FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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| | |
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| Application / Proposal / other work | Proposal P1030<br>Health Claims – Formulated Supplementary Sports Foods & Electrolytes Drinks |
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## Proposal P1030: Health Claims – Formulated Supplementary Sports Foods & Electrolytes Drinks

Thank you for the opportunity to provide comment and feedback on Proposal P1030 "*Health Claims – Formulated Supplementary Sports Foods and Electrolyte Drinks".*

Health World Limited, is a GMP approved manufacturer of complementary medicines and foods, including Formulated Supplementary Sports Foods, across Australia and New Zealand.

We broadly support the goals of this proposal, transferring electrolyte drinks and electrolyte drink bases from 'Standard 2.6.2 – Non-Alcoholic Beverages and Brewed Soft Drinks' to 'Standard 2.9.4 – Formulated Supplementary Sports Foods', and permitting specific exercise, electrolyte and physical performance health claims for this product category, will allow for greater flexibility within the sports food market.

This submission address concerns we have from an industry perspective, we believe some points presented in the proposal are restrictive to the overall classification and marketability of Formulated Supplementary Sports Foods (FSSFs) and Energy Drinks (EDs), as outlined below:

| **Section in P1030** | **Details from Proposal** | **Health World Limited's Response** |
|---|---|---|
| Section 1.3 | Prescribed composition of electrolyte drinks and electrolyte drink bases<br>**Table 1: Prescribed composition of electrolyte drinks and electrolyte drink bases**<br>Carbohydrate (specified sugars) 50-100 g/L<br>Sodium >10 mmol/L<br>Osmolality (isotonic only) 250-340 milliOsmol/L | Under the current EDs regulatory framework - Standard 2.6.2, the composition of electrolyte drinks does not permit electrolyte drinks which contain less than 50g/L of carbohydrates. We are concerned that low carbohydrate, or no carbohydrate, electrolyte drinks will also not be permitted under the proposed changes (P1030) to Standard 2.9.4 – FSSF & EDs.<br><br>We find the prescribed composition for electrolyte drinks limiting, preventing athletes with particular carbohydrate needs to be excluded under the current legislative framework.<br><br>Given the range and variety of athletes, some sports have particular carbohydrate to protein dietary intake requirements. Not all sports people want to 'carb-load' when training for or during a sporting event. Depending on the sport, some athletes are actively dropping weight through reduced calorie diets.<br><br>Some athletes require electrolyte replacements, while also being conscious of their carbohydrate and nutrition intake. A low carbohydrate or no carbohydrate electrolyte dink would provide athletes with fluids and electrolytes needed to rehydrate during times of heavy sweating and intense exercise. We are proposing an electrolyte drink option with little to no carbohydrates which would be acceptable for the 'carb-conscious' athlete. Dietary context needs to be factored into electrolyte / carbohydrate claims. |

| Section in P1030 | Details from Proposal | Health World Limited's Response |
|---|---|---|
| Section 2.1 | The Code recognises that FSSFs and EDs are foods each formulated to achieve a specific purpose: FSSFs to help sports people achieve nutritional or performance goals; and EDs to help with rapid replacement of fluid, carbohydrates and electrolytes. Where these foods meet a prescribed composition, a limited number of claims relating to the purpose of the food are permitted.<br><br>Standard 1.2.7 regulates nutrition content claims as well as health claims. The draft variation makes a minor amendment to Schedule 1 of Standard 1.2.7 to provide the conditions that must be met when making pre-approved health claims about specific vitamins or minerals in relation to FSSFs. | Under the current guidelines, Standard 1.2.7 and the proposed changes to Standard 2.9.4, the following claims would be prohibited:<br><br>"to prevent muscular cramps"<br><br>"improves endurance and stamina"<br><br>The Australia Institute of Sport (AIS) recognise electrolyte replacement supplements as "*Electrolyte replacements are powders, tablets or ready to drink products which allow targeted replacement of the electrolytes (in particular, sodium and potassium) lost through sweat*."<br><br>Evidence presented by the AIS support the use of electrolyte supplements, which have been shown to be beneficial for athletes with exercise associated muscle cramps. See appendix 1 - Australian Institute of Sport – Facts Sheet: Electrolyte replacement supplements.<br><br>Maintaining optimal sodium intake during situations of intense exercise, has been shown to improve endurance and performance for those individuals who require replacement of electrolytes lost through heavy sweating.<br><br>The effect of magnesium on muscular cramps, aches and pains was explored by Gaby (2007), who found magnesium supplementation was shown to improve muscular cramps in 2 double-blind trials and 1 uncontrolled trial. Patients reported symptomatic improvements as magnesium significantly reduced leg cramps in 75% of patients receiving magnesium supplementation, see appendix 2.<br><br>We believe the proposed health claims are appropriate to the nutritional and performance goals of athletes and sports professionals requiring electrolyte replacements and expect to see a number of new permitted health claims, specific to sports people, included under the Standard 1.2.7 – Nutrition, Health and Related Claims.<br><br>We suggest the following health claims are included under Standard 1.2.7 – Nutrition, Health and Related Claims:<br>Magnesium to prevent muscular cramps<br>Magnesium to relieve muscular cramps and spasms<br>Magnesium to manage muscular cramps and spasms<br>Magnesium can assist in muscular cramps and spasms<br>Magnesium can improves endurance and stamina<br>Magnesium to support endurance and stamina<br>Magnesium can supports sports endurance<br>Magnesium and sodium can assist muscular cramps and spasms. |

| Section in P1030 | Details from Proposal | Health World Limited's Response |
|---|---|---|
| Section 2.2.1 | The proposed new definition is: ***electrolyte drink*** *means a drink formulated for the rapid replacement of fluid, carbohydrates and electrolytes lost as a result of sustained strenuous physical activity.* | Under the current EDs regulatory framework - Standard 2.6.2, the composition of electrolyte drinks does not permit electrolyte drinks which contain less than 50g/L of carbohydrates. We are concerned that low carbohydrate, or no carbohydrate, electrolyte drinks will also not be permitted under the proposed changes (P1030) to Standard 2.9.4 – FSSF & EDs.<br><br>The Australian Institute of Sport (AIS) acknowledges electrolyte replacement supplements as "powders or tablets which can be added to fluids varying in carbohydrate content according to the athlete's needs." A low carbohydrate or no carbohydrate electrolyte drink or electrolyte drink base would fit within this classification. See appendix 1 - Australian Institute of Sport – Facts Sheet: Electrolyte replacement supplements.<br><br>We suggest the following definition:<br>***Electrolyte drinks*** *means a drink formulated for the rapid replacement of fluid and electrolytes lost as a result of sustained strenuous physical activity.* |
| Section 2.2.2 | Transferring regulation of EDs from Standard 2.6.2 to Standard 2.9.4 does not change the composition requirements for EDs nor does it change the existing labelling requirements. | The following Standards; 1.1.1, 2.6.2 & 2.9.4, allow a variety of vitamin and minerals forms to be added to foods. This framework, currently does not allow for amino acid chelates or bisglycinates as permitted forms of minerals (Calcium, Copper, Iron, Magnesium, Manganese).<br><br>Bioavailability studies conducted by Abrams, et al.6, and Schuette, et al., have shown that Magnesium Bisglycinate Chelate is a highly bioavailable form of magnesium. It was demonstrated that magnesium from Magnesium Bisglycinate Chelate was absorbed at a rate 228% higher than that of the magnesium from magnesium chloride. As the amino acid chelate approaches the intestinal wall, it remains the same molecule that was ingested. The chelate does not require digestion prior to absorption due to size. The glycine amino acid chelate for example is stable and does not become disassociated in the stomach. For this reason, bisglycinates and amino acid chelate are preferred forms of magnesium, and other minerals, for use in FSSFs and EDs, as they cause less adverse effects such as stomach upsets and gastric emptying when used by athletes during sporting events, see appendix 2.<br><br>In the European Union, bisglycinates; calcium bisglycinate, magnesium bisglycinate, ferrous bisglycinate and manganese bisglycinate are permitted forms of minerals that can be added to foods under the Commission Regulation 1170/2009/EC "*list of vitamins and minerals and their forms that can be added to foods, including food supplements*", see appendix 3.<br><br>Additionally, bisglycinate supplementation has received a positive opinion as a nutritional mineral source from EFSA "*Opinion on certain bisglycinates as sources of copper, zinc, calcium, magnesium and glycinate nicotinate as source of chromium in foods intended for the general population (including food supplements) and foods intended for particular nutritional uses*", see appendix 4.<br><br>We expect to see bisglycinate and amino acid forms of magnesium and other minerals included under the proposed changes to Standard 2.9.4, compositional requirement for EDs. |

| Section in P1030 | Details from Proposal | Health World Limited's Response |
|---|---|---|
| Appendix 1 | Definition<br>***Electrolyte drinks*** *means a drink formulated for the rapid replacement of fluid, carbohydrates and electrolytes lost as a result of sustained strenuous physical activity.* | The proposed Electrolyte drink definition currently does not permit electrolyte drinks which contain less than 50g/L of carbohydrates. We are concerned that low carbohydrate, or no carbohydrate, electrolyte drinks will also not be permitted under the proposed changes (P1030) to Standard 2.9.4 – FSSF & EDs.<br><br>We suggest the following definition<br>***Electrolyte drinks*** *means a drink formulated for the rapid replacement of fluid and electrolytes lost as a result of sustained strenuous physical activity.* |
| Attachment A "1 interpretation (1) In this Code - | **Electrolyte drink** means a drink formulated for the rapid replacement of fluid, carbohydrates and electrolytes lost as a result of sustained strenuous physical activity.<br><br>**Electrolyte drink base** means a solid or liquid which when made up, makes an electrolyte drink. | The proposed Electrolyte drink definition currently does not permit electrolyte drinks which contain less than 50g/L of carbohydrates. We are concerned that low carbohydrate, or no carbohydrate, electrolyte drinks will also not be permitted under the proposed changes (P1030) to Standard 2.9.4 – FSSF & EDs.<br><br>We suggest the following definition:<br>***Electrolyte drinks*** *means a drink formulated for the rapid replacement of fluid and electrolytes lost as a result of sustained strenuous physical activity.* |
| Attachment A "14 (2) composition of electrolyte drinks and electrolyte drink bases | (2) An electrolyte drink, or an electrolyte drink base when made up according to directions, must contain –<br>(a) no less than 50 g/L and no more than 100 g/L total –<br>(i) dextrose; and<br>(ii) fructose; and<br>(iii) glucose syrup; and<br>(iv) maltodextrin; and<br>(v) sucrose; and<br>(b) no more than 50 g/L fructose. | The proposed Electrolyte drink definition currently does not permit electrolyte drinks which contain less than 50g/L of carbohydrates. We are concerned that low carbohydrate, or no carbohydrate, electrolyte drinks will also not be permitted under the proposed changes (P1030) to Standard 2.9.4 – FSSF & EDs.<br><br>We suggest the following changes:<br><br>(2) An electrolyte drink, or an electrolyte drink base when made up according to directions, must contain –<br>(a) no more than 110 g/L total –<br>(i) dextrose; and<br>(ii) fructose; and<br>(iii) glucose syrup; and<br>(iv) maltodextrin; and<br>(v) sucrose; and<br>(b) no more than 50 g/L fructose.<br><br>Increasing the total carbohydrate compositional limit from 100 g/L to 110 g/ L, for electrolyte drinks and electrolyte drink bases more accurately captures those athletes which are taking electrolyte drinks specifically made up and labelled to be consumed as a hypertonic electrolyte beverage, in preparation for exercise and carbohydrate loading. To prevent confusion amongst consumers, the directions for use of these products should be labelled as a hypotonic electrolyte drink. |

| Section in P1030 | Details from Proposal | Health World Limited's Response |
|---|---|---|
| Attachment A "14 (3) composition of electrolyte drinks and electrolyte drink bases | (3) An electrolyte drink, or an electrolyte drink base when made up according to directions, may contain –<br>(a) calcium phosphates; and<br>(b) potassium phosphates; and<br>(c) calcium citrates; and<br>(d) potassium citrates; and<br>(e) sodium citrates; and<br>(f) potassium carbonates, including potassium bicarbonate; and<br>(g) potassium chloride; and<br>(h) calcium chloride; and<br>(i) sodium chloride; and<br>(j) calcium lactate; and<br>(k) magnesium lactate; and<br>(l) magnesium sulphate. | The following Standards; 1.1.1, 2.6.2 & 2.9.4, allow a variety of vitamin and mineral forms to be added to foods. This framework, currently does not allow for amino acid chelates or bisglycinates as permitted forms of minerals.<br><br>Bioavailability studies conducted by Abrams, et al.6, and Schuette, et al., have shown that Magnesium Bisglycinate Chelate is a highly bioavailable form of magnesium. It was demonstrated that magnesium from Magnesium Bisglycinate Chelate was absorbed at a rate 228% higher than that of the magnesium from magnesium chloride. As the amino acid chelate approaches the intestinal wall, it remains the same molecule that was ingested. The chelate does not require digestion prior to absorption due to size. The glycine amino acid chelate for example is stable and does not become disassociated in the stomach. For this reason, bisglycinates and amino acid chelate are preferred forms of magnesium, and other minerals, for use in FSSFs and EDs, as they cause less adverse effects such as stomach upsets and gastric emptying when used by athletes during sporting events, see appendix 2.<br><br>We suggest the following under the proposed changes to Standard 2.9.4, compositional requirement for EDs:<br><br>An electrolyte drink, or an electrolyte drink base when made up according to directions, may contain –<br>(a) calcium phosphates; and<br>(b) potassium phosphates; and<br>(c) calcium citrates; and<br>(d) potassium citrates; and<br>(e) sodium citrates; and<br>(f) potassium carbonates, including potassium bicarbonate; and<br>(g) potassium chloride; and<br>(h) calcium chloride; and<br>(i) sodium chloride; and<br>(j) calcium lactate; and<br>(k) magnesium lactate; and<br>(l) magnesium sulphate; and<br>(m) magnesium bisglycinate; and<br>(n) magnesium amino acid chelate; and<br>(o) calcium bisglycinate; and<br>(p) calcium amino acid chelate |

Yours Faithfully,
Health World Limited.

[redacted]

# Appendix 1

# Australian Institute of Sport – Facts Sheet: Electrolyte replacement supplements

# Electrolyte replacement supplements

## Supplement Overview

- Electrolyte replacements are powders, tablets or ready to drink products which allow targeted replacement of the electrolytes (in particular, sodium and potassium) lost through sweat. May be used during exercise to address high electrolyte loss and/or after exercise to allow restoration of fluid balance.
- Used as an alternative to standard sports drinks (10–25 mmol/L sodium and 3–5 mmol/L potassium) when these drinks are not adequate to replace large electrolyte losses during and after exercise. May also be used by athletes to restore fluid/electrolyte deficits caused by other factors such as the dehydration techniques undertaken to "make weight" for competition or gastrointestinal upsets (vomiting/diarrhea etc)
- Although plasma sodium concentrations are typically tightly regulated at ~ 135–150 mmol/L, mild hyponatremia (<135 mmol/L) can occur in some sports, often without overt symptoms. Risk factors include:
  - An overall fluid gain over exercise – i.e. when the athlete consumes fluid at a rate that is higher than their sweat losses and/or
  - An overall sodium loss during prolonged exercise – i.e. when the athlete replaces fluid losses with low sodium beverages (e.g. water and soft drinks) or meets fuel needs with low sodium foods (e.g. fruit, lollies, some sports gels and bars)
- Severe hyponatremia (plasma sodium < 130 mmol/L) is associated with confusion, nausea, headaches and the potentially fatal outcome of cerebral oedema. It is comparatively rare in sport and occurs when an athlete consumes fluid at a rate that is substantially higher than actual sweat losses, and particularly, the rate of urine excretion. This condition may be exacerbated in individuals who have inappropriate responses of the renal hormone that reduces urine production (vasopressin or ADH) and attenuated by the replacement of sodium during exercise. **Nevertheless, problematic hyponatremia is essentially a problem caused by excessive fluid intake.**
- Guidelines for the optimal sodium intake during endurance exercise (> 1 hour) are still unclear. General recommendations include 0.5–0.7 g per litre of fluid (21–30 mmol/L) [American College of Sports Medicine 1996] however it is important to note individual differences when making recommendations. However, there are suggestions that in situations of large sweat sodium losses e.g. ultra-endurance exercise, individuals who have "salty" sweat or combination of these factors; a more proactive approach to sodium intake during exercise may be needed
  - Exercise associated muscle cramps may be caused by multiple factors, with primary risk factors including fatigue due to unaccustomed volume/intensity of exercise and previous history of cramps. There is some evidence, although controversial, that whole body sodium depletion may be a cause of specific types of cramps in some individuals. Electrolyte supplementation may be beneficial in these athletes.
- During post-exercise rehydration, the replacement of electrolyte losses, particularly sodium, must occur to fully restore fluid balance. Rehydrating with fluids low in electrolytes (e.g. water) can lower plasma sodium levels causing a reduced thirst and increased urine output resulting in decreased voluntary fluid intake and inadequate fluid retention.
- Although sodium can be replaced by eating salty foods (e.g. bread, breakfast cereal, cheese & crackers, Vegemite™) or adding salt to meals, electrolyte supplements or sports drinks with higher sodium content can be useful for rapidly restoring fluids and electrolytes with a more targeted approach.

# Electrolyte replacement supplements

## Products and protocols

- Pharmaceutical Oral Rehydration Solutions (ORS) and sports-related Electrolyte Replacement Supplements are available in ready to drink and powdered forms. Products come in a wide range of flavours and vary according to the their carbohydrate (CHO) and electrolyte content as well as the addition of other ingredients
- In general, ORS are manufactured according to the World Health Organisation guidelines for the treatment and prevention of dehydration associated with diarrhoea and gastroenteritis. ORS are focused on electrolyte/fluid replacement; the low-moderate carbohydrate content is present to contribute to intestinal sodium/fluid absorption.
- Sports-related electrolyte supplements include:
  - electrolyte-only powders and tablets which can be added to fluids varying in carbohydrate content according to the athlete's needs.
  - sports drinks with high electrolyte concentrations (i.e. electrolytes + carbohydrate) **[see also Sports Drinks Fact sheet]**.

| Electrolyte Replacement Product | Presentation | Composition | | Flavours |
|---|---|---|---|---|
| | | Carb g/100 ml | Sodium mmol/L | |
| Gastrolyte | Ready to Drink (250 ml/1L) | 1.6 | 60 | Strawberry, Orange |
| Gastrolyte | Effervescent tablets: 2 tablets added to 200 ml fluid | 1.6 | 60 | Blackcurrant, Lemon, Raspberry |
| Gastrolyte | Powder: 1 sachet added to 200 ml fluid | 1.6 | 60 | Orange |
| Hydralyte | Ready to Drink | 1.4 | 45 | Orange, Apple-Blackcurrant |
| Hydralyte | Effervescent Powder (1 sachet added to 200 ml fluid) and Tablets (2 tablets added to 200 ml fluid) | 1.5–1.6 | 15 | Orange, Apple-Blackcurrant |
| Hydralyte | Ice blocks | 1.6 | 43 | Orange, Apple-Blackcurrant |
| Hydralyte Sports | Sachets (1 sachet added to 600 ml fluid) | 2 | 50 | Orange, Lemon |
| Restore ORS | Powder: 1 sachet added to 200 ml fluid | 1.6 | 60 | Orange |
| **Sports Products** | | | | |
| Shotz Electrolyte (E Shotz) | Effervescent tablet: 1 tablet added to 500mL fluid | - | 37 | Lemon, Vanilla Orange |
| Gu Brew Electrolyte Tablets | Effervescent tablet: 1 tablet added to 500mL fluid | - | 29 | Lemon Lime, Pink Grapefruit, Orange, Peach Tea |
| High 5 Zero Electrolyte Tablets | Effervescent tablet: 1 tablet added to 750mL fluid | - | 15 | Citrus, Berry, Cherry-Orange, Neutral, Pink Grapefruit |
| High 5 Zero X'Treme Electrolyte Tablets | Effervescent Tablet (contains 65mg caffeine per tablet) | - | 15 | Berry, Pink Grapefruit |
| Nunn | Effervescent tablet: 1 tablet added to 500mL fluid | - | 31 | Cherry Limeade, Lemonade, Watermelon, Lemon-Lime, Tri-Berry, Strawberry Lemonade, Grape, Orange, Fruit Punch, Kona Cola, Citrus Fruit, Lemon Tea, Tropical, Banana |

## Situations for Use in Sport

- Situations may occur in sport where focussed replacement of electrolytes is warranted including:
  - Rapid rehydration following moderate-large fluid deficits incurred during exercise or other dehydrating activities (e.g. "making weight").
  - Replacement of large sodium losses during ultra-endurance activities.
  - Replacement of large electrolyte losses during exercise in certain individuals with high rates of sweat loss and/or high sweat content of electrolytes.
  - Replacement of large electrolyte losses due to environmental conditions.
  - For situations when electrolyte replacement is required without carbohydrate intake (e.g. train low protocol)
- Rapid rehydration following moderate-large fluid deficits incurred during exercise or other dehydrating activities (e.g. "making weight").
  - The athlete with a moderate-large fluid deficit should follow a rehydration plan tailored to meet their estimated fluid loss. Specifically, over 2–4 hours the athlete should consume a volume of fluid equal to ~ 1.2–1.5 times their estimated fluid deficit.
  - When the rehydration period prior to an exercise bout is less than 1–2 hours (e.g. weigh-in prior to competition, recovery between repeated training or competition sessions), gastrointestinal discomfort may prevent the athlete from achieving this fluid intake target. In this situation the athlete should consume the greatest volume of their target intake that can be comfortably tolerated.
  - Fluid intake should be accompanied by electrolyte replacement, particularly sodium, to optimise fluid retention. When an athlete has a restricted food intake or is limited to low-sodium sports foods and snacks, it can be useful to consume sports drinks with higher sodium content, ORS electrolyte supplements to ensure sodium replacement
  - A higher sodium level reduces the palatability of most drinks. If the palatability of the drink is reduced, the athlete should be reminded to meet a fluid intake target rather than rely on voluntary intake. Many athletes may prefer to slightly dilute ORS rather than follow the manufacturer's instructions.
  - The carbohydrate content of ORS and some sports electrolyte supplements is negligible and will not contribute substantially to the athlete's refueling goals.
- Replacement of large sodium losses during ultra-endurance activities or for individuals with high sweat rates and/or high sweat sodium concentration.
  - Individualised recommendations for sodium supplementation during exercise should be made under the supervision of a Sports Dietitian or Physician. It may include the use of higher sodium sports drinks, the addition of electrolyte supplements to other fluids and the use of salt-rich everyday foods and drinks (e.g. vegemite sandwiches, stock cubes, instant potato, chicken noodle soup).
  - Education that promotes individualised hydration practices before and during exercise should be provided to athletes to reduce excessive fluid intake and the risk of hyponatremia through over-hydration.
- Prevention and treatment of dehydration during diarrhoea and gastro-enteritis.
  - Guidance for use of electrolyte supplements during illness should be provided by a Sports Physician.
  - ORS are recommended in the treatment or prevention of dehydration associated with diarrhoea and gastro-enteritis. The priority for athletes suffering from gastrointestinal upset is rehydration, rather than refueling.

## Concerns Associated with Supplement Use

- There is no consensus regarding the value of sodium replacement during exercise.
- In some situations, excessive salt supplementation during exercise may lead to gastrointestinal problems or cause further impairment of fluid balance.
- Excessive fluid intake during exercise (substantially greater than sweat losses) is the major cause of serious cases of hyponatremia in susceptible people. Sodium replacement during exercise does not address this problem and may provide a false sense of security.
- Increasing the sodium content of a drink generally reduces the drink palatability and may interfere with the voluntary consumption of fluid.
- The Dietary Guidelines for Australians promote a reduction in sodium/salt intake by the community, due to the link between salt intake and hypertension in susceptible people. Electrolyte replacement during and after sport may be considered as a special situation for a specific sub-group of the population, however, general guidelines for healthy eating should not be overlooked.

## Further Reading

Bergeron M. Exertional heat cramps: Recovery and return to play. *J Sport Rehabil* 2007; 16:190-196.

Schwellnus MP. Cause of exercise associated muscle cramps (EAMC) – altered neuromuscular control, dehydration or electrolyte depletion? *B J Sports Med* 2009; 43:401-408.

Sawka M, Burke L, Eichner R, Maughan R, Montain S, Stachenfeld N. Exercise and Fluid Replacement. Position Stand. *Med Sci Sports Exerc* 2007; 39:377-390.

This Fact Sheet was prepared by AIS Sports Nutrition as part of the Sports Supplement Framework (www.ausport.gov.au/ais/nutrition/supplements). Note that a Fact Sheet with additional information on this topic is available for Members of the Sports Supplement Framework via the Clearinghouse.



Last updated May 2014

## Appendix 2

**Gaby AR., 2007, 'Nutritional Interventions for Muscle Cramps.'** ***Integrative Medicine*****. 6(6):20-23**

**Sally A. Schuette, Bret A. Lashner and Morteza Janghorbani, 1994, 'Bioavailability of Magnesium Diglycinate vs Magnesium Oxide in Patients with Ileal Resection',** ***JPEN J Parenter Enteral Nutrition,*****18: 430**

**Steven A Abrams, Ian J. Griffin, Michelle A. Lopez, Christopher M. Branner, Michelle Brand, 2001, 'Assessment of Magnesium Absorption Using Stable Isotopes',** ***Advances in Magnesium Research: Nutrition and Health, pp. 109-114***

# Nutritional Interventions for Muscle Cramps

*Alan R. Gaby, MD*

**Abstract**

A cramp is a painful, involuntary contraction or spasm of a muscle or group of muscles. Cramps occur more commonly in the muscles of the legs and feet than in other parts of the body and happen most often at night or while a person is at rest. Older people are affected more frequently than younger individuals; as many as 70% of elderly people (an age delineation undefined by the literature) have experienced nocturnal leg cramps at some time. The cause of muscle spasms is not well understood, but certain risk factors have been identified, including dehydration, electrolyte imbalances, diabetes, and pregnancy.

The conventional approach to preventing leg cramps and other muscle spasms includes staying well hydrated and doing stretching exercises regularly. The drug quinine sulfate is effective for preventing leg cramps in some cases, but it can cause tinnitus and other adverse effects.

This article reviews dietary and nutritional factors found to be beneficial for preventing and treating muscle cramps (other than leg cramps of pregnancy). Heat cramps are discussed separately at the end of the article.

## Reactive Hypoglycemia

Of 131 patients with reactive hypoglycemia seen in 1 doctor's practice, 55% experienced leg cramps. This symptom usually improved following dietary modifications designed to stabilize blood glucose levels.[1] Nutritional treatments of reactive hypoglycemia include avoiding refined carbohydrates, caffeine, and alcohol; eating small, frequent meals; consuming adequate amounts of protein; and supplementing with chromium, B vitamins, magnesium, and other nutrients.

## Magnesium

Hypomagnesemia, which is usually indicative of relatively severe magnesium deficiency, is a recognized cause of muscle cramps. In case reports, hypomagnesemia was detected in 3 patients with recurrent cramps. In each case, the symptoms improved following magnesium supplementation. The presumed cause of magnesium deficiency was excessive exercise in 2 patients and diuretic use in the third.[2,3]

Magnesium deficiency not severe enough to cause hypomagnesemia appears to be relatively common in Western societies. Magnesium supplementation was shown to improve muscle cramps in 2 double-blind trials and 1 uncontrolled trial, explained below.

In a double-blind study, 64 patients (aged 18-65 years) with chronic, frequent muscle cramps and paresthesias (another sign of possible magnesium deficiency) were randomly assigned to receive 366 mg/day of magnesium or placebo for 4 weeks. Symptomatic improvement was reported by 75% of the patients receiving magnesium and by 32% of those receiving placebo ($P$ <.01).[4]

In another trial, 46 volunteers suffering from recurrent leg cramps were randomly assigned to receive, in double-blind fashion, 300 mg of magnesium or placebo each night for 6 weeks, and then the other treatment for an additional 6 weeks. The number of cramps was assessed in the final 4 weeks of each treatment period. There was a trend toward fewer cramps with magnesium than with placebo ($P$ = 07). Significantly more subjects experienced improvement during magnesium treatment than during placebo treatment (78% vs 54%; $P$ = 03).[5]

Fourteen trained swimmers who experienced muscle cramps when swimming received 65 mg of magnesium, from either magnesium ascorbate or magnesium aspartate, on 3 consecutive days, just before starting their swimming workout. The frequency of muscle cramps was reduced by 86% in the group receiving magnesium ascorbate and 44% in the group receiving magnesium aspartate. Both treatments reduced the severity of symptoms.[6]

## Potassium

Hypokalemia, usually indicative of relatively severe potassium deficiency, can also cause muscle cramps.[7] Hypokalemia may occur in patients using potassium-depleting diuretics or in association with persistent diarrhea or certain disease states. Potassium deficiency not severe enough to cause hypokalemia is probably common in Western societies as a result of inadequate intake of fruits and vegetables. Suboptimal potassium status may be a contributing factor in some cases of muscle cramps.

**Case report**: A 76-year-old man presented with a multi-year history of recurrent spasms in the calf muscles, which recently had become more severe. Serum potassium and magnesium levels were normal. He was advised to take 2 g/day of potassium magnesium aspartate (a preparation containing 50% potassium aspartate and 50% magnesium aspartate). This form of potassium and magnesium was recommended because of evidence that aspartate enhances intracellular uptake of potassium and magnesium.[8] The patient's leg cramps disappeared within 36 hours and did not return over a 5-month follow-up period, during which he continued to take potassium magnesium aspartate.

## Calcium

Calcium supplementation has been used with some success to treat leg cramps during pregnancy.[9] While calcium supplementation has not been studied as a treatment for other types of muscle cramps, it would be prudent to ensure that any patient experiencing cramps is consuming adequate amounts of calcium.

## Vitamin E

Several investigators have reported that vitamin E is an effective treatment for nocturnal leg cramps and other types of cramps.[10-14]

Of 125 patients with nocturnal leg cramps treated with vitamin E (d-alpha-tocopheryl acetate), 103 had complete or nearly complete symptom relief, an additional 13 had a moderate or good response, and 2 did not improve. Half of the patients responded to 300 IU/day or less, and half required 400 IU/day or more. Symptoms usually improved within a week of starting vitamin E but recurred when treatment was stopped.[11]

In a study of about 100 patients, vitamin E at a dose of 300 IU/day relieved leg cramps in almost all cases, whereas lower doses were frequently ineffective. Some patients who discontinued vitamin E experienced leg cramps that were more severe than usual for several days.[10]

In another study, approximately 50 patients with muscle cramps were treated with vitamin E. A dose of 300 IU/day was sufficient to control most cramps, whereas lower doses were only partially effective. Cramps usually recurred as soon as vitamin E was discontinued.[9]

In a small double-blind trial, vitamin E given at a dose of 800 IU per day for 4 weeks was not significantly more effective than a placebo in patients with nocturnal leg cramps.[15] However, a large proportion of the patients in that study had medical conditions that are associated with magnesium deficiency (diabetes, 70%; coronary artery disease, 41%; hypertension, 52%), and 37% of the patients were taking diuretics, which can deplete magnesium and potassium. Vitamin E would not be expected to be an effective treatment for cramps caused primarily by magnesium or potassium deficiency.

Leg cramps are a common occurrence in people with hepatic cirrhosis. In a study of cirrhotic patients, the mean serum vitamin E concentration was significantly lower in those with leg cramps than in those without cramps (6.3 µg/ml vs 11.5 µg/ml). Among those who experienced leg cramps, vitamin E at a dose of 600 IU per day reduced the frequency and duration of cramps. Patients with subnormal vitamin E levels seemed to benefit most from treatment.[16]

## Vitamin $B_{12}$ and Vitamin B Complex

One practitioner reported that 16 consecutive elderly patients had dramatic relief of nocturnal cramps for 4 to 6 weeks or longer after a single injection of 500 µg of vitamin $B_{12}$.[17] In a study of vitamin $B_{12}$ as a treatment for migraine, 1 woman experienced a resolution of leg cramps while receiving 1,000 µg per day of hydroxocobalamin (a form of vitamin $B_{12}$) intranasally. The cramps returned after treatment was discontinued.[18]

In a double-blind trial, supplementation with a vitamin B-complex preparation produced substantial improvement in nocturnal leg cramps in a group of elderly hypertensive patients. Twenty-eight patients older than age 65 with hypertension (controlled on medication) and severe nocturnal leg cramps were randomly assigned to receive, in double-blind fashion, 1 capsule of a vitamin B-complex preparation 3 times per day or placebo for 3 months. Each capsule contained 50 mg of fursultiamine (a thiamine derivative), 250 µg of hydroxocobalamin, 30 mg of pyridoxal phosphate, and 5 mg of riboflavin (vitamin $B_2$). At the start of the study, none of the patients had evidence of electrolyte abnormalities.

The severity of leg cramps was assessed on a 10-point visual analogue scale, with 10 indicating intolerable cramps and 0 indicating no cramps. In the placebo group, the mean score increased from 7.9 at baseline to 8.2 after 3 months. In the vitamin group, the mean score decreased from 7.9 at baseline to 2.6 after 3 months ($P$ <.01 compared with baseline and compared with the change in the placebo group). The difference between groups became significant after 4 weeks of treatment. Among patients taking the vitamins, 28% reported almost complete remission of leg cramps, and an additional 57% reported significant improvement.[19]

Further research is needed to determine whether the beneficial effect of this vitamin preparation was due entirely to the vitamin $B_{12}$ or to some combination of vitamins.

## Taurine

Muscle cramps are common in patients with cirrhosis of the liver. Cirrhotic patients with muscle cramps have been found to have significantly lower plasma taurine concentrations than cirrhotic patients without muscle cramps.[20] Since taurine is a membrane stabilizer, taurine depletion might increase membrane hyperexcitability, resulting in muscle cramps. In uncontrolled trials, taurine at a dose of 3 g per day in 1 study and 18 g per day in another study relieved muscle cramps in patients with cirrhosis.

Thirty-five patients with cirrhosis and recurrent painful muscle cramps received 3 g per day of taurine for 4 weeks. Marked improvement was seen in 71.4% of the patients, including complete disappearance of cramps in 37.1%. Improvement was seen after as little as 3 days in some cases. Cramps returned after taurine was discontinued.[21]

Twelve nonalcoholic patients with muscle cramps associated with cirrhosis received taurine for 6 months at a dose of 6 g 3 times per day after meals. After 1 month, the cramps had disappeared almost completely in 8 patients and were improved in the other 4 patients. No significant side effects were seen. The improvement was maintained during the 6-month treatment period, but symptoms recurred in 1 patient when taurine was temporarily discontinued.[22]

While taurine did not cause any significant side effects in these studies, long-term treatment with high doses of taurine could conceivably cause amino acid imbalance, an abnormality to which patients with liver disease are particularly susceptible. Therefore, in patients with muscle cramps associated with cirrhosis, the lowest effective dose of taurine should be used.

## Vitamin C and Hesperidin

In an uncontrolled trial, a combination of vitamin C and hesperidin (a citrus flavonoid) relieved nocturnal leg cramps in a group of postmenopausal women. Fourteen postmenopausal women with nocturnal leg cramps were treated with 200 mg each of vitamin C and hesperidin 4 times per day for 2 weeks,

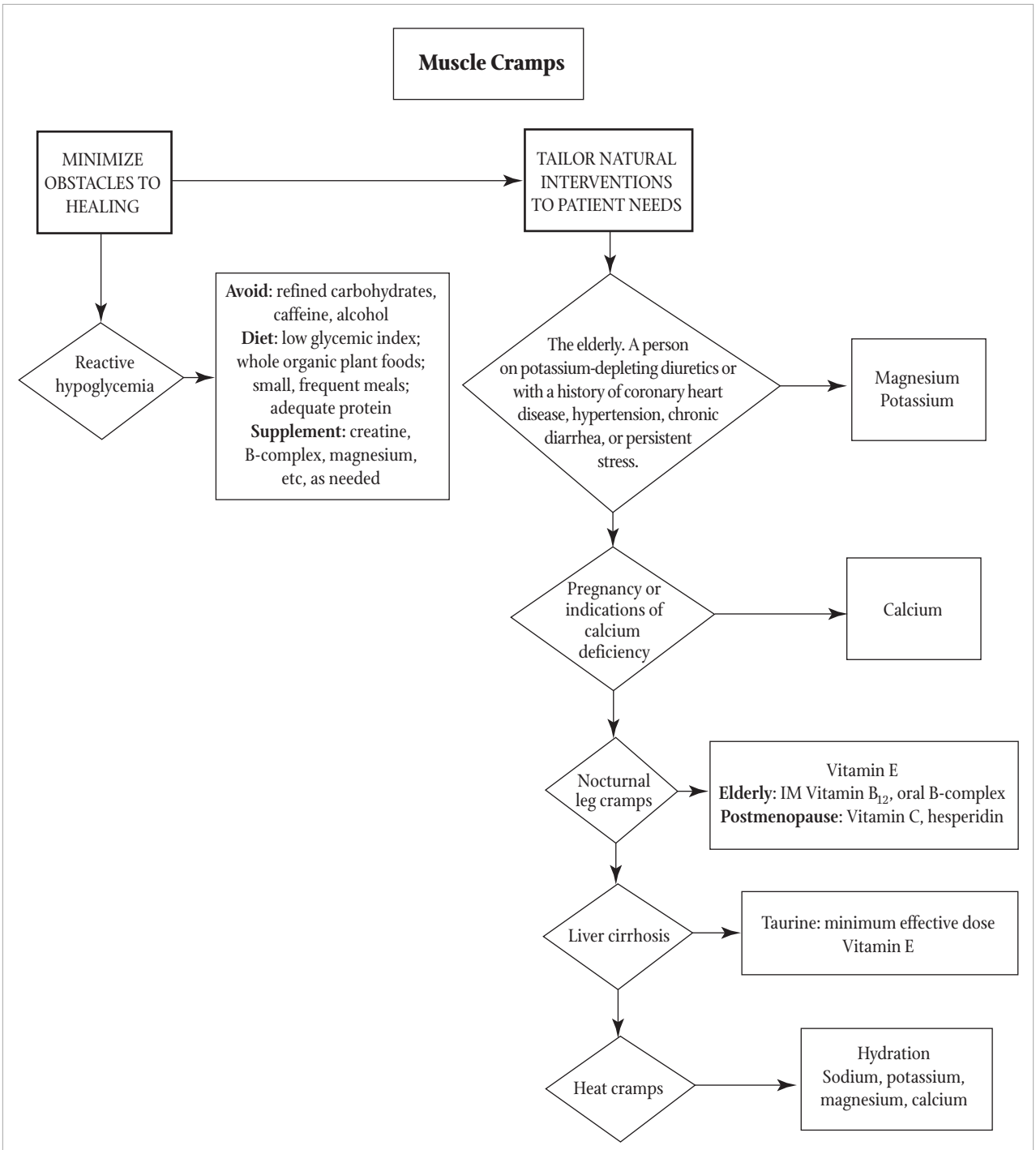


**Figure.** Muscle Cramps Treatment Protocol
Diagram provided by Herb Joyner-Bey, ND

followed by 100 mg of each 4 times per day for at least 4 weeks. All women experienced a resolution of leg cramps within 2 to 12 weeks. In some but not all cases, continued treatment was necessary to maintain the benefit.[23]

### Heat Cramps

Heat cramps are muscle spasms that occur during or after vigorous activity in a hot environment. They appear to be due to the loss of electrolytes, primarily sodium chloride (table salt), that results from excessive sweating. Rehydrating with water or with other fluids that contain insufficient amounts of sodium chloride may lead to hyponatremia, which can cause muscle cramps. Heat cramps can often be prevented by remaining well-hydrated and increasing sodium chloride intake [24,25]

While sodium chloride deficiency is a key factor in the etiology of heat cramps, the importance of potassium, magnesium,

and calcium deficiency should not be overlooked, particularly because Western diets are often low in these minerals. Profuse sweating can cause substantial losses of each of these minerals, as much as 6 g per day for potassium[26] and 1 g per day for calcium.[27] Long-term heat exposure may exacerbate sweating-induced potassium and magnesium depletion, because acclimatization to heat is accompanied by increased aldosterone secretion,[28] which increases urinary losses of these minerals. In addition, ingestion of large amounts of sodium chloride to compensate for losses in sweat may increase urinary excretion of potassium, calcium, and possibly magnesium.[29,30]

There is one case report of a 24-year-old woman who engaged in 6 hours of tennis daily and suffered bouts of post-exercise carpopedal spasm. She was found to have hypomagnesemia, and after receiving magnesium supplements her serum magnesium became normal and her symptoms resolved.[3] Other than that report, there is little published research on potassium, magnesium, and calcium deficiency as factors in the etiology of heat cramps. Nevertheless, it is reasonable to recommend a higher intake of these nutrients for people who are sweating a great deal or who are susceptible to heat cramps.

## Conclusion

Nutritional interventions are frequently beneficial for patients with muscle cramps. Potentially effective therapies include identifying and treating reactive hypoglycemia and supplementing with magnesium, potassium, vitamin E, vitamin $B_{12}$, vitamin B complex, and vitamin C plus citrus flavonoids.

Magnesium and potassium supplementation should be considered as first-line therapy for patients who are at risk of deficiency for one or both of these minerals. These include patients taking diuretics, elderly individuals, and those with diabetes, coronary heart disease, hypertension, chronic diarrhea, or persistent stress. Taurine and vitamin E may be effective for patients with muscle cramps associated with cirrhosis of the liver. For patients with heat cramps, effective prophylaxis and treatment include staying well hydrated and supplementing with sodium chloride and possibly potassium, magnesium, and calcium. Vitamin $B_{12}$, vitamin B complex, and vitamin C plus citrus flavonoids have been found to relieve nocturnal leg cramps.

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**References**

1. Roberts HJ. Spontaneous leg cramps and "restless legs" due to diabetogenic hyperinsulinism: observations on 131 patients. *J Am Geriatr Soc.* 1965 Jul;13:602-638.
2. Bilbey DL, Prabhakaran VM. Muscle cramps and magnesium deficiency: case reports. *Can Fam Physician.* 1996 Jul;42:1348-1351.
3. Liu L, Borowski G, Rose LI. Hypomagnesemia in a tennis player. *Phys Sportsmed.* 1983;11(5):79-80.
4. Fehlinger R, Kemnitz C, Stephan A, et al. Clinical study of the effectiveness of pyrrolidone carboxylic acid magnesium in the treatment of patients with chronic tetanic syndrome. *Curr Ther Res.* 1988;43:160-170.
5. Roffe C, Sills S, Crome P, Jones P. Randomised, cross-over, placebo controlled trial of magnesium citrate in the treatment of chronic persistent leg cramps. *Med Sci Monit.* 2002;8(5):CR326-CR330.
6. Zeana C, Zeana C, Radu G. Magnesium in preventing muscular cramps in swimmers. *Magnes Res.* 1993;6:73.
7. Davies JS, Aguirre G, Cassidy DM, Griffiths BE. Latent tetany associated with the hypokalaemia of Conn's syndrome. *Int J Clin Pract.* 1998;52(5):347-348.
8. Ring K, Grimm E, Schwarz M. Interrelationship between the transport of L-aspartate and potassium ions into the cell [in German]. *Arzneimittelforschung.* 1976;26(6):1195-1201.
9. Hammar M, Larsson L, Tegler L. Calcium treatment of leg cramps in pregnancy. Effect on clinical symptoms and total serum and ionized serum calcium concentrations. *Acta Obstet Gynecol Scand.* 1981;60(4):345-347.
10. Lotzof L. Vitamin E controls muscle cramps. *Med J Aust.* 1977;1:904.
11. Cathcart RF 3rd. Leg cramps and vitamin E. *JAMA.* 1972;219(2):216-217.
12. Ayres S Jr, Mihan R. Nocturnal leg cramps (systremma): a progress report on response to vitamin E. *South Med J.* 1974;67(11):1308-1312.
13. Jacques WH. Vitamin E in chronic poliomyelitis. *Can Med Assoc J.* 1959;81:129-130.
14. Cochrane T. Letter: Post-herpes zoster neuralgia: response to vitamin E therapy. *Arch Dermatol.* 1975;111(3):396.
15. Connolly PS, Shirley EA, Wasson JH, Nierenberg DW. Treatment of nocturnal leg cramps. A crossover trial of quinine vs vitamin E. *Arch Intern Med.* 1992;152(9):1877-1880.
16. Konikoff F, Ben-Amitay G, Halpern Z, et al. Vitamin E and cirrhotic muscle cramps. *Isr J Med Sci.* 1991;27(4):221-223.
17. Aitchison WR. Nocturnal cramps. *N Z Med J.* 1974;80(521):137.
18. van der Kuy PH, Merkus FW, Lohman JJ, ter Berg JW, Hooymans PM. Hydroxocobalamin, a nitric oxide scavenger, in the prophylaxis of migraine: an open, pilot study. *Cephalalgia.* 2002;22(7):513-519.
19. Chan P, Huang TY, Chen YJ, Huang WP, Liu YC. Randomized, double-blind, placebo-controlled study of the safety and efficacy of vitamin B complex in the treatment of nocturnal leg cramps in elderly patients with hypertension. *J Clin Pharmacol.* 1998;38(12):1151-1154.
20. Yamamoto S, Ohmoto K, Ideguchi S, et al. Painful muscle cramps in liver cirrhosis and effects of oral taurine administration [in Japanese]. *Nippon Shokakibyo Gakkai Zasshi.* 1994;91(7):1205-1209.
21. Yamamoto S. Oral taurine therapy for painful muscle cramp in liver cirrhosis. *Am J Gastroenterol.* 1994;89(3):457-458.
22. Matsuzaki Y, Tanaka N, Osuga T. Is taurine effective for treatment of painful muscle cramps in liver cirrhosis? *Am J Gastroenterol.* 1993;88(9):1466-1467.
23. Horoschak A. Nocturnal leg cramps, easy bruisability and epistaxis in menopausal patients: treated with hesperidin and ascorbic acid. *Del Med J.* 1959;31(1):19-22.
24. Bergeron MF. Heat cramps during tennis: a case report. *Int J Sport Nutr.* 1996;6(1):62-68.
25. Cunha JP. Heat cramps. eMedicineHealth. Available at: http://www.emedicinehealth.com/heat_cramps/article_em.htm. Accessed September 18, 2007.
26. Lane HW, Cerda JJ. Potassium requirements and exercise. *J Am Diet Assoc.* 1978;73(1):64-65.
27. Hegsted DM. Present knowledge of calcium, phosphorus, and magnesium. *Nutr Rev.* 1968;26(3):65-70.
28. Knochel JP, Vertel RM. Salt loading as a possible factor in the production of potassium depletion, rhabdomyolysis, and heat injury. *Lancet.* 1967;1(7491):659-661.
29. Castenmiller JJ, Mensink RP, van der Heijden L, et al. The effect of dietary sodium on urinary calcium and potassium excretion in normotensive men with different calcium intakes. *Am J Clin Nutr.* 1985;41(1):52-60.
30. Hills AG, Parsons DW, Webster GD Jr, Rosenthal O, Conover H. Influence of the renal excretion of sodium chloride upon the renal excretion of magnesium and other ions by human subjects. *J Clin Endocrinol Metab.* 1959 Oct;19:1192-1211.

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# Chapter 15

# Assessment of Magnesium Absorption Using Stable Isotopes

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## Summary

Stable isotope techniques permit a unique approach to the assessment of magnesium metabolism, including absorption, excretion, pool sizes, and turnover. However, because of difficulties associated with the use of either radioactive or stable magnesium tracers, few studies of whole body magnesium kinetics, especially in children and adolescents. Recent improvements in analytical techniques have made magnesium stable isotope studies more practical. Using high-precision analytical techniques, an intravenous dose of $^{25}Mg$ of approximately 0.2–0.3 mg/kg would be adequate for absorption measurements. We have shown that after oral and intravenous dosing of magnesium stable isotopes, a complete 72-h urine collection will allow for determination of fractional magnesium absorption. We have recently used this technique to evaluate differences in bioavailability of magnesium bisglycinate chelate (Mg-bgly) compared with magnesium oxide (MgO). Our studies suggest a small benefit to Mg-bgly in young adults with low habitual Mg intakes. As endogenous fecal magnesium excretion is small relative to urinary magnesium excretion, measurement of endogenous fecal magnesium excretion is not needed to make a reasonable estimate of net magnesium retention for most studies. Findings from our recent studies of magnesium kinetics indicate close relationships among the mass of the magnesium exchangeable pool, efflux from this pool, and body weight. The cost and availability of stable isotopes and their analysis are such that it should be feasible for increasing numbers of investigators to make use of these techniques, Further studies to evaluate these relationships are indicated in situations where either magnesium status or body composition is abnormal.

**Key words:** Magnesium absorption, stable isotopes, amino acid chelates, mineral supplements, bone, adolescent nutrition.

## Introduction

The techniques usable to assess magnesium metabolism have not kept pace with the increased attention paid to this important mineral. In particular, non- or minimally invasive approaches to determine dietary magnesium requirements and magnesium status are lacking. Although mass balance studies may be utilized to measure net magnesium absorption and retention, they are not only difficult to perform, but they do not measure the rate of endogenous secretory losses of minerals and cannot be used to assess mineral status or mineral interactions very readily.[1–3]

For other minerals, such as iron and calcium, metabolic studies are frequently performed using radioactive or stable isotopes as tracers. Unfortunately, because of difficulties associated with the use of either radioactive or stable Mg tracers, there are few human studies of whole-body Mg metabolism using these approaches.[4–9] These difficulties include the short half-life, limited availability, and unsuitability for pediatric use of the radioactive isotope, $^{28}$Mg. Although stable isotopes obviate these safety concerns, a true low-abundance Mg stable isotope does not exist. Use of the lowest-abundance isotope available ($^{25}$Mg, natural abundance 10 per cent) requires that relatively high levels of analytical precision be obtained for isotope ratio measurements in studies utilizing Mg stable isotopes. This problem has substantially limited the use of magnesium stable isotopes relative to those of other minerals.

However, this situation has substantially changed in the last several years. Recent improvements in analytical techniques have made Mg stable isotope studies more feasible, and they are increasingly becoming part of the approach to evaluating Mg metabolism in both human and animal research.[4,8]

In this report, we describe the methods used to assess magnesium absorption in humans using stable isotopes and some of the issues involved in performing these studies. Specific examples used are from a study performed to measure magnesium absorption from magnesium supplement pills in adults and a study to assess magnesium absorption and endogenous secretion in healthy adolescents.

## Methods

### Clinical methods

#### *Study of magnesium supplements in adults*

We enrolled and studied 15 healthy young women, 19–24 years of age in this protocol. The study was performed as a randomized double-blinded protocol. After enrollment, each subject received dietary counseling designed to keep her dietary Ca and Mg intake near recommended intake levels for these minerals (approximately 1000 mg/d calcium and 300 mg/d for Mg). Subjects were instructed to aim for these dietary goals for 2 weeks prior to the study and throughout the 2-week study period. The Institutional Review Board of Baylor College of Medicine approved this protocol, and informed written consent was obtained for the study from the subjects prior to enrollment.

At the time of the first study, subjects came to the Metabolic Research Unit of the Children's Nutrition Research Center. After arrival, they were asked to void and then received an infusion of 10.5–12.0 mg of $^{25}$Mg intravenously. After the infusion, half of the subjects received MgO, 100 mg, with breakfast, and half received magnesium bisglycinate chelate, (Mg-bgly), 100 mg, with breakfast (see Fig. 1).

The Mg-bgly (CAS # 14783-68-7) was provided by Albion Laboratories, Inc. (Clearfield, UT). Magnesium bisglycine chelate (Mg-bgly) is the reaction product of Mg and glycine in which the coordinate compound is covalently bonded in a tetrahedral arrangement forming a heterocyclic ring structure through the alpha-amino and carbonyl groups of the glycine and with the Mg as the closing member of the ring structure.

Each subject received the same breakfast which contained about one-third the RDA for Ca and Mg. The supplements included 25 mg of $^{26}$Mg and 75 mg of unenriched Mg. Following the infusion, subjects collected all of their urine for 72 h in 8-h aliquots. Each aliquot was separately analysed for total Mg and for enrichment of the magnesium stable isotopes. Dietary records were maintained by the study subjects for the 72 h of the study. Mg intake was calculated using a standard database system.

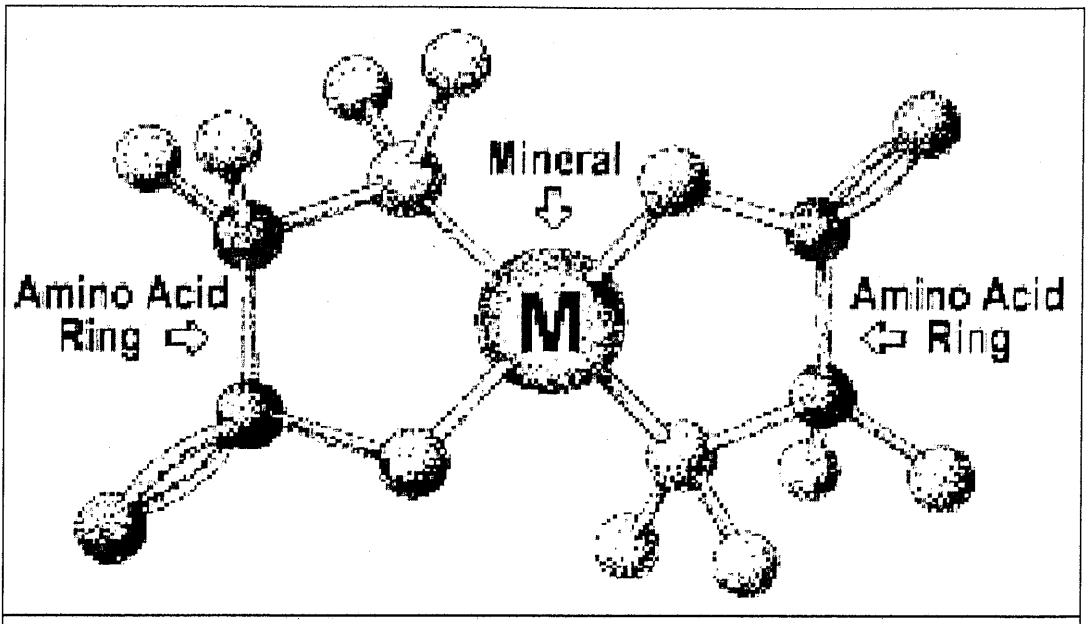


*Fig. 1. Magnesium bisgycinate chelate used in the study in adults.*

*Magnesium studies in young adolescents*

We enrolled and evaluated a group of 25 subjects who were 9 to 14 years of age.[4,5] The Institutional Review Board of Baylor College of Medicine approved this protocol, and informed written consent was obtained for the study from the subjects and their parents. Magnesium intake averaged 261 ± 40 mg/day, with a range of 194 to 321 mg/day.

Studies were conducted by mixing the isotope to be given orally ($^{26}Mg$) with 4 ounces of milk 24 h in advance of the study. These milk-isotope doses were given on the study day with each of the three main meals of the day. The intravenous isotope ($^{25}Mg$) was administered at the time of the first oral doses. All urine and stools were collected throughout the study. A dose of 1.0 mg/kg $^{25}Mg$ was infused over 6–8 min. This dose was higher than would be needed for studies in which measurement of magnesium absorption only was made. For these studies, a dose of 0.2–0.3 mg/kg would be adequate to obtain measurable enrichments. The reason for the higher dose administered in this study was to ensure adequate enrichment of fecal samples and urine and serum samples (used for kinetic analysis not presented in this manuscript). After the initial isotope infusions, each subject completed a 7-day urine and fecal collection.

**Analytical methods**

Magnesium isotopes are purchased as the oxide usually from material originally produced in Russia. The $^{26}Mg$ was 95.8 per cent pure and $^{25}Mg$ was 95.6 per cent pure. All isotopes were prepared for human use and tested for sterility and pyrogenicity prior to use.

Urine and fecal samples were prepared for mass spectrometric analysis by preparation using ion-exchange chromatography method.[4] Some samples were prepared using a simplified precipitation method previously described,[10] but increased purification and improved mass spectrometric analysis were achieved by performing ion-exchange chromatography.

Samples were analysed for isotope ratios using thermal ionization mass spectrometry (TIMS) using a Finnigan 261 magnetic sector mass spectrometer. To minimize fractionation, all samples were analysed for the $^{26}Mg/^{24}Mg$ and $^{25}Mg/^{24}Mg$ ratios at a fixed temperature. Our

measurement precision (for nonenriched samples) is 0.2 per cent or better (usually 0.05–0.15 per cent) for all measured ratios.[7,13] Baseline enrichments of serum and urine are within 0.2 per cent of the accepted naturally occurring values.[4]

Recently, we have begun utilizing a magnetic sector inductively coupled plasma mass spectrometer (Element2, Finnigan Thermoquest, Bremen, Germany) for magnesium sample analysis. Typical sample settings for this equipment include a run-time of 4–5 min and allow for samples to be analysed based on acid digestion with or without precipitation. Ion-exchange chemistry should not be necessary. We have currently found typical precison of 0.2–0.3 per cent for these samples with a mass bias of 2–3 per cent.

The enrichment of Mg isotope was determined as the 'percentage excess' of the sample. This value is the difference between the measured sample $^{25}Mg/^{24}Mg$ or $^{26}Mg/^{24}Mg$ ratio and the naturally occurring ratio determined on a daily basis for our mass spectrometer. To account for the relatively high natural fraction of $^{25}Mg$ and $^{26}Mg$, it is necessary to correct the enrichments measured in the mass spectrometer. The endogenous fecal excretion of Mg was calculated as the ratio of urine vs. fecal recovery of the intravenously administered isotopes. Fractional absorption of Mg was calculated from the total urinary recovery of the $^{26}Mg$ relative to the $^{25}Mg$ similar to the method we recently described.[5]

## Results

### Study of magnesium supplements in adults

Magnesium intake, fractional absorption, and urinary excretion are shown for each study (Tables 1). Differences in intakes were not significant between Mg supplement type (difference = 11.9 mg/d, $t = 0.5$, $P = 0.64$). Mean fractional Mg absorption was 29.3 ± 8.0 per cent from Mg-bgly and 26.7 ± 7.0 per cent from MgO (difference = 2.6 per cent, $t = 1.8$, $P = 0.097$). In the 10 subjects whose mean dietary Mg intake was < 300 mg/day, the difference in fractional absorption was 3.9 per cent favouring Mg-bgly ($t = 2.0$, $P = 0.076$) whereas in the five subjects whose dietary Mg intake was > 300 mg/day, no difference in absorption was seen (difference = 0.1 per cent favouring MgO, $t = 0.08$, $P = 0.94$). No further increase in difference was seen in looking at subjects with the lowest intakes (i.e. those with intakes < 240 mg/d). Mg absorption between the two supplements was significantly correlated, $r = 0.73$, $P = 0.0022$.

**Table 1. Magnesium suppplementation and absorption in healthy young adults**

| | MgO (mg/d) | Mg-bgly (mg/d) | *P*-value of difference |
|---|---|---|---|
| Mg intake (mg/d) | 285 ± 70 | 297 ± 96.0 | 0.64 |
| Mg absorption (%) | 26.7 ±7.0 | 29.3 ± 8.0 | 0.097 |
| Urinary Mg (mg/d) | 102 ± 32 | 91 ± 31 | 0.12 |

Urinary Mg excretion was also similar between the two studies (difference = 10 mg/d). Values for urinary excretion in the two studies were closely correlated ($r = 0.72$, $P = 0.0025$) strongly suggesting that there was no significant change in dietary Mg intake or Mg status between the two studies.

### Magnesium studies in young adolescents

Eleven of the 25 subjects (six girls and five boys) were in negative Mg balance (Table 2). There were no significant differences between males and females in the absorption (fractional or total) of magnesium.

**Table 2. Magnesium balance in children aged 9–14 years**

| | Females ($n$=13) | Males ($n$ = 12) | $P$-value |
|---|---|---|---|
| Absorption (%) | 42.8 ± 11.4 | 45.3 ±15.4 | 0.65 |
| Urinary excretion (mg/d) | 100.7 ± 11.0 | 80.0 ± 20.2 | 0.08 |
| Balance (mg/d) | −0.9 ± 41.2 | 15.6 ± 36.8 | 0.31 |

For the whole group of 25 subjects, the per cent absorption of magnesium from milk was 44.0 ± 13.3 per cent. Magnesium retention averaged 7 ± 39 mg/d from Mg intakes averaging 261 ± 40 mg/d (6.4 ± 1.2 mg/kg/d).

Endogenous fecal Mg excretion averaged 0.4 ± 0.2 mg/kg/d. There was no significant relationship between age, gender, and endogenous fecal magnesium excretion ($r < 0.2$ for each). There was no significant relationship ($r < 0.1$, $P > 0.5$) between magnesium intake and endogenous fecal magnesium excretion.

## Discussion

There are minimal previous data regarding Mg absorption using stable isotopes in humans, and virtually no such data in children.[8] We found that although there was an average positive magnesium balance on intakes approximating currently typical intakes of magnesium, a significant number of young adolescents were in negative balance. Although the requirement for magnesium retention during childhood and adolescence is unknown, it is likely to be at least 5–10 mg/d[5,8] and may increase during the pubertal growth spurt to support the more rapid rate of bone formation during this time period. Although the mean net magnesium retention in this study was 7 mg/day, the significant number of subjects in negative balance suggests that greater intakes may need to be encouraged to enhance magnesium retention.

Complete evaluation of mineral balance using isotopic techniques requires assessment of both mineral absorption and endogenous fecal excretion. The latter is difficult and time-consuming to measure, requiring a prolonged period (7–10 d) of fecal collection.[1–3] In this study, we performed these measurements for magnesium in a larger group of children than previous studies in children.

Our results in young adults suggest a small benefit to Mg-bgly in young adults with low habitual Mg intakes. A larger study targeting this group, possibly employing larger supplement doses, would be needed to further evaluate this benefit. The study demonstrated differences in absorption between supplements that were most apparent at low dietary Mg intakes. To identify a larger effect in this population, we would need to consider studying subjects at very low dietary intake levels in which a larger portion of the intake would come from the supplements and be maintained for several weeks.

It is important to consider the practicality of performing these isotope-based studies, in terms of cost and availability of isotopes and analytical resources to conduct the studies. At one time several years ago, mineral stable isotopes were not readily available and their cost was rapidly escalating.[1] This does not, however, appear to be a problem at the present time. Magnesium isotopes produced in Russia are readily available for purchase, and their price has stabilized. High enrichments (exceeding 95 per cent) are readily available for $^{25}$Mg and $^{26}$Mg. Cost of these isotopes fluctuates, but is generally between $5 to $10/mg of isotope for both $^{25}$Mg and $^{26}$Mg (Darren Brown, Trace Sciences, Inc., Toronto, Canada, personal communication).

Although few nutrition laboratories routinely perform magnesium stable isotope measurements, the analytical equipment (high-precision ICP-MS and magnetic sector TIMS) used for these measurements is widely available in geology and other research laboratories. Taken

together, the increased availability of analytical sites and isotopes suggests that an era of increased use of Mg stable isotopes may be upon us.

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## References

1. Abrams, S.A. (1999): Using stable isotopes to assess mineral requirements in children. *Am. J. Clin. Nutr.* **70,** 955–964.
2. Andon, M.B., Ilich, J.Z., Tzagournis, M.A. & Matkovic, V. (1996): Magnesium balance in adolescent females consuming a low- or high-calcium diet. *Am. J. Clin. Nutr.* **63,** 950–953.
3. Schwartz, R., Walker, G., Linz, M.D. & MacKellar, I. (1973): Metabolic responses of adolescent boys to two levels of dietary magnesium and protein. I. Magnesium and nitrogen retention. *Am. J. Clin. Nutr.* **26,** 510–518.
4. Abrams, S.A. & Wen, J.P. (1999): Methodologies for using stable isotopes to assess magnesium absorption and secretion in children. *J. Am. Coll. Nutr.* **18,** 30–35.
5. Abrams, S.A., Grusak, M.A., Stuff, J. & O'Brien, K.O. (1997): Calcium and magnesium balance in 9–14-year-old children. *Am. J. Clin. Nutr.* **66,** 1172–1177.
6. Aikawa, J.K., Gordon, G.S. & Rhoades, E.L. (1960): Magnesium metabolism in human beings: studies with $^{28}$Mg. *J. Appl. Physiol.* **15,** 503–507.
7. Schwartz, R., Spencer, H. & Welsh, J.J. (1984): Magnesium absorption in human subjects from leafy vegetables, intrinsically labeled with stable $^{26}$Mg. *Am. J. Clin. Nutr.* **39,** 571–576.
8. Sojka, J., Wastney, M., Abrams, S., Froese, S., Martin, B. , Weaver, C. & Peacock, M. (1997): Magnesium kinetics in adolescent girls determined using stable isotopes: Effects of high and low calcium intake. *Am. J. Physiol.* **273,** R710–R715.
9. Stegman, W. & Karbach, U. (1993): Plasma kinetics of magnesium and calcium stable isotopic tracers in a human subject after simultaneous oral ingestion of $^{25}$Mg and $^{44}$Ca determined by thermal ionization mass spectrometry. *Biol. Mass. Spect.* **22,** 441–446.
10. Vieira, N.E., Yergey, A.L. & Abrams, S.A. (1994): Extraction of magnesium from biological fluids using 8-hydroxyquinoline and cation exchange chromatography. *Anal. Biochem.* **218,** 92–97.

# Bioavailability of Magnesium Diglycinate vs Magnesium Oxide in Patients with Ileal Resection

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# Bioavailability of Magnesium Diglycinate *vs* Magnesium Oxide in Patients with Ileal Resection

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**ABSTRACT.** *Background*: Patients who have undergone ileal resection are at risk for developing magnesium depletion/deficiency because of poor absorption and decreased intake as well as increased endogenous losses. Magnesium repletion is difficult to accomplish because of the cathartic action of most oral magnesium supplements at therapeutic doses. The results of *in vitro* and *in situ* studies show that magnesium diglycinate (chelate) represents a highly available form of magnesium that is absorbed in part as an intact dipeptide in the proximal small intestine. *Methods*: We conducted a double-blind, randomized crossover trial with 12 patients who had ileal resections in order to compare the bioavailability of a 100-mg dose of $^{26}$Mg-labeled chelate with MgO in this patient population. *Results*: For the patient group as a whole, $^{26}$Mg absorption was low but was not different for the two supplements (23.5% *vs* 22.8% for magnesium chelate and MgO, respectively). However, $^{26}$Mg absorption was substantially greater from the chelate (23.5% *vs* 11.8%; $p < .05$) in the four patients who showed the greatest impairment of magnesium absorption with MgO and was better tolerated by all patients. Peak isotope enrichment also occurred significantly earlier after $^{26}$Mg chelate than after $^{26}$MgO ingestion (mean difference 3.2 ± 1.3 hours; $p < .05$), and the area under the enrichment *vs* time curve was greater after chelate ingestion ($p < .05$). *Conclusions*: Data from this study support the suggestion that some portion of magnesium diglycinate is absorbed intact, probably via a dipeptide transport pathway. Magnesium diglycinate may be a good alternative to commonly used magnesium supplements in patients with intestinal resection. (*Journal of Parenteral and Enteral Nutrition* **18:**430–435, 1994)

Approximately 80% of patients with Crohn's disease eventually undergo at least one small-bowel resection for their disease, with ileal resection the most prevalent.[1] Patients who have undergone ileal resection are at high risk for developing magnesium depletion/deficiency because of poor absorption and decreased intake as well as increased endogenous losses.[2,3] The prevalence of overt magnesium deficiency, or hypomagnesemia, in patients with inflammatory bowel disease ranges from 9% to 86%, depending on the population studied,[3,4] and is strongly associated with the presence of ileal resection.[4,5] The incidence of magnesium depletion without hypomagnesemia in this patient group is believed to be much higher.[6] Clinically speaking, magnesium depletion significant enough to result in hypomagnesemia can cause hypokalemia and hypocalcemia resistant to replacement therapy without prior or concomitant reversal of the underlying magnesium deficit. Magnesium deficiency can also result in neuromuscular symptoms such as Trousseau's and Chvostek's signs, muscle fasciculations, tremor, and muscle spasms, as well as other abnormalities such as anorexia, nausea, vomiting, and personality changes. Frank tetany, convulsions, and coma have been noted, but they occur primarily in acutely deficient infants.[7]

Magnesium repletion in many patients with ileal resection is difficult to accomplish with oral supplements because of the cathartic action of magnesium therapy, which exacerbates their diarrhea. Although intravenous magnesium is an effective therapy in the acute setting, the availability of a well absorbed and tolerated oral magnesium preparation would be invaluable in the long-term management of these patients. Such a supplement could be used both to prevent and to treat magnesium depletion.

*In vitro* absorption[8] and *in situ* perfusion studies[9,10] and analogy with other mineral amino acid chelates[11–13] show that magnesium diglycinate (chelate) represents a highly available form of magnesium absorbed at least in part as an intact dipeptide in the upper small intestine. Such a route of absorption would offer obvious benefit to patients who have undergone ileal resection.

The goal of the present investigation was to determine whether magnesium diglycinate is sufficiently bioavailable to represent a significant improvement in magnesium therapy for patients with ileal resections. We compared the absorption and retention of magnesium administered as $^{26}$Mg-labeled magnesium diglycinate with that of $^{26}$Mg-labeled MgO in patients who had at least one ileal resection. The use of isotopic techniques allowed us to

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measure magnesium absorption after a single oral dose of $^{26}$Mg-labeled chelate or $^{26}$Mg-labeled MgO and to compare postabsorptive retention.

## SUBJECTS AND METHODS

### *Subjects*

Twelve adults were studied under this protocol, which was approved by the Institutional Review Board of The University of Chicago. (One additional subject was studied but was inadvertently given $^{26}$MgO without unlabeled carrier MgO; data from this subject are not included.) Informed written consent was obtained from all subjects. All subjects had undergone at least one intestinal resection involving the ileum for the treatment of their underlying disease. Ten subjects had been diagnosed as having Crohn's disease, one had radiation-induced enteritis, and one had surgery for intestinal obstruction. All had been stable for at least 2 months before participation. Relevant clinical data for each subject is shown in Table I. All continued their usual medications throughout the study.

### *Experimental Design and Procedures*

The study was conducted as a double-blind, crossover, randomized clinical trial in which the absorption in each subject was determined after a 100-mg dose of $^{26}$Mg-labeled chelate and $^{26}$Mg-labeled MgO; the magnesium supplements were administered in random order with a washout period of 2 weeks between absorption studies. The subjects entered the Clinical Research Center of the University of Chicago the night before the beginning of the study and fasted until the following morning. At ~8 A.M., each subject received a 50-mg dose (49.7 ± 0.1) of $^{26}$Mg as $^{26}$MgO or $^{26}$Mg-chelazome (magnesium diglycinate, Albion Laboratories, Clearfield, UT) plus 50 mg (50.3 ± 0.3) of unlabeled oxide or chelate, all in gelatin capsules taken with deionized water. At the same time, each subject ingested 6 mg (5.57 ± 0.10) of dysprosium (Dy) as dysprosium chloride administered in a capsule that also contained ~0.5 g of glucose. Dy was administered as a nonabsorbable quantitative fecal marker.[14] Shortly after ingestion of the isotope dose, lean body mass and percentage of body fat were determined by bioelectrical impedance analysis.[15]

At 10:30 A.M. the subjects received a low-magnesium snack (up to 23 mg of magnesium), and at 12:30 P.M. they received a low-magnesium lunch (up to 41 mg of magnesium); distilled water was allowed throughout. At dinner and for the rest of the study the patients ate their normal diets. Complete urine and stool collections were initiated in the Clinical Research Center (for 12 hours) and then continued at home for a total length of time of 120 hours; a fasting urine sample was also collected before isotope administration. Two weeks after the initial visit, each subject returned to the Clinical Research Center and repeated the protocol with the alternative magnesium supplement.

Timed blood samples were obtained just before isotope ingestion and at 30 minutes and 1, 2, 4, 8, 12, 24, and 120 hours after isotope ingestion. Plasma and packed red blood cells were saved for $^{26}$Mg enrichment and for total magnesium content determinations. Fasting plasma samples were also saved for 25-hydroxyvitamin D (25 OHD) and creatinine determinations.

Stools were collected individually and frozen until prepared for analysis. For most subjects, individual stools were homogenized with deionized water, as described previously,[16] and accurately weighed fractions were saved for analysis. For a few of the patients who had a large number of bowel movements, a small number of stools were sometimes combined in order to facilitate homogenization and sampling. Immediately after isotope dosing, urine was collected in 4-hour aliquots for 12 hours, followed by one 12-hour and four 24-hour collections. All were analyzed for total magnesium, $^{26}$Mg, and creatinine.

TABLE I
*Summary of clinical data*

| Subject | Age | Sex | Condition | % Body fat | Medications |
|---|---|---|---|---|---|
| 1 | 28 | M | Crohn's, multiple resections, total length unknown | 18 | None |
| 2 | 34 | M | Crohn's, multiple resections, ~100 cm of small bowel remaining | 24 | None |
| 3 | 31 | M | Crohn's, 5 cm of terminal ileum and 40 cm of large bowel resected | 23 | Metronidazole, loperamide hydrochloride |
| 4 | 32 | M | Crohn's, ~15 cm of small bowel resected but diffuse involvement | 19 | Prednisone, sulfasalazine |
| 5 | 20 | M | Crohn's, ~15 cm of terminal ileum resected | 11 | Prednisone, metronidazole, (Cortifoam enema) |
| 6 | 36 | F | Crohn's, ~15 cm of terminal ileum and 5 cm of large bowel resected | 22 | Prenatal vitamins |
| 7 | 72 | M | Crohn's, ~15 cm of terminal ileum and 5 cm of large bowel resected | 24 | None |
| 8 | 51 | F | Crohn's, two resections, unknown length | 35 | Prednisone, vitamins $B_{12}$ and E Mercaptopurine |
| 9 | 62 | F | Radiation enteritis, small-bowel resection, unknown length | 28 | Thyroxine, cimetidine, Triamterene, MgO* |
| 10 | 65 | M | Rectal hernia with adhesions, multiple resections | 11 | Total parenteral nutrition† |
| 11 | 40 | F | Crohn's, chronic diarrhea, ~40 cm of terminal ileum resected | 35 | Cholestyramine |
| 12 | 50 | F | Crohn's, three small-bowel resections, ~40 cm of small bowel remaining | 19 | Prednisone, ranitidine hydrochloride, magnesium sulfate‡ |

* No MgO taken on the days in which isotope was administered.
† Total parenteral nutrition was discontinued for the evenings just before and just after each absorption study.
‡ Twice weekly magnesium sulfate injections; last treatment before each study administered 48 hours (before) isotope dose.

$^{26}$Mg-labeled diglycinate was synthesized from $^{26}$Mg metal (99.20 atom% $^{26}$Mg purchased from Oak Ridge National Laboratory, Oak Ridge, TN) by Albion Laboratories under the supervision of one of the authors (S.A.S.). Both labeled and unlabeled chelate contained 10.2% magnesium by weight. $^{26}$MgO was purchased as $^{26}$MgO (Oak Ridge National Laboratory; 99.53 atom% $^{26}$Mg). For the MgO absorption study, the subjects received three gelatin capsules filled with microcrystalline cellulose along with the $^{26}$MgO and MgO capsules so that the total number of capsules ingested was equal for the two absorption studies.

### *Analytical Procedures*

*Feces.* The magnesium and Dy content of natural isotopic composition was determined by inductively coupled plasma mass spectrometry (ICP-MS) using isotope dilution procedures as previously described[14,17]; $^{25}$Mg and $^{164}$Dy were used, respectively, as *in vitro* spikes. $^{26}$Mg excess was also determined by ICP-MS on the same samples used for isotope dilution.

*Urine and plasma.* Total magnesium content was determined by atomic absorption spectrometry (Perkin Elmer 5000, Norwalk, VA) on fresh plasma samples and on urine samples that had been acidified with nitric acid and stored at room temperature. $^{26}$Mg content in excess of natural isotopic composition was determined by ICP-MS. Baseline fasting plasma samples and all urine samples were also analyzed for creatinine content by autoanalyzer (Astra-4 automated analyzer, Beckman Instruments, Inc, Fullerton, CA). Plasma 25-OHD levels were measured by competitive binding assay on a single blood sample taken at baseline for each of the absorption studies using normal human serum as the standard[18] (analyses were performed by the Vitamin/Bone Mineral Assay Laboratory, Clinical Nutrition Research Unit, University of Chicago).

*Red blood cells.* One-gram samples of packed red blood cells were frozen until ready for analysis. The total magnesium content was determined by isotope dilution and ICP-MS using $^{25}$Mg as the *in vitro* spike; $^{26}$Mg content in excess of natural isotopic composition was determined in the same samples.

All chemicals were reagent grade and were used as obtained from chemical supply houses. High purity water (>15 MΩ) was used throughout.

### *Calculations*

Absorption of $^{26}$Mg label was estimated using fecal isotope balance procedures described previously.[16,19] The method involves determination of the amount of any orally administered stable isotope appearing in stool collected for 5 days after the ingestion of the labeled supplement with the data having been corrected for contributions from sources with natural isotopic composition. Dy-marker excretion was determined in the same stool composites. Absorption data were corrected for Dy recovery as follows: $^{26}$Mg absorption (%) = [1-(% $^{26}$Mg excreted/% Dy excreted)] × 100 where excretion of $^{26}$Mg and Dy are both expressed as percentage of administered dose. In a few cases, Dy recoveries were >100% because of the small errors inherent in the administration and quantification of the fecal marker; mineral absorption data from these collections were not adjusted for Dy recovery.

Urinary excretion of $^{26}$Mg label was also determined for each urine collection using $^{26}$Mg excess and total magnesium excretion data and was totaled for the 5-day collection period. The amount of $^{26}$Mg label retained was calculated and expressed as mg $^{26}$Mg or as percentage of absorbed dose.

$^{26}$Mg enrichment of plasma, red blood cells, and urine at various times after isotope ingestion was calculated as: % $^{26}$Mg enrichment$_t$ = $[(R_{26/24,t} - R_{baseline})/R_{baseline}] \times 100$ in which $R_{26/24,t}$ = $^{26}$Mg/$^{24}$Mg (wt/wt) of samples collected at time $t$ and $R_{baseline}$ = $^{26}$Mg/$^{24}$Mg of baseline samples collected just before isotope administration for each study. For plasma, the area under the curve of percentage enrichment *vs* time was then approximated as the sum of the trapezoids.

$^{26}$Mg present in stool, urine, plasma, or red blood cells in excess of natural isotopic composition is designated as the specific isotope throughout the manuscript.

The total number and weight of the stools passed in the first 24 hours after dosing were calculated as a measure of tolerability and compared for the two magnesium supplements.

### *Statistical Evaluation*

$^{26}$Mg absorption, retention, and isotope enrichment data (urine, plasma, and red blood cells) were compared using a $t$ test for paired observations. Comparisons between subgroups of the subjects or with magnesium-absorption data from the literature were made using an unpaired Student's $t$ test. Correlations were performed using least squares regression. All statistical calculations were performed using Systat Version 2.1. Data are presented as mean ± SEM throughout, except for isotope-dose information.

## RESULTS

Percentage of $^{26}$Mg absorption for all subjects from both magnesium supplements is shown in Table II. For the patient group as a whole, magnesium absorption from magnesium chelate (23.5% ± 1.8%) was not different from the value observed for MgO (22.6% ± 2.8%). However, for the four patients whose absorption of magnesium from MgO was the lowest, absorption increased dramatically with the chelate from a mean of 11.8% to 23.5% ($p < .05$). This was especially apparent in subject 12, who was known to have only 40 cm of small bowel; magnesium absorption in this patient increased from a value of 4.3% for MgO to a value of 12.0% for the chelate. For the remaining eight subjects with more normal magnesium absorption, absorption tended to be lower with the chelate, but the difference between the supplements was not significant.

Magnesium absorption from both supplements was significantly less ($p < .05$) in this patient group than in the healthy subjects studied by Roth and Werner[20] under similar experimental conditions; magnesium absorption averaged 29.0% ± 1.5% in 11 healthy subjects who received a tracer dose of $^{28}$Mg plus a 100-mg dose

TABLE II
*$^{26}$Mg absorption from both supplements*

| Subject | $^{26}$MgO | $^{26}$Mg Diglycinate |
|---|---|---|
| 1 | 22.8 | 16.0 |
| 2 | 15.9 | 25.2 |
| 3 | 13.3 | 33.2 |
| 4 | 21.2 | 30.6 |
| 5 | 20.4 | 16.8 |
| 6 | 13.7 | 23.4 |
| 7 | 29.4 | 21.0 |
| 8 | 28.6 | 25.1 |
| 9 | 30.5 | 24.4 |
| 10 | 36.4 | 28.7 |
| 11 | 35.2 | 25.3 |
| 12 | 4.3 | 12.0 |
| Mean | 22.6 | 23.5 |
| ± SEM | 2.8 | 1.8 |

All data are shown as percentage of administered dose.

of $MgCl_2$. Only 1 of 11 healthy subjects had an absorption value less than 20%, whereas 4 of 12 patients in our study had absorption values less than 16%, one had values less than 5% for MgO, and three had a value less than 20% for magnesium chelate.

There was a statistically significant difference ($p < .05$) between the two supplements in the number of stools passed during the first 24 hours after magnesium dose; on average, 2.4 ± 0.4 stools were passed after chelate ingestion *vs* 3.7 ± 0.6 stools after the MgO. The weight of the stools passed during this time period was not significantly different between treatments, but was greater in 8 of 12 subjects after the MgO dose. In addition, the 100-mg dose of magnesium ($^{26}$Mg plus unlabeled Mg) caused only one subject to have severe diarrhea and only after the MgO was consumed.

Five-day urinary excretion and retention of $^{26}$Mg were not different for the two supplements, whether the data were expressed as absolute milligrams of $^{26}$Mg or as percentage of absorbed dose. Mean urinary $^{26}$Mg excretion was 0.84 ± 0.17 mg or 8.5% ± 1.8% and 0.99 ± 0.18 mg or 8.1% ± 1.3% for $^{26}$MgO and $^{26}$Mg diglycinate, respectively. Urinary $^{26}$Mg excretion (peak, 24-hour or 5-day total) was not significantly correlated with $^{26}$Mg absorption. Five-day $^{26}$Mg retention averaged 10.4 ± 1.3 mg and 10.7 ± 0.8 mg for the oxide and chelate, respectively, or 91.5% ± 1.8% and 91.9% ± 1.3% of the absorbed dose.

For each individual we compared the magnitude and timing of plasma peak isotope enrichment as well as the area under the enrichment *vs* time curve (0 to 24 hours) between supplements. The magnitude of peak plasma isotope enrichment was not significantly different between treatments; the mean values were 6.6% ± 0.7% and 8.3% ± 1.0%, respectively, for the $^{26}$MgO and $^{26}$Mg chelate treatments. However, peak isotope enrichment occurred significantly earlier after $^{26}$Mg chelate than after $^{26}$MgO ingestion (mean difference 3.2 ± 1.3 hours; $p < .05$). The area under the enrichment *vs* time curve was also significantly greater after chelate ingestion ($p < .05$). The latter point is illustrated in Figure 1, which depicts the group average values of plasma isotope enrichment *vs* time. Percentage of $^{26}$Mg absorption was not correlated with peak plasma isotope enrichment or with the area under the enrichment *vs* time curve. Isotope enrichment of red blood cells was too low to permit comparisons between treatment groups.

Fasting plasma magnesium and 25-OHD levels, erythrocyte magnesium content, and urinary magnesium/creatinine ratios for all subjects are shown in Table III. Because there was no treatment effect on these parameters, the data from both absorption studies were pooled for each subject, and the mean value was listed. Plasma magnesium levels were near or slightly below normal for 9 of 12 subjects, and three subjects were clearly hypomagnesemic. The latter was true for subject 12, despite her twice weekly intramuscular $MgSO_4$ injections. Erythrocyte magnesium content was normal in nine subjects but low in three. The lowest values were observed for subject 9 who seemed to have renal wasting, as evidenced by a high urinary magnesium/creatinine ratio in the face of hypomagnesemia, and for subject 12, who had only 40 cm of small bowel. Plasma 25-OHD levels fell within the normal range for 9 of 12 subjects but was low in three. None of these parameters were significantly correlated with magnesium absorption.

The fasting urinary magnesium/creatinine ratios were within the range reported by other investigators.[21] Four of 12 subjects had ratios <0.025, which has been reported to be indicative of magnesium depletion.[21] Mean magnesium absorption (mean of the values obtained from the MgO and magnesium chelate period) was lower in the four subjects with magnesium/creatinine ratios <0.025 than in the remaining subjects ($p < .05$), exclusive of data from subject 12 who received intramuscular $MgSO_4$. Magnesium absorption averaged 20.8% in the low-ratio subjects *vs* 25.4% in the remaining subjects. Low plasma or erythrocyte magnesium content was not predictive of low magnesium absorption.

## DISCUSSION

As expected, magnesium absorption for this group of patients as a whole was low, but it varied greatly among individuals. With the exception of subject 12, who was known to have only 40 cm of small bowel remaining, clinical evaluation did not predict those individuals whose absorption of magnesium was very low.

On average, magnesium absorption from the two supplements was similar, indicating that for most individuals the bioavailability of magnesium chelate is equivalent to that of MgO. However, there were some differences in response to the two supplements, which may have important clinical implications. In the four patients whose magnesium absorption from MgO was less than 16%, magnesium absorption from the chelate was substantially greater. For these same subjects, the number of stools passed during the 24 hours after magnesium supplement ingestion was reduced, and for three of the four, fecal weight was 36% to 58% lower after chelate ingestion. Thus for those patients with the greatest impairment in magnesium absorption, magnesium chelate seems to offer both greater bioavailability and tolerability. The chelate was also absorbed more rapidly and seemed to be cleared from the plasma compartment more slowly. The latter conclusion is made

on the basis of the observations that absorption and retention of $^{26}Mg$ from the two supplements was not different for the group as a whole, yet the area under the plasma enrichment *vs* time curves was consistently greater for the chelate. Whether more rapid absorption and sustained elevation of plasma magnesium after larger, more therapeutic doses of magnesium chelate would be of benefit in repleting body magnesium stores remains to be established.

Data from *in vitro* studies[8-13] suggest that magnesium diglycinate and other metal amino acid chelates may be absorbed via dipeptide absorption pathways in the upper small intestine. That the absorption of magnesium in the form of magnesium diglycinate occurred, at least in part, by a different mechanism than did the absorption of inorganic MgO is supported by our observations that timing of plasma appearance and the area under the enrichment *vs* time curves were both significantly different for the two forms of magnesium. Previous investigators have shown that a sizable portion of diglycine peptide is absorbed intact when the human small intestine is perfused with a solution containing glycylglycine.[22] We also observed that magnesium chelate absorption was less variable with a smaller range of values and coefficient of variation for this group of patients. Dipeptide absorption occurs predominately in the jejunum and has been shown to be relatively unaffected in a number of gastrointestinal disease states.[23]

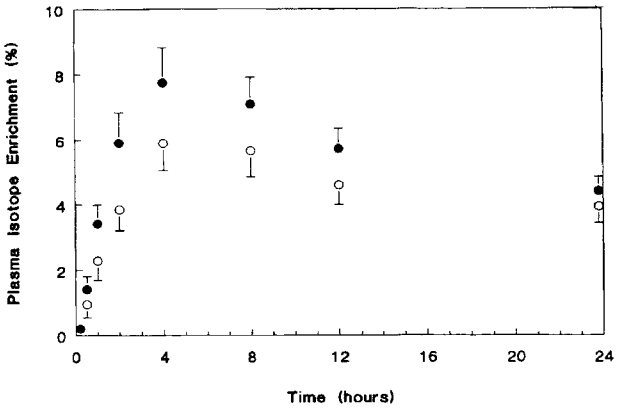


FIG. 1. Plasma isotope enrichment *vs* time for the first 24 hours after $^{26}Mg$ ingestion. Data for both $^{26}Mg$-labeled chelate (●) and $^{26}MgO$ (○) are shown as the group average ± SEM for all time points. The area under the curve was significantly greater after $^{26}Mg$ chelate ingestion ($p < .05$). Although not well depicted in the group average values, on a paired basis, peak isotope enrichment occurred significantly earlier ($p < .05$) after chelate ingestion, but the magnitude of peak isotope enrichment was not different between treatments.

We were concerned about the undercollection of stool and thus the overestimation of absorption in this patient group because of the large number of stools passed (mean, 11; range, 3 to 29 stools per 120 hours) and the episodes of diarrhea. To circumvent these concerns, we used dysprosium chloride as a quantitative fecal marker and corrected estimates of magnesium absorption for Dy recovery. Dy has been shown to be nonabsorbed both in mice[24] and in humans.[14] In addition, Dy and magnesium have been shown to follow similar excretion kinetics.[14]

For our patient group as a whole, Dy recovery averaged 85% and was ≥90% for 16 of the 24 individual absorption studies. For three of the subjects, however, Dy recovery was low for both test periods, averaging 73%, 43%, and 56% for subjects 10, 11, and 12, respectively. Subject 10 excreted close to 500 g of stool per day as three to five individual stools, subject 11 lost some diarrheal stool after both magnesium supplements, and subject 12 excreted >800 g of stool per day as three to four stools. For these three subjects, the mean corrected and uncorrected estimates of $^{26}Mg$ absorption were 22.0% *vs* 44.1% and 25.3% *vs* 60.6% for the magnesium chelate and MgO treatments, respectively, a twofold to threefold difference. The corrected values fell within the range of values observed for the remainder of the patient group and represent accurate estimates of true $^{26}Mg$ absorption. For the remaining subjects, Dy recovery averaged 95%. In these subjects, the use of Dy minimized any overestimation of $^{26}Mg$ absorption, but the magnitude of the correction was relatively small; corrected and uncorrected $^{26}Mg$ absorption values for subjects 1 through 9 were 24.0% *vs* 30.4% and 21.8% *vs* 24.8%, respectively, for the magnesium chelate and MgO treatments.

TABLE III
*Pertinent blood and urine parameters related to Mg and vitamin D status**

| Subject | Plasma Mg* (mmol/L) | Erythrocyte Mg† (mmol/L packed cells) | Mg/creatinine‡ (mg/mg) | 25-Hydroxyvitamin D† (ng/mL) |
|---|---|---|---|---|
| 1 | 0.85 | 2.49 | .023 | 37 |
| 2 | 0.79 | 2.12 | .037 | 32 |
| 3 | 0.77 | 2.25 | .022 | 28 |
| 4 | 0.81 | 2.03 | .032 | 15 |
| 5 | 0.77 | 1.85 | .014 | <5 |
| 6 | 0.81 | 1.89 | .056 | 30 |
| 7 | 0.73 | 2.05 | .071 | 24 |
| 8 | 0.65 | 1.97 | .018 | 22 |
| 9 | 0.63 | 1.43 | .069 | 34 |
| 10 | 0.78 | 1.82 | .035 | 6 |
| 11 | 0.74 | 2.32 | .088 | 19 |
| 12 | 0.65 | 1.73 | .044 | 16 |

* Data are shown as mean of fasting values obtained from both absorption studies.
† The normal ranges for plasma and erythrocyte Mg as well as for plasma 25-hydroxyvitamin D are 0.80–1.20 mmol/L, 1.82–2.78 mmol/L packed cells,[25] and 16–60 ng/mL, respectively.
‡ A Mg/creatinine ratio of <0.025 mg/mg has been suggested to be indicative of Mg deficiency or depletion.[21]

Although on average magnesium absorption was lower in those patients with low magnesium/creatinine ratios, poor magnesium absorption was not strictly predictive of magnesium status. In addition, as has been observed by other investigators,[21,25] the correspondence between "indicators" of magnesium status was poor. In this patient group, 6 of the 12 subjects had some evidence of magnesium deficiency/depletion regardless of whether it was the result of poor absorption, poor intake, increased urinary excretion, or a combination of factors. As a whole then, these patients would likely benefit from magnesium supplementation.

In conclusion, magnesium absorption in patients who have had ileal resection was generally lower than has been reported for healthy controls, but it varied greatly among individuals and was low in some patients. In most patients, magnesium diglycinate and MgO were found to be of similar bioavailability. However, the magnesium diglycinate was better tolerated by the group as a whole and better absorbed in those patients with the greatest impairment of magnesium absorption. Data from this *in vivo* study also support the suggestion that some portion of magnesium diglycinate (chelate) is absorbed intact, probably via a dipeptide transport pathway.

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## REFERENCES

1. Farmer RG, Whelan G, Fazio VW: Long-term follow-up of patients with Crohn's disease. Gastroenterology 88:1818–1825, 1985
2. Rosenberg IH, Bengoa JM, Sitrin MD: Nutritional aspects of inflammatory bowel disease. Ann Rev Nutr 5:463–484, 1985
3. Galland L: Magnesium and inflammatory bowel disease. Magnesium 7:78–83, 1988
4. Sjogren A, Floren C-H, Nilsson A: Evaluation of magnesium status in Crohn's disease as assessed by intracellular analysis and intravenous magnesium infusion. Scand J Gastroenterol 23:555–561, 1988
5. Heaton FW, Clark CG, Galigher JC: Magnesium deficiency complicating intestinal surgery. Br J Surg 54:41–45, 1967
6. Hessov I, Hasselblad C, Fasth S, et al: Magnesium deficiency after ileal resection for Crohn's disease. Scand J Gastroenterol 18:643–649, 1983
7. Shils ME: Magnesium. IN Modern Nutrition in Health and Disease, Shils ME, Olson JA, Shike M (eds). Lea & Febiger, Philadelphia, 1994, pp 164–184
8. Ashmead H, Ashmead DW, Jensen N: Chelation does not guarantee mineral metabolism. J Appl Nutr 26:5–21, 1974
9. Ashmead HDW, Graff DJ, Ashmead HH: Intestinal Absorption of Metal Ions and Chelates. Charles C Thomas, Springfield IL, 1985
10. Wapnir RA: Protein digestion and the absorption of mineral elements. Adv Exp Med Biol 249:95–111, 1989
11. Wapnir RA, Khani DE, Bayne MA, et al: Absorption of zinc by the rat ileum: Effects of histidine and other low molecular weight ligands. J Nutr 113:1346–1354, 1983
12. Taylor PG, Martinez-Torres C, Romano EL, et al: The effect of cysteine-containing peptides released during meat digestion on iron absorption in humans. Am J Clin Nutr 43:68–71, 1986
13. Wapnir RA: The role of protein breakdown products in the absorption of essential trace elements. IN: Nutrition for Special Needs in Infancy, Lifshitz F (ed). Marcel Dekker, New York, 1985, pp 37–57
14. Schuette SA, Janghorbani M, Young VR, et al: Dysprosium as a nonabsorbable marker for studies of mineral absorption with stable isotope tracers in human subjects. J Am Coll Nutr 12:307–315, 1993
15. Kushner RF, Schoeller DA: Estimation of total body water by bioelectrical impedance analysis. Am J Clin Nutr 44:417–424, 1986
16. Janghorbani M, Ifstan NW, Pagounes JO, et al: Absorption of dietary zinc in man: Comparison of intrinsic and extrinsic labels using a triple stable isotope method. Am J Clin Nutr 36:537–545, 1982
17. Schuette SA, Vereault D, Ting BTG, et al: Accurate measurement of stable isotopes of magnesium in biological materials with inductively coupled plasma mass spectrometry. Analyst 113:1837–1842, 1988
18. Belsey R, DeLuca HF, Potts JT: Competetive binding assay for vitamin D and 25-hydroxyvitamin D. J Clin Endocrinol Metab 33:554–557, 1971
19. Schuette SA, Ziegler EE, Nelson SE, et al: Feasibility of using the stable isotope $^{25}$Mg to study Mg metabolism in infants. Pediatr Res 27:36–40, 1990
20. Roth P, Werner E: Intestinal absorption of magnesium in man. Intl J Appl Radiat Isot 30:523–526, 1979
21. Ryzen E, Elbaum N, Singer FR et al: Parenteral magnesium tolerance testing in the evaluation of magnesium deficiency. Magnesium 4:137–147, 1985
22. Adibi SA. Intestinal transport of dipeptides in man: Relative importance of hydrolysis and intact absorption. J Clin Invest 50:2266–2275, 1971
23. Adibi SA, Kim YS. Peptide absorption and hydrolysis. IN Physiology of the Gastrointestinal Tract, Johnson LR (ed). Raven Press, New York, 1981, pp 1073–1095
24. Hutcheson DP, Gray DH, Venugopal B, et al: Studies of nutritional safety of some heavy metals. J Nutr 105:670–675, 1975
25. Elin RJ: Assessment of magnesium status. IN Magnesium in Health and Disease, Itokawa Y, Durlach J (eds). John Libbey, London, 1989, pp 137–46
26. Durlach J: Magnesium in Clinical Practice. John Libbey, London, 1988.

# Appendix 3

# EUROPEAN COMMISSION REGULATION 1170/2009 the lists of vitamin and minerals and their forms that can be added to foods, including food supplements

# COMMISSION REGULATION (EC) No 1170/2009

## of 30 November 2009

## amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements

**(Text with EEA relevance)**

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements [1], and in particular Article 4(5) thereof,

Having regard to Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods [2], and in particular Article 3(3) thereof,

After consulting the European Food Safety Authority,

Whereas:

(1) Annexes I and II to Directive 2002/46/EC establish the lists of vitamins and minerals, and for each of them the forms, that may be used for the manufacture of food supplements. Modifications to these lists are to be adopted in compliance with the requirements laid down in Article 4 of that Directive and in accordance with the procedure referred to in its Article 13(3).

(2) Annexes I and II to Regulation (EC) No 1925/2006 establish the lists of vitamins and minerals, and for each of them the forms, that may be added to food. Modifications to these lists are to be adopted in compliance with the requirements laid down in Article 3 of that Regulation and in accordance with the procedure referred to in its Article 14(3).

(3) New vitamin and mineral forms have been evaluated by the European Food Safety Authority. The substances which have received a favourable scientific opinion and for which the requirements laid down in Directive 2002/46/EC and in Regulation (EC) No 1925/2006 are complied with should be added to the respective lists in those acts.

(4) Interested parties were consulted and the provided comments were taken into consideration.

(5) Following the scientific evaluation by the European Food Safety Authority, it is appropriate to introduce specifications for some vitamin and mineral substances for their identification.

(6) Directive 2002/46/EC and Regulation (EC) No 1925/2006 should therefore be amended accordingly.

(7) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS REGULATION:

*Article 1*

Annexes I and II to Directive 2002/46/EC are replaced respectively by the texts in Annex I and II to this Regulation.

*Article 2*

Regulation (EC) No 1925/2006 is amended as follows:

1) In Annex I, the word 'Boron' is added in the list in point 2.

2) Annex II is replaced by the text in Annex III to this Regulation.

*Article 3*

This Regulation shall enter into force on the 20th day following its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 30 November 2009.

*For the Commission*
Androulla VASSILIOU
*Member of the Commission*

[1] OJ L 183, 12.7.2002, p. 51.
[2] OJ L 404, 30.12.2006, p. 26.

*ANNEX I*

*'ANNEX I*

**Vitamins and minerals which may be used in the manufacture of food supplements**

1. **Vitamins**

Vitamin A (µg RE)

Vitamin D (µg)

Vitamin E (mg a-TE)

Vitamin K (µg)

Vitamin B1 (mg)

Vitamin B2 (mg)

Niacin (mg NE)

Pantothenic acid (mg)

Vitamin B6 (mg)

Folic acid (µg) (*)

Vitamin B12 (µg)

Biotin (µg)

Vitamin C (mg)

2. **Minerals**

Calcium (mg)

Magnesium (mg)

Iron (mg)

Copper (µg)

Iodine (µg)

Zinc (mg)

Manganese (mg)

Sodium (mg)

Potassium (mg)

Selenium (µg)

Chromium (µg)

Molybdenum (µg)

Fluoride (mg)

Chloride (mg)

Phosphorus (mg)

Boron (mg)

Silicon (mg)

(*) Folic acid is the term included in Annex I of Commission Directive 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions for nutrition labelling purposes and covers all forms of folates.'

*ANNEX II*

*'ANNEX II*

**Vitamin and mineral substances which may be used in the manufacture of food supplements**

A. **Vitamins**

1. VITAMIN A
   (a) retinol
   (b) retinyl acetate
   (c) retinyl palmitate
   (d) beta-carotene
2. VITAMIN D
   (a) cholecalciferol
   (b) ergocalciferol
3. VITAMIN E
   (a) D-alpha-tocopherol
   (b) DL-alpha-tocopherol
   (c) D-alpha-tocopheryl acetate
   (d) DL-alpha-tocopheryl acetate
   (e) D-alpha-tocopheryl acid succinate
   (f) mixed tocopherols (*)
   (g) tocotrienol tocopherol (**)
4. VITAMIN K
   (a) phylloquinone (phytomenadione)
   (b) menaquinone (***)
5. VITAMIN B1
   (a) thiamin hydrochloride
   (b) thiamin mononitrate
   (c) thiamine monophosphate chloride
   (d) thiamine pyrophosphate chloride
6. VITAMIN B2
   (a) riboflavin
   (b) riboflavin 5′-phosphate, sodium
7. NIACIN
   (a) nicotinic acid
   (b) nicotinamide
   (c) inositol hexanicotinate (inositol hexaniacinate)
8. PANTOTHENIC ACID
   (a) D-pantothenate, calcium
   (b) D-pantothenate, sodium
   (c) dexpanthenol
   (d) pantethine
9. VITAMIN B6
   (a) pyridoxine hydrochloride
   (b) pyridoxine 5′-phosphate
   (c) pyridoxal 5′-phosphate
10. FOLATE
   (a) pteroylmonoglutamic acid
   (b) calcium-L-methylfolate
11. VITAMIN B12
   (a) cyanocobalamin
   (b) hydroxocobalamin
   (c) 5′-deoxyadenosylcobalamin
   (d) methylcobalamin
12. BIOTIN
   (a) D-biotin
13. VITAMIN C
   (a) L-ascorbic acid
   (b) sodium-L-ascorbate
   (c) calcium-L-ascorbate (****)
   (d) potassium-L-ascorbate
   (e) L-ascorbyl 6-palmitate
   (f) magnesium L-ascorbate
   (g) zinc L-ascorbate

B. **Minerals**

calcium acetate

calcium L-ascorbate

calcium bisglycinate
calcium carbonate
calcium chloride
calcium citrate malate
calcium salts of citric acid
calcium gluconate
calcium glycerophosphate
calcium lactate
calcium pyruvate
calcium salts of orthophosphoric acid
calcium succinate
calcium hydroxide
calcium L-lysinate
calcium malate
calcium oxide
calcium L-pidolate
calcium L-threonate
calcium sulphate
magnesium acetate
magnesium L-ascorbate
magnesium bisglycinate
magnesium carbonate
magnesium chloride
magnesium salts of citric acid
magnesium gluconate
magnesium glycerophosphate
magnesium salts of orthophosphoric acid
magnesium lactate
magnesium L-lysinate
magnesium hydroxide
magnesium malate
magnesium oxide
magnesium L-pidolate
magnesium potassium citrate
magnesium pyruvate
magnesium succinate
magnesium sulphate
magnesium taurate
magnesium acetyl taurate
ferrous carbonate
ferrous citrate
ferric ammonium citrate
ferrous gluconate
ferrous fumarate
ferric sodium diphosphate
ferrous lactate
ferrous sulphate
ferric diphosphate (ferric pyrophosphate)
ferric saccharate
elemental iron (carbonyl + electrolytic + hydrogen reduced)
ferrous bisglycinate
ferrous L-pidolate
ferrous phosphate
iron (II) taurate
cupric carbonate
cupric citrate
cupric gluconate
cupric sulphate
copper L-aspartate
copper bisglycinate
copper lysine complex
copper (II) oxide
sodium iodide
sodium iodate
potassium iodide
potassium iodate
zinc acetate
zinc L-ascorbate
zinc L-aspartate
zinc bisglycinate
zinc chloride
zinc citrate
zinc gluconate
zinc lactate
zinc L-lysinate
zinc malate
zinc mono-L-methionine sulphate
zinc oxide
zinc carbonate
zinc L-pidolate
zinc picolinate
zinc sulphate
manganese ascorbate
manganese L-aspartate
manganese bisglycinate
manganese carbonate
manganese chloride
manganese citrate

| | |
|---|---|
| manganese gluconate | L-selenomethionine |
| manganese glycerophosphate | selenium enriched yeast (*****) |
| manganese pidolate | selenious acid |
| manganese sulphate | sodium selenate |
| sodium bicarbonate | sodium hydrogen selenite |
| sodium carbonate | sodium selenite |
| sodium chloride | chromium (III) chloride |
| sodium citrate | chromium (III) lactate trihydrate |
| sodium gluconate | chromium nitrate |
| sodium lactate | chromium picolinate |
| sodium hydroxide | chromium (III) sulphate |
| sodium salts of orthophosphoric acid | ammonium molybdate (molybdenum (VI)) |
| potassium bicarbonate | potassium molybdate (molybdenum (VI)) |
| potassium carbonate | sodium molybdate (molybdenum (VI)) |
| potassium chloride | calcium fluoride |
| potassium citrate | potassium fluoride |
| potassium gluconate | sodium fluoride |
| potassium glycerophosphate | sodium monofluorophosphate |
| potassium lactate | boric acid |
| potassium hydroxide | sodium borate |
| potassium L-pidolate | choline-stabilised orthosilicic acid |
| potassium malate | silicon dioxide |
| potassium salts of orthophosphoric acid | silicic acid (******) |

(*) alpha-tocopherol < 20 %, beta-tocopherol < 10 %, gamma-tocopherol 50-70 % and delta-tocopherol 10-30 %

(**) Typical levels of individual tocopherols and tocotrienols:
- 115 mg/g alpha-tocopherol (101 mg/g minimum),
- 5 mg/g beta-tocopherol (< 1 mg/g minimum),
- 45 mg/g gamma-tocopherol (25 mg/g minimum),
- 12 mg/g delta-tocopherol (3 mg/g minimum),
- 67 mg/g alpha-tocotrienol (30 mg/g minimum),
- < 1 mg/g beta-tocotrienol (< 1 mg/g minimum),
- 82 mg/g gamma-tocotrienol (45 mg/g minimum),
- 5 mg/g delta-tocotrienol (< 1 mg/g minimum),

(***) Menaquinone occurring principally as menaquinone-7 and, to a minor extent, menaquinone-6.

(****) May contain up to 2 % of threonate.

(*****) Selenium-enriched yeasts produced by culture in the presence of sodium selenite as selenium source and containing, in the dried form as marketed, not more than 2,5 mg Se/g. The predominant organic selenium species present in the yeast is selenomethionine (between 60 and 85 % of the total extracted selenium in the product). The content of other organic selenium compounds including selenocysteine shall not exceed 10 % of total extracted selenium. Levels of inorganic selenium normally shall not exceed 1 % of total extracted selenium.

(******) In the form of gel.'

*ANNEX III*

*'ANNEX II*

**Vitamin formulations and mineral substances which may be added to foods**

1. **Vitamin formulations**

VITAMIN A

retinol

retinyl acetate

retinyl palmitate

beta-carotene

VITAMIN D

cholecalciferol

ergocalciferol

VITAMIN E

D-alpha-tocopherol

DL-alpha-tocopherol

D-alpha-tocopheryl acetate

DL-alpha-tocopheryl acetate

D-alpha-tocopheryl acid succinate

VITAMIN K

phylloquinone (phytomenadione)

menaquinone (*)

VITAMIN B1

thiamin hydrochloride

thiamin mononitrate

VITAMIN B2

riboflavin

riboflavin 5′-phosphate, sodium

NIACIN

nicotinic acid

nicotinamide

PANTOTHENIC ACID

D-pantothenate, calcium

D-pantothenate, sodium

dexpanthenol

VITAMIN B6

pyridoxine hydrochloride

pyridoxine 5′-phosphate

pyridoxine dipalmitate

FOLIC ACID

pteroylmonoglutamic acid

calcium-L-methylfolate

VITAMIN B12

cyanocobalamin

hydroxocobalamin

BIOTIN

D-biotin

VITAMIN C

L-ascorbic acid

sodium-L-ascorbate

calcium-L-ascorbate

potassium-L-ascorbate

L-ascorbyl 6-palmitate

2. **Mineral substances**

calcium carbonate

calcium chloride

calcium citrate malate

calcium salts of citric acid

calcium gluconate

calcium glycerophosphate

calcium lactate

calcium salts of orthophosphoric acid

calcium hydroxide

calcium malate

calcium oxide

calcium sulphate

magnesium acetate

magnesium carbonate

magnesium chloride

magnesium salts of citric acid

magnesium gluconate

magnesium glycerophosphate

magnesium salts of orthophosphoric acid

magnesium lactate

magnesium hydroxide

magnesium oxide

magnesium potassium citrate

magnesium sulphate

ferrous bisglycinate

ferrous carbonate

ferrous citrate

ferric ammonium citrate

ferrous gluconate

ferrous fumarate

ferric sodium diphosphate

ferrous lactate

ferrous sulphate

ferric diphosphate (ferric pyrophosphate)

ferric saccharate

elemental iron (carbonyl + electrolytic + hydrogen reduced)

cupric carbonate

cupric citrate

cupric gluconate

cupric sulphate

copper lysine complex

sodium iodide

sodium iodate

potassium iodide

potassium iodate

zinc acetate

zinc bisglycinate

zinc chloride

zinc citrate

zinc gluconate

zinc lactate

zinc oxide

zinc carbonate

zinc sulphate

manganese carbonate

manganese chloride

manganese citrate

manganese gluconate

manganese glycerophosphate

manganese sulphate

sodium bicarbonate

sodium carbonate

sodium citrate

sodium gluconate

sodium lactate

sodium hydroxide

sodium salts of orthophosphoric acid

selenium enriched yeast (**)

sodium selenate

sodium hydrogen selenite

sodium selenite

sodium fluoride

potassium fluoride

potassium bicarbonate

potassium carbonate

potassium chloride

potassium citrate

potassium gluconate

potassium glycerophosphate

potassium lactate

potassium hydroxide

potassium salts of orthophosphoric acid

chromium (III) chloride and its hexahydrate

chromium (III) sulphate and its hexahydrate

ammonium molybdate (molybdenum (VI))

sodium molybdate (molybdenum (VI))

boric acid

sodium borate

(*) Menaquinone occurring principally as menaquinone-7 and, to a minor extent, menaquinone-6.

(**) Selenium-enriched yeasts produced by culture in the presence of sodium selenite as selenium source and containing, in the dried form as marketed, not more than 2,5 mg Se/g. The predominant organic selenium species present in the yeast is selenomethionine (between 60 and 85 % of the total extracted selenium in the product). The content of other organic selenium compounds including selenocysteine shall not exceed 10 % of total extracted selenium. Levels of inorganic selenium normally shall not exceed 1 % of total extracted selenium.'

# Appendix 4

# Opinion on certain bisglycinates as sources of copper, zinc, calcium, magnesium and glycinate nicotinate as source of chromium in foods intended for the general population (including food supplements) and foods intended for particular nutritional uses

European Food Safety Authority

*The EFSA Journal* (2008) 718, 1-26

# Opinion on certain bisglycinates as sources of copper, zinc, calcium, magnesium and glycinate nicotinate as source of chromium in foods intended for the general population (including food supplements) and foods for particular nutritional uses[1]
## Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

**(Question No EFSA-Q-2005-035, EFSA-Q-2005-133, EFSA-Q-2005-034, EFSA-Q-2005-038, EFSA-Q-2005-166, EFSA-Q-2005-033, EFSA-Q-2005-132, EFSA-Q-2005-036, EFSA-Q-2005-130)**

**Adopted on 22 May 2008**

**PANEL MEMBERS**

F. Aguilar, H. Autrup, S. Barlow, L. Castle, R. Crebelli, W. Dekant, K.-H. Engel, N. Gontard, D. Gott, S. Grilli, R. Gürtler, J.-C. Larsen, C. Leclercq, J.-C. Leblanc, F. X. Malcata, W. Mennes, M.-R. Milana, I. Pratt, I. Rietjens, P. Tobback, F. Toldrá.

**SUMMARY**

Following a request from the European Commission, the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) was asked to deliver a scientific opinion on the use of copper bisglycinate chelate and chromium glycinate nicotinate as sources for respectively copper and chromium added for nutritional purposes to food supplements, and of calcium bisglycinate chelate and magnesium bisglycinate chelate as sources for respectively calcium and magnesium added for nutritional purposes to foods for particular nutritional uses and food supplements, and of zinc bisglycinate chelate when used as a source for zinc in foods intended for the general population (including food supplements) and foods for particular nutritional uses.

The mineral amino acid chelates considered in this application are intended for use as a direct replacement for the permitted respective mineral forms of copper and chromium for nutritional purposes in food supplements according Council Directive 2002/46/EC, for calcium and magnesium for nutritional purposes in food supplements and the categories of PARNUTS other than for baby foods and infant formula according to Council Directive 89/398/EEC, and for zinc in foods intended for the general population (including food supplements) and foods for particular nutritional uses.

[1] For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission on certain bisglycinates and glycinate nicotinate as sources for copper, zinc, calcium, magnesium and chromium. *The EFSA Journal* (2008) 718, 1-266.

The present opinion deals only with the safety of bisglycinate chelates of copper, zinc, calcium, magnesium, and of glycinate nicotinate as sources of the nutrient cations of respectively copper, zinc, calcium, magnesium and chromium and with the bioavailability of the nutrient cations from these sources. The safety of the nutrient cations themselves (copper, chromium, zinc, calcium and magnesium), in terms of amounts that may be consumed, is outside the remit of this Panel.

The bisglycinates considered in this opinion consist of a bivalent metal ion, namely $Cu^{+2}$, $Zn^{+2}$, $Ca^{+2}$, and $Mg^{+2}$, linked to two molecules of glycine. The metal is bound to the carboxyl group and to the α-amino group of glycine with coordinate covalent bonds to form two heterocyclic rings. This 1:2 metal to ligand ratio restricts reaction with dietary inhibitors of the metal absorption and does not participate in oxidation reactions.

Chromium(III) glycinate nicotinate chelate is also considered in this opinion.

No specific use levels for the mineral bisglycinates under consideration in this opinion have been given. However, it is assumed that under the intended conditions of use, the daily intake would not exceed those levels anticipated through existing supplementation of the listed minerals and would be similar to other forms of copper, zinc, calcium, magnesium and chromium that are already approved for use in foods in the EU.

Regarding the bioavailability of the different cations from their sources, data are provided showing that the minerals are bioavailable after oral administration.

No genetic toxicity studies have been conducted on the compounds; however the Panel has no concern on the genotoxicity aspects of glycine or nicotinic acid.

Due to the similarity in chemical structure between the metal glycinates considered in the present application and ferrous bisglycinate it is anticipated that the glycine part of these glycinates will exhibit similar toxicological characteristics as their ferrous bisglycinate counterpart, the safety of which was already evaluated and accepted by the AFC Panel in 2006. The Panel agrees that the subchronic studies on ferrous bisglycinate can be used to assess the subchronic toxicity of the glycinates. From the studies a NOAEL of 500 mg/kg body weight/day for ferrous bisglycinate in rats (the highest dose tested) was derived, corresponding to approximately 400 mg glycinate/kg body weight/day.

Specific chronic toxicity or carcinogenicity studies are not available.

Specific reproductive toxicity and developmental toxicity studies on the bisglycinates are also not available. However, in longer-term feeding studies with livestock (female pigs) receiving dietary supplementation with mineral glycinates throughout a period covering multiple litters, no adverse effects on reproduction or on the resulting offspring were observed.

The Panel noted that these longer-term feeding studies are of limited value for the assessment of either chronic toxicity or carcinogenicity of the chelates, due to the relatively short duration of the studies relative to the life span of the pig and the small numbers of animals used in the studies.

A conservative estimate of the dietary exposure was made based on a hypothetical intake from all sources (PARNUTS, food supplements and foods intended for the general population) at the tolerable upper intake level for copper (5 mg/day), zinc (25 mg/day), calcium (2500 mg/day) and magnesium (250 mg/day). The equivalent exposure to glycine would be around 12 mg glycine/day for copper bisglycinate, 57 mg glycine/day for zinc bisglycinate, 9239 mg glycine/day for calcium bisglycinate and 1523 mg glycine/day for magnesium bisglycinate. The Panel noted that this estimated exposure is lower than the NOAEL of 400 mg glycinate/kg bw/day, the highest dose tested.

In addition the normal (mean) intake of glycine in proteins from both food of animal origin, and vegetable origin was calculated to be about 26 mg/kg bw/day for adults (>15 years) and to about 43 mg/kg bw/day for children (< 15 years).

Glycine (synthetic or natural) is already permitted in the EU for use in foods under Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses (PARNUTS). Glycine and its salts (E640) have an ADI not specified and are permitted as food additives in the EU under Directive 95/2/EC on food additives other than colours and sweeteners.

The Panel concludes that the use of copper bisglycinate chelate as a source for copper added for nutritional purposes to food supplements, and of calcium bisglycinate chelate and magnesium bisglycinate chelate as sources for respectively calcium and magnesium added for nutritional purposes to foods for particular nutritional uses and food supplements, and of zinc bisglycinate chelate when used as a source for zinc in foods intended for the general population (including food supplements) and foods for particular nutritional uses, is not of safety concern

As regards chromium glycinate nicotinate complex, due to lack of information on the specific identity of its components, the Panel is unable to reach a conclusion on the safety of this source and on the bioavailability of chromium from this source.

**Keywords:**

Copper bisglycinate chelate, CAS N° 13479-54-4; Zinc bisglycinate chelate, CAS N° 14281-83-5; Zinc Glycinate, CAS N° 14281-83-5; Calcium bisglycinate chelate; CAS N° 56960-17-9; Magnesium bisglycinate chelate, CAS N° 14738-68-7; Chromium glycinate nicotinate hydrochloride.

## TABLE OF CONTENTS

## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients. The Commission has received a request for the evaluation of certain amino acid chelates as sources of certain minerals in foods for particular nutritional uses and in foods for the general population (including food supplements).

The relevant European legislative measures identified by the petitioner are:

- Commission Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses[2].
- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements[3].
- Regulation EC 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods[4].

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide scientific opinions, based on its consideration of the safety and bioavailability of:

- copper bisglycinate and chromium glycinate nicotinate hydrochloride when added to food supplements,
- calcium bisglycinate and magnesium bisglycinate when added for nutritional purposes in foods for particular nutritional uses and food supplements,
- zinc bisglycinate when used as a source for zinc in foods intended for the general population (including food supplements) and foods for particular nutritional uses.

## ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion:

F. Aguilar, D. Boskou, D. Gott, S. Grilli, R. Guertler, K. Hulshof, J.C. Larsen, J.C. Leblanc, C. Leclercq, A. Mortensen, D. Parent-Massin, I. Pratt, I. Rietjens, P. Tobback, G. Speijers, F. Toldra.

[2] OJ No L 52, 22.2.2001, p.19.
[3] OJ No L 183, 12.7.2002, p. 51.
[4] OJ No L 404, 30.12.2006, p. 26.

## ASSESSMENT

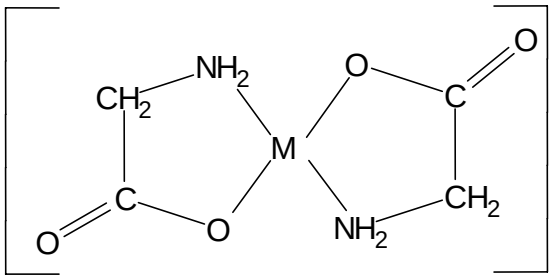
### 1. Introduction

The present opinion deals only with the safety of bisglycinate chelates of copper, zinc, calcium, magnesium, and of glycinate nicotinate as sources of the nutrient cations of respectively copper, zinc, calcium, magnesium and chromium and with the bioavailability of the nutrient cations from these sources. The safety of the nutrient cations themselves (copper, chromium, zinc, calcium and magnesium), in terms of amounts that may be consumed, is outside the remit of this Panel.

### 2. Chemistry

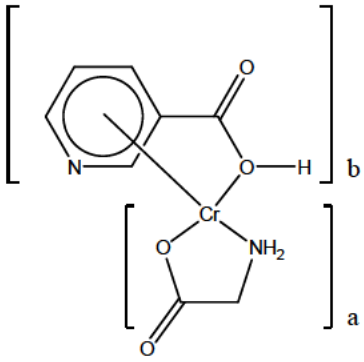
Metal bisglycinates consist of a bivalent metal ion ($M^{+2}$) linked to two molecules of glycine. The metal is bound to the carboxyl group and to the α-amino group of glycine with coordinate covalent bonds as described by McMurray and Fay (1995), to form two heterocyclic rings (Atkins and Beran, 1992; Ashmead, 2001). According to Jeppsen (2001) and to Allen (2002), this 1:2 metal to ligand ratio restricts reaction with dietary inhibitors of the metal absorption and prevents the metal from participating in oxidation reactions.

***General molecular Formula:*** *$M(COOCH_2NH_2)_2$*




In the structural formula, 'M', represents the ions Copper(II), Zinc(II), Calcium(II) or Magnesium(II).

Unlike the bisglycinates with bivalent cations, in chromium glycinate nicotinate chelate two molecules of chromium(III) are linked to three molecules of glycine and three molecules of nicotinic acid, chromium being a trivalent cation Cr(III). The structural formula as proposed by the petitioner is as follows:




In this formula 'b' represents 1-3 moles of niacin and 'a' represents 1-3 moles of glycine.

## 2.1 Identity of the substances

### 2.1.1. Copper bis(glycinate-N,O), CAS Number 13479-54-4

***Molecular Formula:*** $Cu(COOCH_2NH_2)_2$; molecular weight (Mw): 211.68 Dalton

Two commercial food-grade products of copper bisglycinate were considered by the Panel. Product 1 is a formulation of copper bisglycinate with approved food additives. The exact composition of the formulation is known to the Panel. The copper content in this product is not less than 10%.

Product 2 is at least 98% pure copper bisglycinate on a dry weight basis.

Copper bisglycinate and the commercially available products are hygroscopic blue powders, freely soluble in water and practically insoluble in ethanol and acetone.

***Proposed Chemical Specifications***

The limits for heavy metals for the products as proposed by the petitioners are given in Table 2.1.1

Table 2.1.1 **Proposed limits for heavy metals for copper bisglycinate**

| Test | Product 1 | Product 2 |
|---|---|---|
| Lead | Less than 1.5 mg/kg | Less than 5 mg/kg |
| Cadmium | Less than 0.5 mg/kg | Not given |
| Arsenic | Not given | Less than 3 mg/kg |
| Mercury | Not given | Less than 1 mg/kg |

For Product 1 analysis data of 7 non-consecutive batches of the commercial formulation of copper bisglycinate shows that the copper content varies between 10.7 and 11.5 %, lead between < 0.1 and 0.26 mg/kg and that the cadmium content is always < 0.1 mg/kg.

For Product 2 no analysis data of batches have been provided by the petitioner.

### 2.1.2. Zinc bisglycinate, CAS number, 14281-83-5,

***Molecular Formula:*** $Zn(COOCH_2NH_2)_2$ ; molecular weight (Mw): 213.53 Dalton

Three commercial food-grade products of zinc bisglycinate were considered by the Panel. Product 1 and Product 2 are a formulation of zinc bisglycinate with approved food additives. The exact composition of the formulations is known to the Panel. The zinc content in Product 1 is not less than 20% and not less than 10% in Product 2.

Product 3 is at least 98% pure zinc bisglycinate on a dry weight basis.

Zinc bisglycinate is freely soluble in water and practically insoluble in ethanol and acetone.

***Proposed Chemical Specifications***

The limits for heavy metals for the products as proposed by the petitioners are given in Table 2.1.2

Table 2.1.2 **Proposed limits for heavy metals for Zinc bisglycinates**

| Test | Product 1 | Product 2 | Product 3 |
|---|---|---|---|
| Lead (mg/kg) | Less than 5 mg/kg | Less than 2 mg/kg | Less than 5 mg/kg |
| Cadmium (mg/kg) | Less than 5 mg/kg | Less than 1 mg/kg | Not given |
| Arsenic | Not given | Not given | Less than 5 mg/kg |
| Mercury | Not given | Not given | Less than 1 mg/kg |

Analysis data of 7 non-consecutive batches of the commercial products show that, for Product 1, the zinc content varies between 20.3 and 22.6 %, lead, between 0.64 and 1.37 mg/kg and that the cadmium content is always < 0.1 mg/kg.

For Product 2 the zinc content varies between 10.9 and 11.1 %, lead between 0.64 and 1.63 mg/kg and that the cadmium content is also always < 0.1 mg/kg. All 7 batches analysed also pass for the test on colour, texture and scent.

For Product 3 no analysis data of batches have been provided by the petitioner.

### 2.1.3. Calcium bis(glycinate-N,O), CAS Number 56960-17-9

***Molecular Formula:*** $Ca(COOCH_2NH_2)_2$; Molecular weight (Mw): 188.11 Dalton

Four commercial food-grade products of calcium bisglycinate were considered by the Panel. Products 1, 2 and 3 are formulations of calcium bisglycinate with approved food additives. The exact composition of the formulations is known to the Panel. The calcium content in these products is respectively not less than 18%, not less than 13% and not less than 13%.

Product 4 is at least 98% of calcium bisglycinate on a dry weight basis.

Calcium bisglycinate is soluble in water, and practically insoluble in ethanol and acetone.

***Proposed Chemical Specifications***

The limits for heavy metals for the products as proposed by the petitioners are given in Table 2.1.3

Table 2.1.3 **Proposed limits for heavy metals for Calcium bisglycinates**

| Test | Product 1 | Product 2 | Product 3 | Product 4 |
|---|---|---|---|---|
| Lead | Less than 0.5 mg/kg | Less than 0.5 mg/kg | Less than 0.5 mg/kg | Less than 5 mg/kg |
| Cadmium | Less than 0.5 mg/kg | Less than 0.5 mg/kg | Less than 0.5 mg/kg | Not given |
| Mercury | Not given | Not given | Not given | Less than 1 mg/kg |
| Arsenic | Not given | Not given | Not given | Less than 3 mg/kg |

Analyses data of 7 non-consecutive batches of the commercial products show that, for Product 1 the calcium content varies between 19.0 and 21.1 %, and the content of lead and cadmium always <0.1 mg/kg.

For Product 2 the calcium content varies between 13.9 and 16 %; for lead and cadmium the content is always <0.1 mg/kg.

For Product 3 the calcium content varies between 26.8 and 30.0%, lead between 0.16 and 0.2 mg/kg and for cadmium between < 0.1 and 0.27 mg/kg.

For Product 4 no analysis data of batches have been provided by the petitioner.

### 2.1.4. Magnesium bis(glycinate-N,O), CAS Number 14783-68-7

***Molecular Formula:*** $Mg(COOCH_2NH_2)_2$; molecular weight (Mw) : 172.44 Dalton.

Four commercial food-grade products of magnesium bisglycinate were considered by the Panel. Products 1, 2 and 3 are formulations of magnesium bisglycinate with approved food additives. The exact composition of the formulations is known to the Panel. The magnesium content in these products is respectively not less than 10%, not less than 8% and not less than 18%.

Product 4 is at least 98% of magnesium bisglycinate on a dry weight basis.

Magnesium bisglycinate is freely soluble in water and practically insoluble in ethanol and acetone.

***Proposed Chemical Specifications***

The limits for heavy metals for the products as proposed by the petitioners are given in Table 2.1.4

Table 2.1.4 **Proposed limits for heavy metals for Magnesium bisglycinates**

| Test | Product 1 | Product 2 | Product 3 | Product 4 |
|---|---|---|---|---|
| Lead | Less than 0.3 mg/kg | Less than 1.5 mg/kg | Less than 1.5 mg/kg | Less than 5 mg/kg |
| Cadmium | Less than 0.5 mg/kg | Less than 0.5 | Less than 0.5 mg/kg | Not given |

| | | | | |
|---|---|---|---|---|
| Mercury<br>Arsenic | Not given<br>Not given | mg/kg<br>Not given<br>Not given | Not given<br>Not given | Less than 1 mg/kg<br>Less than 3 mg/kg |

Analysis data of 7 non-consecutive batches of the commercial products show that, for Product 1, the magnesium content varies between 10.9 and 11.9 %, for lead between < 0.1 and 0.14 mg/kg and for cadmium between <0.1 and 0.15 mg/kg.

For Product 2 the magnesium content varies between 8.28 and 8.93%, lead between <0.1 and 0.15 mg/kg and for cadmium always <0.1 mg/kg.

For Product 3 the magnesium content varies between 18.3 and 19.5%, for lead between < 0.1 and 0.12 mg/kg and for cadmium between < 0.1 and 0.19 mg/kg.

For Product 4 no analysis data of batches have been provided by the petitioner.

### 2.1.5. Chromium glycinate nicotinate chelate hydrochloride, CAS Number: None established

***Molecular Formula:*** $Cr_2(COOCH_2NH_2)_3(C_6H_5NO_2)_3$ 6HCl, Mw 914.3 Dalton. The molecular weight of the active ingredient [i.e. $Cr_2(COOCH_2NH_2)_3(C_6H_5NO_2)_3$] is 659.52 Dalton.

One commercial food-grade product was considered by the Panel. The product is a formulation of chromium glycinate nicotinate chelate hydrochloride with approved food additives. The composition of the formulation is known to the Panel, but the exact identity of the active ingredient is not known. The chromium content in this product is not less than 2.5%.

The commercially available chromium glycinate nicotinate chelate is a hygroscopic powder, soluble in water and practically insoluble in ethanol and acetone.

#### *Proposed Chemical Specifications*

The limits for heavy metals for the products as proposed by the petitioners are given in Table 2.1.5

Table 2.1.5 **Proposed limits for heavy metals for chromium glycinate nicotinate chelate**

| Test | Specification |
|---|---|
| Lead (mg/kg)<br>Cadmium (mg/kg) | Less than 1.5 mg/kg<br>Less than 0.5 mg/kg |

Analysis data of 7 non-consecutive batches of the commercial product shows that the chromium content varies between 2.66 and 2.99 % and lead and cadmium are always < 0.1 mg/kg.

## 2.2 Manufacturing processes

### 2.2.1. Introduction

The petitioners indicate that glycine and nicotinic acid used to chelate the different metal ions meets USP grade specifications and is equivalent to food-grade material. Glycine (synthetic or natural) is permitted in the EU for use in foods under Directive 2001/15/EC (EC, 2001) on substances that may be added for specific nutritional purposes in foods for particular nutritional uses (PARNUTS) (EC, 2001) for the foods for special medical purposes. Glycine and its salts (E640) are also permitted as food additives in the EU under Directive 95/2/EC on food additives other than colours and sweeteners (EC, 1995). The glycine used in the production of the bisglycinates conforms to the EU specification as declared within Commission Directive 2000/63/EC (EC, 2000) amending Directive 96/77/EC laying down specific purity criteria of food additives other than colours and sweeteners (EC, 1996, 2000). Other processing chemicals (pH adjustment, flow agents, *etc*.) meet specifications appropriate for food-use. All other ingredients used in the formulation of the commercial products, meet specifications appropriate for food-use.

### 2.2.2. Methods of Manufacturing

The details of the manufacturing processes for the commercial formulation of the chelates are given by the petitioners in the application dossiers. This is considered by the Panel to be sufficient.

## 2.3 Methods of analysis

The respective chelates are first digested under strong acidic conditions at temperatures between 95 and 130°C. The sample digest is then assayed for the different metals content using a calibrated and validated Inductively Coupled Plasma/Atomic Emission Spectrometry (ICP/AES). The methods are described in detail by the petitioner in the application dossier. Glycine can be identified as indicated in EU Commission Directive 2000/63/EC (EC, 2000).

## 2.4 Reaction and fate in foods

The petitioners provided only information related to the stability of the sources in food supplements. As regards copper bisglycinate, in a 17-month assay, there was no interaction reported between the contents of a capsule, which contained 0.45 to 0.55 mg copper as copper bisglycinate and other metal amino acid chelates of zinc, and iron at levels of 1.37 to 1.67, 0.86 to 1.05, and 1.8 to 2.2 mg, respectively, and 36 to 44 µg of chromium (as chromium glycinate nicotinate chelate).

Similarly, in a 17-month assay, there was no interaction reported between the contents of a capsule, which included 1.37 to 1.67 mg zinc as zinc bisglycinate and other metal amino acid chelates of copper, and iron (supplied as ferrous bisglycinate) at levels respectively of 0.45 to 0.55, 0.86 to 1.05, and 1.8 to 2.2 mg and of 36 to 44 µg of chromium as chelate product

In both stability studies also a multivitamin preparation (Vitamins A, C, D and E, as well as the Vitamin B series) was included. The stability of the chelated products was demonstrated by a lack of interaction between the metal amino acid chelates and any of the components of the capsules, including the vitamins, which were reported to retain levels within the specifications of the USP for the duration of the study period (Ashmead and Ashmead, 1995).

There are no specific data on stability in foods. However, based on the results of the stability studies of the sources in food supplements, interaction of the sources with food components is not to be expected.

## 3. Case of Need and Proposed Uses

According to the petitioner the forms or sources of minerals have a bearing on their relative bioavailability and toxicology/safety. A study by Hillman (1996) indicates that e.g., mineral oxides are less soluble than other sources of minerals and resist further digestion. They are usually less bioavailable than the more soluble/ionisable inorganic salts. Organic forms of minerals are often more bioavailable than inorganic salts.

The naturally occurring amino acids are ligands that confer both increased bioavailability and complete utilisability once within the body. Such chelates provide advantages over other synthetic ligands, such as EDTA, where it has been observed that the ligated material occurs intact in human urine or alternatively ligated to essential elements in the body, which can lead to the unnecessary removal of these essential compounds (Pineda *et al.*, 1994).

Amino acid chelates are better absorbed than many other mineral sources (Heaney *et al.*, 1990; Pineda *et al.*, 1994; Bovell-Benjamin *et al.*, 2000).

The mineral bisglycinates considered in this application are intended for use as a direct replacement for the permitted respective mineral forms of copper and chromium for nutritional purposes in food supplements according to Council Directive 178/2002, calcium and magnesium for nutritional purposes in food supplements and the categories of PARNUTS other than for baby foods and infant formula according to Council Directive 89/398/EEC and for zinc for nutritional purposes in foods for the general population (including food supplements) and PARNUTS.

According to the petitioners the amino acid chelates are used by food supplement manufacturers as an ingredient in tablets, caplets, capsules, chewable tablets, effervescent powders and liquids that are food supplements. The method of incorporation is determined by the individual manufacturers as appropriate for the particular type of finished products.

## 4. Exposure

No specific use levels for the amino acid chelates under consideration in this opinion have been given. Tolerable Upper Intake Levels have been established in adults for four of the minerals considered: 5 mg/day for copper (SCF, 2003a), 25 mg/day for zinc (SCF, 2003c), 2500 mg/day for calcium (SCF, 2003b), 250 mg/day for magnesium was established for readily dissociable magnesium salts (SCF, 2001). No Tolerable Upper Intake Level was established for chromium(III) but the SCF (SCF, 2003e) noted that no adverse effects were associated with supplementary intake of chromium up to 1 mg/day.

Anticipated levels of exposure to glycine were calculated based on these Tolerable Upper Intake Levels for copper, zinc, calcium and magnesium by considering that they would entirely be ingested in the bisglycinate form: 11.7 mg glycine/day for copper bisglycinate, 56.6 mg glycine/day for zinc bisglycinate, 9239 mg glycine/day for calcium bisglycinate and 1523 mg glycine/day for magnesium bisglycinate. An intake of 1 mg/day of chromium in the form of chromium glycinate nicotinate would give rise to an intake of about 3.3 mg nicotinic acid/day and to 2.2 mg glycine/day.

The intake of glycine from proteins in meat and seeds (soya, flax, canola) was also considered. Average percentages of glycine in proteins of 1% for meat and 6% for seeds are reported (Chung *et al.,* 2005; Toldra *et al.,* 1995). Based on these data and on French consumption data

(INCA survey, 2000) the average intake of glycine was calculated for both food of animal (pork and pork-based-products, chicken, fish, beef, poultry and game) and vegetal origin (soya shoots, soya desserts, tofu, soya drinks, soya sauce, canola oil). The Panel estimated a mean intake of 26.2 mg/kg bw/day for adults (≥ 15 years) with a 97.5$^{th}$ percentile of 54.7 mg/kg bw/day and a mean intake of 43.1 mg/kg bw/day for children (3 to 14 years) with a 97.5$^{th}$ percentile of 101.4 mg/kg bw/day was obtained.

## 5. Information on Existing Authorisations and Evaluations

According to the petitioners the amino acid chelates of copper, zinc, calcium, magnesium and chromium (III) are currently utilised for dietary supplements and/or food fortification in North America (United States, Canada); Latin America (Argentina, Brazil, Guatemala); Europe (Austria, Belgium, Czech Republic, Denmark, Finland, France, Portugal, Spain, Sweden, Germany, United Kingdom); and Asia (Taiwan, Thailand); as well as Australia, Pakistan and South Africa.

The substances are freely sold in the United States under the 1994 Dietary Supplement Health and Education Act (DSHEA) due to their long history of safe use.

Ferrous bisglycinate, meeting certain specifications, was evaluated by the EFSA (EFSA, 2006) as a source of iron in foods intended for the general population, food supplements, and foods for particular nutritional uses including foods intended for infants and young children, and it was concluded that these uses do not present a safety concern.

Glycine (synthetic or natural) is already permitted in the EU for use in foods under Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses (PARNUTS). Glycine and its salts (E640) are also permitted as food additives in the EU under Directive 95/2/EC on food additives other than colours and sweeteners.

## 6. Biological and toxicological data

### 6.1 Biological data

One of the petitioners indicates that the metal amino acid chelates used in the studies described below contain, in part, amino acids derived from hydrolysed vegetable protein (soya). The petitioner also indicates that the manufacturing formulae for some of these products may also contain commercially available glycine. Where glycine is added by formulation for part of the chelating ligands, an increased potential to create metal bisglycinate chelates is achieved.

#### 6.1.1. Bioavailability of the mineral-bisglycinates following oral consumption

A study by Kirchhoff (1983) showed that the absorption of minerals presented as chelates is more effective than minerals that are present in the cationic state. This seems to be due to the fact that cationic minerals must be chelated by proteins in the cell wall prior to absorption, thus slowing down the process. No additional chelation of the amino acid chelates is required at the brush border of the cell membrane thus making the membrane transport more rapid.

##### 6.1.1.1. Copper bisglycinate

In *in vitro* studies in rat jenunal slices on the absorption of copper contained in inorganic compounds was compared with chelates from hydrolysed protein sources.

The rats were previously maintained on a commercial diet adequately fortified with copper to ensure no forced tissue uptake of minerals. The slices were incubated with solutions of inorganic copper sources or copper amino acid chelate, utilising ligands derived from plant (soy) and casein proteins. The chelated copper and the inorganic salts contained an equivalent mineral concentration.

The results showed that the uptake of copper from the amino acid chelate (from plant origin) was 35 mg copper/kg as compared to the uptake of 8 mg copper/kg from copper sulphate, 11 mg copper/kg from copper oxide, 12 mg copper/kg from copper carbonate. Control values (trace values) were well below the values obtained for tissue samples incubated with the respective chelated or inorganic mineral solutions (Ashmead *et al*., 1985).

A study in humans (23 patients: 6 males, 17 females) was conducted on the effect of copper amino acid chelate supplementation on superoxide dismutase (SOD) in rheumatoid arthritis patients. The participants ranged in age from 35 to 53 years and were suffering from rheumatoid arthritis. Forty-eight healthy age-matched individuals were also selected to serve as a control group. Blood samples were taken to determine baseline mineral levels. Participants were then given 2 mg of copper amino acid chelate per day for 4 weeks. After the treatment an analysis of Cu-Zn SOD activity was carried out in the blood.

The results showed that the copper amino acid chelate supplementation increased erythrocyte Cu-Zn SOD activity in 18 of 23 rheumatoid arthritis patients. The average increase was 21%, showing that the copper chelate was absorbed and was effective in increasing erythrocyte Cu-Zn SOD activity in the majority of rheumatoid arthritis patients (DiSilvestro *et al*., 1992).

#### 6.1.1.2. Zinc bisglycinate

In an *in vitro* study analogous with the one described above for copper, rat jejunal slices obtained from Sprague-Dawley rats were incubated for equal times with solutions of inorganic zinc sources or with zinc amino acid chelate utilising ligands derived from plant (soy) protein. The chelated zinc and the inorganic zinc salts contained an equivalent mineral concentration. After incubation, washing and drying, the tissue samples were analysed for metal content and were compared to untreated intestinal segment controls.

The results showed that the intestinal uptake of zinc was considerably greater for the zinc amino acid chelate solution (*i.e.* 191 mg Zn/kg) compared to the uptake from zinc sulphate (84 mg Zn/kg), zinc oxide (66 mg Zn/kg) or zinc carbonate (87 mg Zn/kg). Control values (14 mg Zn/kg) were well below the values obtained for tissue samples incubated with the respective chelated or inorganic mineral solutions (Ashmead *et al*., 1985).

Several studies in animals regarding the uptake of zinc from zinc amino acid chelate have been conducted.

In an *in vivo* study with Albino rats the extent of absorption of zinc from radiolabelled zinc ($^{65}$Zn) amino acid chelate was compared to its uptake from zinc chloride. In the study the rats received the same amount of radioactive zinc, either as zinc chloride or as $^{65}$Zn chelate, by gavage. After dosing, the rats were placed in metabolic cages on a normal diet (commercial rat chow) and observed for one week during which time the faeces were collected. The rats were then sacrificed and total radioactivity present in the faeces of each rat was measured.

The results showed that during the 7-day period more than 50% of the $^{65}ZnCl_2$ was excreted in the faeces. In comparison, only 12% of the radiolabelled $^{65}$Zn amino acid chelate was excreted during the same time period. The results indicate that the zinc amino acid chelate was retained within the animal significantly better than the $^{65}ZnCl_2$ group (Ashmead *et al*., 1975).

Another *in vivo* study was carried out to compare the rate of absorption and length of time for incorporation of $^{65}$Zn amino acid chelate versus $^{65}$Zn chloride into the blood of two groups of 4 male Sprague-Dawley rats. The animals received a single oral dose of 5 µg $^{65}$Zn either as $^{65}ZnCl_2$ or $^{65}$Zn bisglycinate under light anesthesia following a 24-hour fast. Blood was taken from each animal by suborbital bleeding at specific intervals for 4 hours post dosing. Blood samples were assayed for $^{65}$Zn.

The results indicated that the rates of absorption of both sources of zinc are rapid. Increases in plasma levels occurred within 30 minutes. The major difference between the two sources however, was that the $^{65}$Zn bisglycinate yielded 30% higher blood levels, indicating an increased level of absorption (Peck and Graff, 1973).

The same authors carried out an *in vivo* study whereby two groups of 4 male Sprague-Dawley rats each received an intra-peritoneal dose of 5.0 µg of $^{65}$Zn as either $^{65}$Zn bisglycinate or as $^{65}$Zn chloride following a 24-hour fast. Four hours post dosing, each animal was sacrificed and a number of tissues were excised including thigh muscle tissue, left ventricle, liver, kidney and right cerebrum of brain. Each tissue sample was assayed for radioactivity. It was shown that the zinc deposition from $^{65}$Zn bisglycinate absorption was greater in muscle, kidney and brain (Peck and Graff, 1973).

In a study with dogs, a zinc amino acid chelate was evaluated to determine the bioavailability compared to zinc oxide with and without a calcium antagonist in the diet. Using a randomized block design, 4 adult beagles were first given a commercial control diet with zinc oxide for a period of 20 days. The animals were then given a diet supplemented with 50 mg/kg bw of Zn as oxide or amino acid chelate in the presence of or absence of 20 g/kg bw calcium. Zinc balance was determined using faecal collection for 5 days. Hair growth and hair zinc content was also determined. The authors found that the negative effect of calcium can be overcome through the use of zinc amino acid chelate, and that the zinc amino acid chelate treatment resulted in more hair and higher zinc content of the hair, suggesting higher bioavailability even in the face of dietary antagonisms (Lowe *et al.*, 1994).

In a study with humans, the level of absorption of zinc from Zn amino acid chelate was measured in ten healthy adult males (mean age: 35 years). Participants underwent a stabilisation period of 45 days during which no nutritional supplements were given. The diet was monitored to ensure that the intake of essential nutrients was within the normal range. Blood and urine were then taken from each participant to determine baseline levels. Participants were then given 25 mg Zn amino acid chelate 3 times per day for a total of 75 mg/day for a period of 90 days. Samples were again taken from participants and the results compared to baseline.

The results showed a rise of over 40% (from 91.63 mg % to 131.25 mg %) in blood serum levels of zinc. In comparison, no rise in urinary zinc levels was detected (Ashmead, Unpublished report cited in the application dossier).

In another study with humans on the bioavailability of analogous amino acid chelates, the bioavailability of zinc from zinc histidine complexes as compared to zinc sulphate in 10 healthy volunteers was determined. Ingestion of zinc complexed with histidine at a ratio of 1:2 or 1:12 increased serum-zinc concentration 25% more than ingestion of zinc sulphate. Calculated uptake was 30 to 40% increased with zinc histidine compared with zinc sulphate. Urinary excretion was not different with any preparation. Application of 15 mg zinc as zinc histidine complex (1:2) gave an identical serum response as 45 mg zinc sulphate suggesting that the zinc histidine complex was better absorbed and subsequently, more bioavailable than zinc sulphate (Schölmerich *et al.*, 1987).

6.1.1.3. Calcium bisglycinate

The petitioners indicate that no *in vitro* studies have been conducted on calcium bisglycinate.

In a study in humans to determine the absorption of calcium from calcium bisglycinate in comparison to other sources of calcium, a group of adult women (age from 20 to 40 years) with normal menstrual periods and no prior history of absorption disorders was selected. Most of the subjects were studied in the follicular phase of their menstrual cycles. The test subjects were given various forms of calcium (calcium bisglycinate, calcium citrate malate, calcium carbonate, tricalcium phosphate, calcium citrate, hydroxyapatite, calcium oxalate), which had been labeled with either $^{45}Ca$ or $^{47}Ca$.

The data show that the percentage absorption of calcium from Ca-bisglycinate was 44. 0 ± 10.4 % which is higher than the absorption of calcium from the other Ca-sources which varied between 16.6 ± 9.0 % and 24.2 ± 4.9 % (Heaney, 1998, Unpublished report cited in the application dossier).

A study conducted by Greger *et al*. (1987) showed similar bioavailability for a number of different calcium sources including milk, dibasic calcium phosphate, oyster shell, calcium carbonate, calcium lactate, calcium bisglycinate and dolomite.

6.1.1.4. Magnesium bisglycinate

The petitioners state that no *in vitro* studies have been conducted on magnesium bisglycinate.

There have been a number of studies with humans on the bioavailability of magnesium from magnesium amino acid chelate.

In a study to assess the absorption and retention characteristics of an oral dosage of magnesium amino acid chelate in human subjects, twenty-one employees (18 women and 3 men, ages ranging from 21 to 37 years) in a hospital in the USA were selected. Eleven subjects were given orally 360 mg/day of Mg-amino acid chelate and 10 subjects were given placebo capsules containing cellulose. The subjects were given an intravenous magnesium infusion at baseline and after 4 and 8 weeks of treatment and urinary magnesium and magnesium retention were measured.

The data show that following the magnesium infusion, urinary magnesium increased significantly (from 16.5+42.5 mg Mg/24h to 146.3+39.8 mg Mg/24h) in the test group but remained relatively constant in the placebo group. Similarly, the amount of magnesium retained from the infusion decreased significantly in the test group but remained constant in the placebo group.

From this the authors concluded that, since in the test group more magnesium from the infusion was excreted and less was retained, the data demonstrated that (i) the oral Mg-amino acid chelate was absorbed, and that (ii) the Mg-amino acid chelate was taken up by body tissues, thus reducing the need for magnesium from the infusion (Yang *et al*., 1989, Unpublished report cited in the application dossier).

6.1.1.5. Chromium glycinate nicotinate chelate

According to the petitioner there are no specific *in vitro* studies currently available for chromium glycinate nicotinate chelate.

In an animal study, designed to determine the absorption of chromium from a chromium amino acid chelate (composition not specified by the petitioner in the application) in comparison to the absorption of chromium from inorganic chromium (III) chloride, two groups of rats (not

further specified) were slightly anesthetised and then intragastrically intubated with equal amounts of chromium as either $^{51}CrCl_3$ or the $^{51}$Cr-amino acid chelate. Blood was drawn at 1 hour intervals for 3 hours and equal volumes (100 µl) were measured for corrected disintegration counts per minute.

Data show that the absorption of chromium nearly doubled when supplied as chromium amino acid chelate, in comparison to inorganic chromic (III) chloride (Graff 1992, Unpublished report cited in the application dossier).

No direct data from studies with humans regarding the absorption of chromium are currently available for chromium glycinate nicotinate chelate. However, based on data obtained with regard to ferrous bisglycinate (EFSA, 2006), the petitioner anticipates that chromium from foods fortified with any chromium fortificant form will be more readily available than chromium from unfortified foods. In addition, chromium as a fortificant in the form of a chromium amino acid chelate will be no less available than chromium from inorganic forms.

A review article by Lukaski (1999) summarised two articles on the absorption of chromium. Lukaski stated that amino acids when chelating the dietary chromium prevent precipitation within the alkaline milieu of the small intestine. Similarly, nicotinic acid when administered with trivalent chromium will enhance absorption. In a radio-isotope study, it was found that $^{51}$Cr as nicotinate had significantly greater short-term retention (1-12 h post-gavage) in muscle, liver, kidney, blood and urine compared to the chromium chloride or chromium picolinate. In another study summarised by Lukaski, it was found that Cr nicotinate promoted Cr accumulation in the kidney and that nicotinate, like picolinate and acetate, increased Cr incorporation into the liver.

## 6.2 Toxicological data

The toxicity of the cations copper, zinc, calcium, magnesium and chromium has already been evaluated, and Tolerable Upper Intake Levels have been established (SCF, 1990; SCF, 2001; SCF, 2003d; EVM, 2003). Also nicotinic acid and nicotinamide have been evaluated and ULs have been derived (SCF, 2002).

As regards glycine, the SCF reviewed the nutritional, biochemical and toxicological information on glycine and concluded that, if used at levels corresponding to good manufacturing practice, no nutritional or toxicological hazards arise to man (SCF, 1990).

### 6.2.1. Subchronic toxicity

Presently, there are no specific studies on the subchronic toxicity of the bisglycinates of copper, zinc, calcium, magnesium or chromium (III). However, due to the similarity in chemical structure between these bisglycinates and ferrous bisglycinate, the petitioner anticipates that the bisglycinates under consideration in this opinion will exhibit similar subchronic toxicological characteristics as their ferrous bisglycinate counterpart. The Panel agrees that these studies can be used to assess the subchronic toxicity of the glycinates. These characteristics have been fully described in the EFSA opinion on ferrous bisglycinate in 2006 (EFSA, 2006). In the study described in the ferrous bisglycinate opinion, no rats subjected to a 13-week study with treatments corresponding to 0, 100, 250, and 500 mg ferrous bisglycinate/kg bw/day died. Histopathological examination revealed no biologically or statistically significant, dose-dependent, macroscopic or microscopic findings that could be attributed to the treatment. Non-linear increases in mean hepatic non-haem iron concentrations indicated the existence of a physiological control on the absorption and distribution of the iron bisglycinate. Jeppsen and Borzelleca (1999) and Mandella (2000) reported a NOAEL of 500

mg/kg bw/day for ferrous bisglycinate in rats (the highest dose tested), corresponding to approximately 100 mg iron/kg bw/day and to approximately 400 mg glycinate/kg bw/day.

### 6.2.2. Genotoxicity

Genetic toxicity studies have not been conducted on calcium bisglycinate or magnesium bisglycinate.

The SCF makes reference to some *in vitro* and *in vivo* genotoxicity tests on other forms of zinc that have shown positive findings at elevated dosages, however, the SCF concluded that the weight of evidence from these studies did not indicate biologically relevant genotoxicity (SCF, 2003c).

The SCF in 2003, concluded that although chromium (III) compounds may bind to DNA and produce DNA-protein cross-links under certain circumstances they generally they did not produce gene mutations, sister chromatid exchanges or cell transformation in cultured mammalian cells. Weak clastogenic effects have been observed in some mammalian *in vitro* systems at relatively high and cytotoxic concentrations. No induction of genetic damage or micronuclei has been observed in experimental animals (SCF, 2003e). Therefore even though in vitro data show that Cr(III) has the potential to react with DNA and to cause DNA damage in cell culture systems, the available *in vivo* evidence suggests that genotoxic effects are not expected to occur in humans or animals exposed to nutritional or recommended supplemental levels of Cr(III) (Eastmond *et al*., 2008).

### 6.2.3 Longer-term feeding studies

The petitioner provided the results of two sets of longer-term feeding studies for the cations, copper, magnesium and zinc (Jeppsen, 1987; Jeppsen, 1990). The studies were carried out with production pigs (sows) that received feed rations containing amino acid chelates of the above mentioned cations, derived from hydrolysed soy protein. The Panel noted that, although this hydrolysate contained approximately 6% glycine, the resultant chelates were not characterised and are not directly equivalent to the chelates covered by this opinion. The dietary metal amino acid chelates were provided as extra supplementation and were not formulated to meet all of the nutritional needs of the pig. Metals in various other natural and non-chelated forms were already present in the dietary rations.

The first set of studies (Jeppsen 1987) were performed on a test farm where the animals had been administered the respective metal-amino acid chelates over the course of several years. They are considered by the petitioner as multigenerational studies representative for various filial generations of sows. The second set of studies (Jeppsen 1990) was conducted to assure that there was no toxicity due to the ingestion of the chelates by sows near the end of their reproductive lifetime.

The results of these studies showed that the respective metal-chelates were devoid of cumulative teratogenic effects and chronic morbidity, based both on practical observations and on observations at the macroscopic and microscopic level (Jeppsen, 1987). There were no significant differences in the gross examinations between the test and control group of sows and no histopathological tissue alterations were observed which could be attributed to metal-amino acid chelate. Haematological examinations likewise did not reveal any abnormalities related to administration of the test product (Jeppsen, 1990).

In a more recent study with pigs by Jeppsen (2005), magnesium amino acid chelate was administered as a dietary supplement to the sows throughout their life spans. The cumulative ingestion of magnesium per individual sow between the start of the study and its age at

sacrifice (range from approximately 38 to 52.5 months) varied between 93.7 g and 124.9 g. In comparison with control sows of similar age and parity, there were no observable gross or microscopic findings attributable to administration of the test product.

There are no specific chronic toxicity or carcinogenicity studies available on the individual metal bisglycinates.

There are also no specific chronic toxicity or carcinogenicity studies available on chromium glycinate nicotinate.

### 6.2.4 Reproductive and Developmental Toxicity

Reproductive toxicity studies in laboratory animals have not been conducted with copper amino acid chelates, zinc amino acid chelates, calcium amino acid chelates and magnesium amino acid chelates. However, in the longer term toxicity studies described above where sows received dietary supplementation with Cu-amino acid chelate throughout a period covering multiple litters, no adverse effects on reproduction or on the resulting offspring were observed.

## Discussion

The bisglycinates considered in this opinion consist of a bivalent metal ion, namely $Cu^{+2}$, $Zn^{+2}$, $Ca^{+2}$, and $Mg^{+2}$, linked to two molecules of glycine. The metal is bound to the carboxyl group and to the α-amino group of glycine with coordinate covalent bonds to form two heterocyclic rings. This 1:2 metal to ligand ratio restricts reaction with dietary inhibitors of the metal absorption and does not participate in oxidation reactions.

No specific use levels for the mineral bisglycinates under consideration in this opinion have been given. However, based on the information provided by the petitioner, under the conditions of intended use, the daily intake would not exceed those levels anticipated through existing supplementation of the listed minerals and would be similar to other forms of copper, zinc, calcium and magnesium and chromium that are already approved for use in foods in the EU.

Regarding the bioavailability of the different cations from their sources, data are provided showing that the minerals are bioavailable after oral administration.

No genetic toxicity studies have been conducted on the compounds considered in this opinion. However the Panel has no concern on the genotoxicity of glycine and nicotinic acid, nor on any of the metal cations under the expected conditions of use.

Due to the similarity in chemical structure between the metal glycinates considered in the present application and ferrous bisglycinate it is anticipated that the glycine part of these glycinates will exhibit similar toxicological characteristics as their ferrous bisglycinate counterpart, the safety of which was already evaluated and accepted by the AFC Panel in 2006. The Panel agrees that the subchronic studies on ferrous bisglycinate can be used to assess the subchronic toxicity of the glycinates. From the studies a NOAEL of 500 mg/kg bw/day for ferrous bisglycinate in rats (the highest dose tested) was derived, corresponding to approximately 400 mg glycinate/kg bw/day.

*In vitro* data show that Cr(III) has the potential to react with DNA and to cause DNA damage in cell culture systems, however, data from *in vivo* studies suggest that genotoxic effects are not expected to occur in humans or animals exposed to nutritional or recommended supplemental levels of Cr(III).

Specific chronic toxicity or carcinogenicity studies are not available.

Specific reproductive toxicity and developmental toxicity studies on the bisglycinates are also not available. However, in the longer-term feeding studies with livestock (female pigs) receiving dietary supplementation with mineral glycinates throughout a period covering multiple litters, no adverse effects on reproduction or on the resulting offspring were observed.

The Panel noted that these longer-term feeding studies are of limited value for the assessment of either chronic toxicity or carcinogenicity of the chelates, due to the relatively short duration of the studies relative to the life span of the pig and the small numbers of animals used in the studies.

A conservative estimate of the dietary exposure was made based on a hypothetical intake from all sources (PARNUTS, food supplements and foods intended for the general population) at the tolerable upper intake levels for copper (5 mg/day), zinc (25 mg/day), calcium (2500 mg/day) and magnesium (250 mg/day). Assuming that the mineral amino acid chelates concerned in this opinion would entirely be ingested in the bisglycinate form the equivalent exposure to glycine would be around 12 mg glycine/day for copper bisglycinate, 57 mg glycine/day for zinc bisglycinate, 9239 mg glycine/day for calcium bisglycinate and 1523 mg glycine/day for magnesium bisglycinate. The Panel noted that this estimated exposure is lower than the NOAEL of 400 mg glycinate/kg bw/day, the highest dose tested.

An intake of 1 mg of chromium under the form of chromium glycinate nicotinate would result in an exposure to approximately 3.3 mg nicotinic acid/day and 2.2 mg glycine/day. The Panel considered that such levels are not of safety concern.

In addition the normal (mean) intake of glycine in proteins from both food of animal origin (beef, pork, poultry, game, fish; glycine present at an estimated level of 1%), and vegetable origin (soya shoots, soya desserts, tofu, soya drinks, soya sauce, canola oil; glycine present at an estimated level of 6%) was calculated to be about 26 mg/kg bw/day for adults (>15 years) and to about 43 mg/kg bw/day for children (< 15 years).

## CONCLUSION

The present opinion deals only with the safety of bisglycinate chelates of copper, zinc, calcium, magnesium, and of glycinate nicotinate as sources of the nutrient cations of respectively copper, zinc, calcium, magnesium and chromium and with the bioavailability of the nutrient cations from these sources. The safety of the nutrient cations themselves (copper, chromium, zinc, calcium and magnesium), in terms of amounts that may be consumed, is outside the remit of this Panel.

The Panel concludes that the use of copper bisglycinate chelate as a source for copper added for nutritional purposes to food supplements, and of calcium bisglycinate chelate and magnesium bisglycinate chelate as sources for respectively calcium and magnesium added for nutritional purposes to foods for particular nutritional uses and food supplements, and of zinc bisglycinate chelate when used as a source for zinc in foods intended for the general population (including food supplements) and foods for particular nutritional uses, is not of safety concern.

As regards chromium glycinate nicotinate complex, due to lack of information on the specific identity of its components, the Panel is unable to reach a conclusion on the safety of this source and on the bioavailability of chromium from this source.

## DOCUMENTATION PROVIDED TO EFSA

1. Application for the approval of Copper bisglycinate (amino acid) chelate as a source for copper for use in the manufacture of foods, July 13, 2006; revised October 15, 2007. Submitted by Albion Laboratories, Clearfield, Utah, USA.
2. Application for the approval of Chromium glycinate nicotinate hydrochloride as a source for chromium for use in the manufacture of food supplements, July 13, 2006; revised October 15, 2007. Submitted by Albion Laboratories, Clearfield, Utah, USA.
3. Application for the approval of Zinc bisglycinate (amino acid) chelate as a source for zinc for use in the manufacture of foods, July 13, 2006; revised October 15, 2007. Submitted by Albion Laboratories, Clearfield, Utah, USA.
4. Application for the approval of Calcium bisglycinate (amino acid) chelate as a source for calcium for use in the manufacture of foods, July 13, 2006; Submitted by Albion Laboratories, Clearfield, Utah, USA.
5. Application for the approval of Magnesium bisglycinate (amino acid) chelate as a source for magnesium for use in the manufacture of foods, July 13, 2006; Submitted by Albion Laboratories, Clearfield, Utah, USA.
6. Dossier on Copper Glycinate Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements; June, 2005; Submitted by Health Food Manufacturers Association UK.
7. Dossier on Zinc Glycinate Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements; June, 2005; Submitted by Health Food Manufacturers Association UK.
8. Dossier on Calcium Glycinate Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements; June, 2005; Submitted by Health Food Manufacturers Association UK.
9. Dossier on Magnesium Glycinate Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements; June, 2005; Submitted by Health Food Manufacturers Association UK.

## REFERENCES

Allen, L.H., 2002. Advantages and limitations of iron amino acid chelates as iron fortificants. *Nutr. Rev.*, 60, S18-S21.

Atkins, P.W., Beran, J.A., 1992. *General Chemistry, 2nd Edition*. Scientific American Books. WH Freeman, New York, pp. 804-809.

Ashmead, H., Jensen, N., Graff, D., 1975. Radioactive study of amino acid chelated zinc. Unpublished report.

Ashmead, Unpublished report cited in the application dossier.

Ashmead, S.D., 2001. The chemistry of ferrous bis-glycinate chelate. *Arch. Latinoam Nutr.*, 51, 7-12.

Ashmead, H., Ashmead, S., 1995. Albion research: Past, present and future. In: *Proceedings of the International Conference on Human Nutrition*, Salt Lake City, Utah, January 21-22, 1995. Albion Laboratories, Inc., pp. 1-8.

Ashmead, H.D., Graff, D.J. Ashmead, H.H., 1985. *Intestinal Absorption of Metal Ions and Chelates*. Charles C Thomas, Springfield, Ill., pp. 118-125.

Bovell-Benjamin, A.C., Viteri, F.E., Allen, L.H., 2000. Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. *Am. J. Clin. Nutr.*, 71, 1563-1569.

Chung, M., Lei, B., Li-Chan, E., 2005. Isolation and structural characterization of the major protein fraction from NorMan flaxseed (*Linum usitatissimum* L.) *Food Chemistry 90, 271-279.*

DiSilvestro, R.A., Marten, J., Skehan, M., 1992. Effects of copper supplementation on ceruloplasmin and copper-zinc superoxide dismutase in free-living rheumatoid arthritis patients. *J. Am. Coll. Nutr.* 11, 177-180.

Eastmond DA, Macgregor JT, Slesinski RS., 2008. Trivalent chromium: assessing the genotoxic risk of an essential trace elementand widely used human and animal nutritional supplement. Crit Rev Toxicol. 38(3):173-90.

EC (European Commission), 1995. European Parliament and Council Directive No. 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners [amended to Nov. 20, 2003]. *Official Journal* 38, L 61, 18.3.1995, P. 1-40.

EC (European Commission), 1996. Commission Directive 96/77/EC of 2 December 1996 laying down specific purity criteria on food additives other than colours and sweeteners. *Official Journal* 39, L 339, 30.12.1996 P. 1–69.

EC (European Commission), 2000. Commission Directive 2000/63/EC of 5 October 2000 amending Directive 96/77/EC laying down specific purity criteria of food additives other than colours and sweeteners. *Official Journal* 43, L 277, 28.10.2000, P.1-61.

EC (European Commission), 2001. Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. *Official Journal* 44, L 52, 22.2.2001, P. 19-25.

EC (European Commission), 2002. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. *Official Journal* 45, L 183, 12.7.2002, P. 51-57.

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact With Food on a Request from the Commission related to Ferrous bisglycinate as a Source of Iron for use in the Manufacturing of Foods and in Food Supplements. European Food Safety Authority (EFSA), Brussels, EFSA Journal, Vol. 299, 1-17. (Question No EFSA-Q-2005-039. Adopted on 6 January 2006 by written procedure).

http://www.efsa.europa.eu/science/afc/afc_opinions/1319/afc_op_ej299_ferrousbisglycinate_opinion_en3.pdf.

EVM (Expert Group on Vitamins and Minerals), 2003. Risk Assessment: Calcium. In: *Safe Upper Levels for Vitamins and Minerals*. Report of the Expert Group on Vitamins and Minerals, London, Engl. pp. 264-273.

http://www.food.gov.uk/multimedia/pdfs/vitmin2003.pdf.

Graff, D., 1992. Improvement in intestinal absorption of Albion® Chromium amino acid chelate as compared to chromium trichloride, Unpublished report cited in the application dossier.

Greger, J.L., Krzykowsky, C.E., Khazen, R.R., Krashoc, C.L., 1987. Mineral utilization by rats fed various commercially available calcium supplements or milk. *J. Nutr.,* 117-724.

Heaney, R.P., 1998. *Calcium: Important for Health and Bioavailability of Various Sources*. Albion International Conference of Human Nutrition (cited in application dossier without reference).

Heaney, R.P., Recker, R.R., Weaver, C.M., 1990. Absorbability of calcium sources: The limited role of solubility. *Calcif. Tissue Int.*, 46, 300-304.

Hillman, R.S., 1996. Hematopoietic agents: growth factors, minerals, and vitamins. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., Gilman, A.G. (Eds.). *Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition*. McGraw-Hill, New York, p.1320.

INCA , 2000. Volatier, J.L. (coordinator), Enquête INCA individuelle et nationale sur les consommations alimentaires, editions TEC&DOC.

Jeppsen, R.B., 1987. Assessment of long-term feeding of Albion Copper Amino Acid Chelate. Unpublished report.

Jeppsen, R.B.; Borzelleca, J.F., 1999. Safety evaluation of ferrous bisglycinate chelate. *Food Chem. Toxicol.*, 37, 723-731.

Jeppsen, R.B., 1990. Examination of long-term feeding of Albion® Copper Amino Acid Chelate in sows achieving 6 or greater parity. Unpublished report.

Jeppsen, R.B., 2001. Toxicology and safety of ferrous bisglycinate chelate and other iron amino acid chelates. *Arch. Latinoam Nutr.*, 51, 26-34.

Jeppsen, R.B., 2005. Examination of long-term feeding of Albion® Magnesium Amino Acid Chelate in sows achieving six or greater parity. Albion Laboratories Inc., Research Report cited in the application dossier.

Kirchhoff, H.W., 1983. The treatment of iron deficiency anemia with iron chelate tablets. *Therapiewoche,* 33/37, 4833-4842.

Lowe, J.A., Wiseman, J. Cole, D.J.A., 1994. Zinc source influences zinc retention in hair and hair growth in the dog. *J. Nutr.* 124, 2575S-2576 S.

Lukaski, H.C., 1999. Chromium as a supplement. *Annu. Rev. Nutr.* 19, 279-302.

Mandella, R.C., 2000. *A Subchronic (3-Month) Toxicity Study of Ferrochel in the Rat Administered via Diet: Final Report*. Submitted to: EFSA by Albion Laboratories, Inc., Clearfield, Utah, 2000, [Huntingdon Life Sciences Inc., Unpublished Report No. 96-2503, East Millstone, New Jersey].

McMurray, J., Fay, R.C., 1995. *Chemistry*. Prentice Hall, New Jersey, pp. 812-817.

Peck, A., Graff, D.J., 1973. The absorption and metabolism of hot zinc chloride versus hot zinc amino acid chelate. Unpublished report.

Pineda, O., Ashmead, H.D., Perez, J.M., Lemus, C.P., 1994. Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. *J. Appl. Nutr.*, 46, 2-13.

SCF (EC, Scientific Committee for Food), 1990. *Report of the Scientific Committee for Food (25th Series)*. Opinion expressed on 18 May 1990.

http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_25.pdf.

SCF (EC, Scientific Committee for Food), 1993. Copper: In: *Nutrient and Energy Intakes for the European Community (Opinion Expressed on 11 December 1992)*. Commission of the European Communities (EEC), Scientific Committee for Food (SCF), Brussels, Belgium. Reports of the Scientific Committee for Food, 31st Series, pp. 196-201. www.europa.eu.int/comm/food/fs/sc/scf/out89.pdf.

SCF (EC, Scientific Committee for Food), 2001. *Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Magnesium (Expressed on 26 September 2001)*. European Commission, Health & Consumer Protection Directorate-General, Directorate C – Scientific Opinions, C2 - Management of Scientific Committees II, Scientific Co-operation and Networks, Scientific Committee on Food, Brussels, Belgium. http://ec.europa.eu/food/fs/sc/scf/out105_en.pdf.

SCF (EC, Scientific Committee for Food), 2002. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Nicotinic Acid and Nicotinamide (Niacin) (Expressed on 17 April 2002). European Commission, Health & Consumer Protection Directorate-General, Directorate C – Scientific Opinions, C2 - Management of Scientific Committees II, Scientific Co-operation and Networks, Scientific Committee on Food, Brussels, Belgium.http://ec.europa.eu/food/fs/sc/scf/out80j_en.pdf.

SCF (EC, Scientific Committee for Food), 2003a. *Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper (Expressed on 5 March 2003)*. European Commission, Health & Consumer Protection Directorate-General, Directorate C - Scientific Opinions, C3 - Management of Scientific Committees II, Scientific Co-operation and Networks, Scientific Committee on Food, Brussels, Belgium.

http://ec.europa.eu/comm/food/fs/sc/scf/out176_en.pdf.

SCF (EC, Scientific Committee for Food), 2003b. *Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Calcium (Expressed on 4 April 2003)*. European Commission, Health & Consumer Protection Directorate-General, Directorate C – Scientific Opinions, C3 - Management of Scientific Committees II, Scientific Co-operation and Networks, Scientific Committee on Food, Brussels, Belgium.

http://ec.europa.eu/food/fs/sc/scf/out194_en.pdf.

SCF (EC, Scientific Committee for Food), 2003c. *Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Zinc (Expressed on 5 March 2003)*. European Commission, Health & Consumer Protection Directorate-General, Directorate C – Scientific Opinions, C3 - Management of Scientific Committees II, Scientific Co-operation and Networks, Scientific Committee on Food, Brussels, Belgium.

http://ec.europa.eu/food/fs/sc/scf/out177_en.pdf.

SCF (EC, Scientific Committee on Food), 2003d. *Opinion of the Scientific Committee on Food on the Revision of Reference Values for Nutrition Labelling* (expressed on 5 March 2003). European Commission, Health & Consumer Protection Directorate-General, Directorate C - Scientific Opinions, C2 - Management of Scientific Committees; Scientific Cooperation and Networks, Scientific Committee on Food, Brussels, SCF/CS/NUT/GEN/18 Final. http://ec.europa.eu/food/fs/sc/scf/out171_en.pdf.

SCF (EC, Scientific Committee for Food), 2003e. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Trivalent Chromium (Expressed on 4 April 2003). European Commission, Health & Consumer Protection Directorate-General, Directorate C – Scientific Opinions, C2 - Management of Scientific Committees II, Scientific Co-operation and Networks, Scientific Committee on Food, Brussels, Belgium. http://ec.europa.eu/food/fs/sc/scf/out197_en.pdf.

Schölmerich, J., Freudemann, A., Köttgen, E.., Wietholtz, H., Steiert, B., Löhle, E., Häussinger, D., Gerok, W., 1987. Bioavailability of zinc from zinc-histidine complexes. I Comparison with zinc sulphate in healthy men. *Am. J. Clin. Nutr.* 45, 1480-1486.

Toldrá, F.; Flores, M. & Aristoy, M-C., 1995. Enzyme generation of free amino acids and its nutritional significance in processed pork meats. In: Food Flavors: Generation, Analysis and Process Influence (G. Charalambous, ed.), Elsevier Science Publishers B.V., Amsterdam, The Netherlands, pp. 1303-1322.

Yang, X.Y., Villagomez, S., Moore, T.J., Salvini, S., Stampfer, M.J., Sacks, F.M., 1989. Effect of magnesium supplementation on mononuclear blood cell and urinary magnesium, retention of intravenous magnesium, and blood pressure in normal persons. Unpublished report cited in the application dossier.

## GLOSSARY / ABBREVIATIONS

| | |
|---|---|
| AFC | Scientific Panel on food additives, flavourings, processing aids and materials in contact with food. |
| ADI | Acceptable Daily Intake |
| bw | Body weight |
| CAS | Chemical Abstract Service |
| DSHEA | Dietary Supplement Health and Education Act |
| EVM | Expert Group on Vitamins and Minerals |
| NOAEL | No Observed Adverse Effect Level |
| PARNUTS | Foods for Particular Nutritional Uses |
| SCF | Scientific Committee on Food |
| SOD | Superoxide Dismutase |
| UL | Upper level |
| USP | U.S. Pharmacopeia |