for calcium lactate/gluconate. Calcium absorption from calcium L-lactate and calcium lactate/gluconate were higher than from tri-calcium-di-phosphate (p-values were respectively 0.018 and 0.026), but no significant differences could be demonstrated with the other calcium sources investigated. Calcium was highly absorbed by postmenopausal women when calcium L-lactate was given with either a Western (31.5%) or an Asian (29.4%) breakfast. Calcium absorption did not differ significantly between the meals. Without breakfast, calcium absorption was significantly higher (45.0%).

Kale, Collard Greens, Brussels Sprouts, Mustard Seeds (Brassicacae Vegetable Family containing Glucosinolates & Isothiocyanates):

Study #4

Drugs New Perspect. 2005 Sep;18(7):445-51

Chemopreventive functions of Isothiocyanates.

Department of Pharmaceutics, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey, USA.

Numerous animal and epidemiological studies have demonstrated that consumption of cruciferous vegetables is inversely correlated with the cancer incidence. Strong anticarcinogenic activities of cruciferous vegetables are attributed to the high abundance of glucosinolates. Upon consumption, glucosinolates are hydrolyzed into iso-thiocyanates (ITCs), which in turn are conjugated with intracellular glutathione and excreted via mercapturic acid pathway. On the basis of the principle that ITCs can interact with 1,2-benzenedithiole to produce 1,3-benzodithiole-2-thione, a simple high-pressure liquid chromatography-based assay (namely cyclocondensation assay) has been devised to carry out the accurate measurement of the intracellular ITC accumulation. This assay has been adopted to assess the pharmacokinetic profiles of ITCs in humans, to evaluate the enzymatic efficiency of glucosinolate breakdown by myrosinase and to investigate the intracellular absorption and elimination mechanisms of ITCs. In addition, LC-MS-MS has recently been introduced as an alternative quantitative method for ITCs and proved to be superior to cyclocondensation assay, in terms of sensitivity as well as selectivity. Among several possible cellular mechanisms, both the modulation of nuclear factor kappaB (NF-kappaB) and activator protein-1 (AP-1) and the induction of phase II cellular detoxification and antioxidant enzymes (in brief phase II enzymes) have been proposed as potential molecular mechanisms of chemoprevention by ITCs. In the present review, we describe how ITCs modulate the activity of NF-kappaB and AP-1 and discuss how Nrf2/ARE module was discovered as the central core in the transcriptional regulation of phase II cellular detoxification and antioxidant enzymes. (c) 2005 Prous Science.

Study #5

Curr Opin Lipidol. 2005 Feb;16(1):47-54.

Dietary antioxidants and cardiovascular disease.

Department of Nutrition, Institute for Basic Medical Sciences, University of Oslo, PO Box 1046, Blindern, N-0316 Oslo, Norway.

PURPOSE OF REVIEW: Oxidative damage is involved in cardiovascular diseases. Intervention with alpha-tocopherol, ascorbic acid and beta-carotene does not appear to reduce pathogenesis. The purpose of this review is to describe alternative antioxidant mechanisms that may be involved. **RECENT FINDINGS:** Antioxidants with different chemical properties may recharge each other in an antioxidant network. The total antioxidant content of dietary plants may therefore be a useful tool for testing the "antioxidant network" hypothesis. Several berries, fruits, nuts, seeds, vegetables, drinks and spices have been found to be high in total antioxidants. Initial studies in animals and humans are supportive as to the beneficial effects of dietary plants rich in total antioxidants. Additionally, antioxidants and other plant compounds may also improve the endogenous antioxidant defence through induction of antioxidant and phase 2 enzymes. Dietary plants rich in such compounds include broccoli, Brussels sprouts, cabbage,

kale, cauliflower, carrots, onions, tomatoes, spinach and garlic. SUMMARY: Although initial studies have indicated that antioxidants may reduce oxidative stress, human intervention studies do not support a beneficial effect of antioxidant supplements. Further research is needed to clarify whether other plant antioxidants, plants rich in a combination of antioxidants, or plant compounds that induce the endogenous antioxidant defence can reduce pathogenesis of cardiovascular disease and other oxidative stress-related diseases.

Study #6

J Med Food. 2003 Spring;6(1):19-26.

Reduction of cancer risk by consumption of selenium-enriched plants: enrichment of broccoli with selenium increases the anticarcinogenic properties of broccoli.

United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034, USA. jfinley@gfhnrc.ars.usda.gov

Plant-based diets and phytochemicals present in plants are associated with decreased risk of cancer. Brassica species, and broccoli in particular, are associated with reduced risk of several important cancers. Selenium (Se) is an essential nutrient that is covalently bound in a number of different chemical forms found in plants. Broccoli accumulates Se many-fold beyond the concentration of Se in the soil, and the chemical form of Se in broccoli is similar to the chemical form in high-Se garlic, a food with unique chemoprotective properties. Se from broccoli grown to accumulate more than 500 micro g Se/g did not accumulate in rat tissues or increase glutathione peroxidase enzyme activity to the same extent as Se salts or seleno-amino acids. Se from high-Se broccoli decreased the incidence of aberrant crypts in rats with chemically induced colon cancer by more than 50%, compared with controls. Se from high-Se broccoli also decreased the incidence of mammary tumors in rats treated with 7,12-dimethylbenz(a)anthracene (DMBA) and tumor number and volume in APC(min) mice. These results suggest that development of methods to increase the natural accumulation of Se in broccoli may greatly enhance its health-promoting properties.

Study #7

Adv Exp Med Biol. 1999;472:159-68.

Brassica vegetables and cancer prevention. Epidemiology and mechanisms.

TNO Nutrition and Food Research Institute, Zeist, The Netherlands.

This paper first gives an overview of the epidemiological data concerning the cancer-preventive effect of brassica vegetables, including cabbages, kale, broccoli, Brussels sprouts, and cauliflower. A protective effect of brassicas against cancer may be plausible due to their relatively high content of glucosinolates. Certain hydrolysis products of glucosinolates have shown anticarcinogenic properties. The results of six cohort studies and 74 case-control studies on the association between brassica consumption and cancer risk are summarized. The cohort studies showed inverse associations between the consumption of brassica's and risk of lung cancer, stomach cancer, all cancers taken together. Of the case-control studies 64% showed an inverse association between consumption of one or more brassica vegetables and risk of cancer at various sites. Although the measured effects might have been distorted by various types of bias, it is concluded that a high consumption of brassica vegetables is associated with a decreased risk of cancer. This association appears to be most consistent for lung, stomach, colon and rectal cancer, and least consistent for prostatic, endometrial and ovarian cancer. It is not yet possible to resolve whether associations are to be attributed to brassica vegetables per se or to vegetables in general. Further

epidemiological research should separate the anticarcinogenic effect of brassica vegetables from the effect of vegetables in general. The mechanisms by which brassica vegetables might decrease the risk of cancer are reviewed in the second part of this paper. Brassicas, including all types of cabbages, broccoli, cauliflower, and Brussels sprouts, may be protective against cancer due to their glucosinolate content. Glucosinolates are usually broken down through hydrolysis catalysed by myrosinase, an enzyme that is released from damaged plant cells. Some of the hydrolysis products, viz. indoles, and isothiocyanates, are able to influence phase 1 and phase 2 biotransformation enzyme activities, thereby possibly influencing several processes related to chemical carcinogenesis, e.g. the metabolism, DNA-binding, and mutagenic activity of promutagens. Most evidence concerning anticarcinogenic effects of glucosinolate hydrolysis products and brassica vegetables has come from studies in animals. In addition, studies carried out in humans using high but still realistic human consumption levels of indoles and brassica vegetables have shown putative positive effects on health. The combination of epidemiological and experimental data provide suggestive evidence for a cancer preventive effect of a high intake of brassica vegetables.

Kale

Kale is a leafy green vegetable that belongs to the Brassica family, a group of vegetables including cabbage, collards and Brussels sprouts that have gained recent widespread attention due to their health promoting, sulfur-containing phytochemicals. It is easy to grow and can grow in colder temperatures where a light frost will produce especially sweet kale leaves.

Health Benefits:

- Organosulfur Compounds that Prevent Cancer
- A Very Good Source of Fiber
- Calcium - For A Lot Less Calories and Minus the Fat in Cow's Milk
- Kale Gets an A+ for its Pro-vitamin A
- A Healthy Dose of Vitamin C for Antioxidant Protection and Immune Support
- Protection against Rheumatoid Arthritis
- Manganese - Energy Production Plus Antioxidant Protection
- Cardiovascular Protection Brought to You By Kale's Vitamin B6 and Riboflavin

Collard Greens

Collards are leafy green vegetables that belong to the same family that includes cabbage, kale and broccoli. While they share the same botanical name as kale, Brassica Oleracea, and some resemblance, they have their own distinctive qualities. Like kale, collards are one of the non-head forming members of the Brassica family. Collards' unique appearance features dark blue green leaves that are smooth in texture and relatively broad. They lack the frilled edges that are so distinctive to their cousin kale. The taste of collards can be described as pleasantly green and bitter.

Health Benefits:

- Calcium--A Mineral for A Lot More than Strong Bones
- Rich in Anti-Cancer Phytonutrients
- Broad Antioxidant Protection
- Optimizing Immune Function
- Cardiovascular Protection

Brussels Sprouts

Brussels sprouts are members of the brassica family and therefore kin to broccoli and cabbage. They resemble miniature cabbages, with diameters of about 1 inch. They grow in bunches of 20 to 40 on the stem of a plant that grows as high as three feet tall. Brussels sprouts are typically sage green in color, although some varieties feature a red hue. They are oftentimes sold separately but can sometimes be found in stores still attached to the stem. Perfectly cooked Brussels sprouts have a crisp, dense texture and a slightly sweet, bright and “green” taste.

Health Benefits:

- Cancer-Fighter Phytochemicals
- Healthy Skin and Immune Function
- Fiber-Rich for a Healthy Colon

Mustard Seeds

Mustard seeds are from the mustard plant, which is a cruciferous vegetable related to broccoli, Brussels sprouts and cabbage. While there are approximately forty different varieties of mustard plants, there are three principal types used to make mustard seeds: black mustard (*Brassica nigra*), white mustard (*Brassica alba*) and brown mustard (*Brassica juncea*). Black mustard seeds have the most pungent taste, while white mustard seeds, which are actually yellow in color, are the most mild and are the ones used to make American yellow mustard. Brown mustard, which is actually dark yellow in color, has a pungent acrid taste and is the type used to make Dijon mustard.

Health Benefits:

- Phytonutrient Compounds Protective Against Gastrointestinal Cancer
- Anti-Inflammatory Effects from Selenium and Magnesium

Sesame Seeds

Not only are sesame seeds a very good source of manganese and copper, they are also a good source of calcium, magnesium, iron, phosphorous, vitamin B1, zinc and dietary fiber. In addition to these important nutrients, sesame seeds contain two unique substances: **sesamin** and **sesamol**. Both of these substances belong to a group of special beneficial fibers called **lignans**, and have been shown to have a cholesterol-lowering effect in humans, and to prevent high blood pressure and increase vitamin E supplies in animals. Sesamin has also been found to protect the liver from oxidative damage. Sesame seeds are highly valued for their high content of sesame oil, an oil that is very resistant to rancidity.

Health Benefits:

- Copper may provide Relief of Rheumatoid Arthritis
- Magnesium Supports Vascular and Respiratory Health
- Calcium Helps Prevent Colon Cancer, Osteoporosis, Migraine and PMS
- Zinc for Bone Health
- Sesame Phytosterols Lower Cholesterol

Necessary Synergistic Co-Factors

Contains all Critical Co-Factors for Proper Calcium Assimilation Including:

- Vitamins A, C, D & E
- Magnesium, Zinc, Selenium, Copper, Manganese, Chromium, & Potassium

- Boron
- Ionic Trace Minerals from Plants

Calcium - What is it?

Calcium is the most abundant mineral found in the human body and it has several key functions that impact the Skeletal, Cardiovascular and Nervous Systems.

- Keeps our Bones & Teeth Strong
- Direct “Trigger” in Muscle & Blood Vessel Contraction
- Necessary for Hormone and Enzyme Secretion and Sending Messages throughout our Intricate Nervous System.

Over our lifespan our bone undergoes a continuous cycle of Bone Loss (Resorption) & Bone Formation. This cycle changes as we age.

- | | |
|-----------------------|---|
| • Childhood | More Bone Formation than Bone Loss |
| • Early-Mid Adulthood | Equal amounts of Bone Formation & Bone Loss |
| • Aging Adults | More Bone Loss than Bone Formation |

Ironically the human body does not make calcium on its own and it is constantly losing this all important mineral through nails, hair, skin, sweat, urine & feces. Therefore, it is of utmost importance to maintain constant calcium levels to insure that all of the bodily systems are functioning at optimal efficiency.

Two ways of insuring that we maintain optimal levels of calcium is to consume high quality, highly bioavailable forms of calcium in the foods we eat and the nutritional supplements we take.

Examples of Foods and Ordinary Supplemental Forms of Calcium

- Dairy (Milk, Yogurt, Cottage Cheese...)
- Dark Green Leafy Vegetables (Kale, Brussels Sprouts, Collard Greens...)
- Calcium Carbonate, Calcium Citrate, Calcium Hydroxyapatite, Oyster Shell, Dolomite, Bone Meal...

Intestinal absorption of calcium occurs via two processes

- Active cellular transport, mainly in the duodenum and jejunum. This process is mediated by calcitriol, the active component of vitamin D.
- Passive paracellular transport, mainly in the jejunum and ileum. Most of the calcium absorbed from foods through passive transport. For this passive absorption process calcium should be solubilized.

Endogenous factors affecting calcium absorption

- Elderly people show lower solubilization due to reduced secretion of gastric acid, which negatively influences calcium absorption
- Infants: increased calcium absorption is associated with increased hormone and calcitriol levels of the mother.
- In postmenopausal women, oestrogen deficiency is associated with increased calcium resorption from the bone.

Exogenous factors affecting calcium absorption

- In general, taking calcium together with a meal enhances the absorption

of calcium, while a meal stimulates gastric acid secretion, which results in increased calcium solubility. The prolonged calcium transport time also benefits calcium absorption. Nevertheless, meals may contain components that inhibit calcium absorption.

- Calcium intake shows an inverse relationship to calcium absorption. Low intakes cause increased PTH and calcitriol levels, promoting the active calcium absorption. The relationship between calcium load and absorption fraction is non-linear. A plateau of absorption is reached when approximately 400-500 mg of calcium was given as single dose.²
- Deficiency in vitamin D (from sunlight or diet) leads to a decrease of active transcellular calcium transport, hypocalcaemia, hyperparathyroidism and bone resorption. It is furthermore required for formation of calcitriol, which stimulates absorption of calcium.
- Phytate (from e.g. outer husks of cereals, beans, pulses) forms insoluble calcium phytate, which is not available for absorption.
- Oxalate (from e.g. rhubarb, spinach) forms insoluble calcium oxalate, which is unavailable for absorption.
- Indigestible oligosaccharides like dietary fiber have a positive influence on absorption, except when insoluble calcium complexes are formed.
- Although calcium can form insoluble complexes with phosphorus in the intestine, no negative influences on absorbed amount of calcium are reported. Excessive phosphate in the diet can decrease the absorption. A positive influence is the fact that phosphorus can reduce excretion.
- Phosphopeptides that are released during the digestion of milk protein may increase passive absorption.
- Fat can increase absorption as it increases gastrointestinal transport time. However, long chain saturated fatty acids can form insoluble complexes, which are not available for absorption.
- Protein stimulates intestinal calcium absorption but also increases urinary excretion. There is no evidence protein plays a significant role in bone loss.
- Sodium increases urinary excretion.
- An acute effect of alcohol intake is extra urinary excretion
- Also caffeine increases urinary excretion
- Some *medications* such as glucocorticoids and anticonvulsants have shown to decrease calcium absorption.

References:

1. TNO Nutrition and Food Research Institute, Zeist, The Netherlands, 2003
2. TNO Nutrition and Food Research Institute, Zeist, The Netherlands, 1991
3. Current Topics in Nutraceutical Research 2003, 1 (2), pp161-168.
4. Drugs New Perspect. 2005 Sep;18(7):445-51
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6. J Med Food. 2003 Spring;6(1):19-26
7. Adv Exp Med Biol. 1999;472:159-68
8. <http://www.whfoods.com/index.html>