General information

1. Iron homeostasis

Iron homeostasis[1] is defined as the correct balance of iron in the body. The balance of iron is associated with a physiological ratio of iron between tissues and blood. Iron deficiency and iron deficiency anemia are disorders of iron homeostasis.

2. Lactoferrin

2.1. Natural occurrence

Lactoferrin is a protein that occurs naturally in human and in cow's milk. Human first milk (colostrum), which is unusually rich in lactoferrin, contains on average 7gr of lactoferrin per liter[2]. One liter of cow's milk contains on average 100mg of lactoferrin[2]. Lactoferrin is also present in other body fluids such as tears, saliva, nasal secretions, bile and pancreatic secretions, as well as in circulating white blood cells[3].

2.2. Lactoferrin and iron homeostasis

Lactoferrin is an important regulator of iron homeostasis. Recent data suggest that this natural protein, capable of interacting with the most important components of iron homeostasis, may represent a valuable alternative to iron supplements. In a number of studies on pregnant women, lactoferrin was consistently shown to be more effective in restoring the iron homeostasis and better tolerated than iron salts.

2.3. Lactoferrin and protection from infections

There is substantial evidence that lactoferrin supports the immune system and protects from infections.

In 2012 lactoferrin was granted GRAS status (Generally Regarded as Safe) by the European Food Safety Authority (EFSA)[4].

1). Body iron delocalization: the serious drawback in iron disorders in both developing and developed countries. *Path Glob Health* **2012**, 106, 200-216.

2). Lactoferrin in human milk: Its role in iron absorption and protection against enteric infection in the newborn infant. *Arch Dis Child* **1980**, 55, 417-421.

3). An iron-binding protein common to many external secretions. *Clin Chim Acta* **1966**, 14, 735-739.

4). EFSA: Scientific Opinion on bovine lactoferrin. EFSA Journal **2012**, 10, 2701-2727.

3. Ferrochel® (ferrous bis-glycinate)

3.1. Nature of ferrous bis-glycinate

EFSA states that: "Ferrous bis-glycinate consists of one molecule of ferrous iron bound to two molecules of glycine. Following oral administration, ferrous bis-glycinate is absorbed intact into the mucosal cells of the intestine and is subsequently hydrolyzed into its iron and glycine components. Following dissociation from ferrous bis-glycinate, the free amino acid glycine, will enter the normal metabolic process of amino acids"[1].

3.2. Tolerability of ferrous bis-glycinate

EFSA also states that: "Iron supplementation using ferrous bis-glycinate, providing 15-120mg iron per day, has been well tolerated by pregnant females, non-pregnant females and more in particular by iron-deficient young children. In all cases substantial improvements in iron status were reported in the groups supplemented with ferrous bis-glycinate compared to the controls"[1].

3.3. Absorption and bioavailability of ferrous bis-glycinate

Many studies have confirmed that ferrous bis-glycinate is better absorbed and exhibits far higher bioavailability compared to ferrous sulfate.

3.4. Effect of acidity on the absorption of ferrous bis-glycinate

The solubility of ferrous bis-glycinate with that of ferrous sulfate was compared in a study[2] testing the effect of pH change from 2 to 6. It was found that at pH 2 ferrous bis-glycinate and ferrous sulfate were completely soluble. When pH was raised to 6, ferrous bis-glycinate remained completely soluble while solubility of ferrous sulfate decreased by 64%. These results indicate that ferrous bis-glycinate remains soluble and highly absorbable even when the pH is changed over a wide range as happens when food moves from the stomach to the small intestine.

The efficacy, safety and tolerability of ferrous bis-glycinate has been acknowledged by EFSA with the following statement: "On the basis of

the available studies on bioavailability, metabolism, toxicity, the use of ferrous bis-glycinate 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, does not present a safety concern"[1].

In 2006 ferrous bis-glycinate was granted GRAS status (Generally Regarded as Safe) by EFSA[1]. Ferrous bis-glycinate is also granted GRAS status by the U.S. Food and Drug Administration (FDA).

EFSA have set a PRI of 16mg/day of iron for premenopausal women[3].

1). EFSA: Scientific Opinion on ferrous bis-glycinate as a source of iron for use in the manufacturing of foods and in food supplements. *EFSA Journal* **2006**, 299, 1-17.

2). The effect of change in pH on the solubility of iron bis-glycinate chelate and other iron compounds *Arch Latin Nutr* **2001**, 51, 35-36.

3). EFSA: Scientific Opinion on Dietary Reference Values for iron. *EFSA Journal* **2015**, 13, 4254-4369.

4. Ascorbic acid

4.1. Ascorbic acid as an iron absorption enhancer

Ascorbic acid is the most potent enhancer of absorption of non-heme iron[1, 2]. Several reports have shown a marked and consistent effect of ascorbic acid in enhancing the absorption of non-heme iron. It is estimated that an addition of 50mg ascorbic acid in the diet will increase iron absorption to about the same extent as an addition of 90-100gr of meat or as a doubling of the iron content of the meal by iron fortification with ferrous sulfate. Ascorbic acid increases the absorption of both iron from foods and iron from food supplements that are soluble in gastric fluid. Ascorbic acid is also useful for reducing the influence of all the recognized inhibitors of non-heme iron absorption including phytates, polyphenols and calcium as well as vegetable and certain animal proteins[1]. Ascorbic acid is effective only when taken with a meal or with food supplements containing iron. In one study, 500mg of ascorbic acid taken with a meal resulted in six-fold increase in iron absorption, whereas the same quantity had little effect when consumed several hours earlier.

The importance of interaction of ascorbic acid and iron is also acknowledged by the European Food Safety Authority (EFSA)[3] with the following statement: "The reducing capacity of vitamin C has been implicated in enhancing gastrointestinal absorption of non-heme iron".

4.2. How ascorbic acid enhances iron absorption

The dominant form of iron in foods is ferric iron, which is much less bioavailable than ferrous iron. One of ascorbic acid's main attributes is its ability to reduce ferric to ferrous iron.

Ascorbic acid acts in the duodenum both by reducing ferric food iron to the ferrous state and by preserving its solubility as the luminal pH rises in the duodenum[1].

4.3. Optimal ratio between ascorbic acid and iron

The presence of low to medium levels of inhibitors of absorption of iron in food require a molar ratio of ascorbic acid to iron of 2:1 for efficient absorption of iron.

1). Iron and ascorbic acid: Proposed fortification levels and recommended iron compounds *J Nutr* **2003**, 133, 2978S–2984S.

2). Enhancers of iron absorption: Ascorbic acid and other organic acids *Int J Vit Nutr Res* **2004**, 74, 403-419.

3). EFSA: Scientific Opinion on Dietary Reference Values for vitamin C. *EFSA Journal* **2013**, 11, 3418-3486.

5. Beta-carotene

5.1. Natural sources

Beta-carotene is a precursor form of vitamin A. Vitamin A is found in animal sources such as liver, eggs, and milk and beta-carotene in green leafy vegetables and yellow or orange fruits such as mangos and papaya. Among carotenoids, beta-carotene has the highest conversion rate to vitamin A.

5.2. Conversion of beta-carotene to vitamin A.

Within the intestinal wall, beta-carotene is partially converted into vitamin A. This mechanism is regulated by the individual's vitamin A status. If the body has enough vitamin A, the conversion of beta-carotene decreases. Therefore, beta-carotene is a safe source of vitamin A and high intakes of beta-carotene will not lead to an

increase beyond normal of the level of vitamin A. It has been shown consistently[1-4], and provided that there is no deficiency, that plasma levels of vitamin A are not affected by beta-carotene supplementation.

5.3. Vitamin A and iron homeostasis

A large number of clinical trials have shown that vitamin A plus iron supplementation can improve hematological status better than iron alone in children and adolescents, pregnant and lactating women. The mechanisms through which vitamin A affects anemia remain largely unclear, though a number of hypotheses have been proposed including: 1) a potential role of vitamin A in mobilizing iron stores from the liver 2) increasing erythropoiesis and 3) increasing iron absorption[5, 6].

The importance of interaction of vitamin A and iron is also acknowledged by the European Food Safety Authority (EFSA) with the following statement: "Iron supplementation combined with vitamin A was more effective than iron alone in improving hemoglobin concentrations in anemic children and pregnant and lactating women"[7].

1). The importance of beta-carotene as a source of vitamin A with special regard to pregnant and breastfeeding women. *Eur J Nutr* **2007**, 46, I1-I20.

2). Serum beta-carotene before and after beta-carotene supplementation. *Eur J Clin Nutr* **1992**, 46, 15-24.

3). Plasma concentrations of beta-carotene, vitamin A and vitamin E after beta-carotene and vitamin E intake. *Biomed Chromatogr* **1993**, 7, 136-138.

4). Vitamins A, E, and carotene: effects of supplementation on their plasma levels. *Am J Clin Nutr* **1983**, 38, 559-566.

5). Vitamin A and carotenoids during pregnancy and maternal, neonatal and infant health outcomes: A systematic review and meta-analysis. *Paediatr Perinat Epidemiol* **2012**, 26 (Suppl 1), 36-54.

6). Interdependence of vitamin A and iron: an important association for programs of anemia control. *Proc Nutr Soc* **1995**, 54, 501-508.

7). EFSA: Scientific Opinion on Dietary Reference Values for vitamin A. *EFSA Journal* **2015**, 13, 4028-4112.

6. Quatrefolic® (L-methyltetrahydrofolate)

6.1. Importance of folate

Folate (vitamin B9) is particularly essential during early stages of human development. Maintaining an adequate folate status throughout pregnancy is important not only for the mother but also for the developing fetus, because folate deficiency in pregnancy has been linked to a serious set of disorders called neural tube defects. Folate is also important for the development of healthy red blood cells. Another implication of folate deficiency is a type of anemia known as megaloblastic anemia. Megaloblastic anemia is characterized by lower than normal number of red blood cells, which also appear large, immature and dysfunctional.

6.2. Differences between folate and folic acid

Folate, also known as vitamin B9, is a general term for a group of water soluble compounds naturally found in foods. The most important form of folate is L-5-methyltetrahydrofolate (in short: L-methyltetrahydrofolate). L-methyltetrahydrofolate is the only form of folate that can enter the metabolic cycle of human cells.

Folic acid is an oxidized synthetic compound not found in foods and used in dietary supplements and food fortification. Folic acid from food supplements is not biologically active and need to be converted to the metabolically active L-methyltetrahydrofolate. Due to genetic variations that impair conversion of folic acid to its active form, L-methyltetrahydrofolate, part of the population may not be able to efficiently assimilate folic acid from food supplements. Genetic variations are estimated to occur in up to 67% of the population[1], leaving many at increased risk for adverse health outcomes[2, 3].

Several studies have reported an increase in serum of unmetabolized folic acid as a result of different conditions such as genetic variations and overdosing due to consumption of high dosages of folic acid from food supplements[4-7]. Daily ingestion of more than 200-300mcg of folic acid leads to the appearance of unmetabolized folic acid in the plasma, which may interfere with normal folate metabolism[6].

A more natural, efficient and cautious approach is the consumption of the biologically active form of folic acid, L-methyltetrahydrofolate. Consumption of L-methyltetrahydrofolate does not lead to unmetabolized folic acid in plasma and also in people who have a genetic enzyme deficiency requires no conversion to become metabolically active[10-12].

6.3. L-methyltetrahydrofolate and iron homeostasis

Clinical trials have shown that L-methyltetrahydrofolate is more effective than folic acid at improving folate status of red blood cells.

EFSA set the adequate intake of folate for pregnant women at 600mcg/day[2].

In 2004 L-methyltetrahydrofolate was granted GRAS status (Generally Regarded as Safe) by EFSA (European Food Safety Authority)[11].

1). Folate nutrigenomics: a convergence of dietary folate metabolism, folic acid supplementation, and folate antagonist pharmacogenetics. *Drug Metab Lett* **2007**, 1, 55-60.

2). EFSA: Scientific Opinion on Dietary Reference Values for folate. *EFSA Journal* **2014**, 12, 3893-3952.

3). Is folic acid good for everyone? *Am J Clin Nutr* **2008**, 87, 517-533.

4). Persistent circulating unmetabolised folic acid in a setting of liberal voluntary folic acid fortification. *BMC Public Health* **2009**, 9, 295-302.

5). Folate Supplementation Too Much of a Good Thing? *Canc Epid Biom Prev* **2006**, 15, 189-193.

6). Folic acid handling by the human gut-implications for food fortification and supplementation *Am J Clin Nutr* **2014**, 100, 593-599.

7). Folic acid metabolism in human subjects revisited: Potential implications for proposed mandatory folic acid fortification in the UK. *B J Nutr* **2007**, 98, 667–675.

8). Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. *Xenobiotics* **2014**, 44, 480-488.

9). L-5-methyltetrahydrofolate an alternative to folic acid for the prevention of neural tube defects. *J Perinat Med* **2013**, 41, 469-483.

10). Folic acid and L-5-methyltetrahydrofolate comparison of clinical pharmacokinetics and pharmacodynamics. *Clin Pharmacokin* **2010**, 49, 535-548.

methyltetrahydrofolate or folic acid on plasma homocysteine a randomized placebo-controlled study. *Am J Clin Nutr* **2003**, 77, 658-662.

11). EFSA: Scientific Opinion on Calcium L-Methyltetrahydrofolate. *EFSA Journal* **2004**, 135, 1-20.

7. Cobalamin

7.1. Natural sources

Cobalamin (vitamin B12), is a water-soluble vitamin, which is essential for proper red blood cell formation, normal function of the brain and the nervous system and synthesis of DNA of the cells. It is naturally present in most foods of animal origin (meat, fish, eggs, milk and milk products).

7.2. Cobalamin and iron homeostasis

In proportion to deficiency of folate, deficiency of vitamin B12 also causes megaloblastic anemia. A diet rich in vitamin B12 is essentially the best prevention of megaloblastic anemia. Supplements containing cobalamin are rarely needed in pregnant women due to large maternal vitamin B12 stores that make virtually impossible a great impact of the developing fetus on vitamin B12 levels. Nevertheless, pregnant women following a vegan diet may benefit from cobalamin supplements[1].

EFSA sets the adequate intake of cobalamin at 4mcg/day for adults, at 4,5mcg/day for pregnant and at 5,0mcg/day for lactating women[2].

1). Anemia in pregnancy. *Best Prac Res Clin Obs Gyn* **2012**, 26, 3-24.

2). EFSA: Scientific Opinion on Dietary Reference Values for cobalamin. *EFSA Journal* **2015**, 13, 4150-4214.

Lactiferon® plus

1.1 What is Lactiferon® plus and what it contains

Lactiferon® plus is a food supplement that contains lactoferrin, ferrous bis-glycinate (Ferrochel®), ascorbic acid, beta-carotene, L-methyltetrahydrofolate (Quatrefolic®) and methylcobalamin.

1.2. Qualitative and quantitative composition

Ingredients with physiological action: Each tablet of Lactiferon® plus contains: 100mg lactoferrin, 125mg Ferrochel® (equivalent to 25mg of elemental ferrous iron, 156%PRI[1]), 160mg ascorbic acid (200%PRI[2]), 2,6mg beta-carotene, 624mcg Quatrefolic® (equivalent to 600mcg of natural folate, 100%PRI[3]) and 9 mcg methylcobalamin (equivalent to cyanocobalamin 9mcg, 200%AI[4]). *Excipients:* microcrystalline cellulose, hydroxypropyl cellulose, magnesium stearate. *Coating agent:* Sepifilm®. Lactiferon® plus tablets are gastro-resistant and therefore iron and the rest of the ingredients are released in the intestine.

[1]EFSA Journal **2015**, 13, 4254-4369. [2]*EFSA Journal* **2013**, 11, 3418-3486. [3]*EFSA Journal* **2014**, 12, 3893-3952. [4]*EFSA Journal* **2015**, 13, 4150-4214.

1.3. Suggested use

As a supplement of the diet with the objective of restoring the iron homeostasis in pregnant and breastfeeding women. Also for restoring the iron homeostasis in women of childbearing age, women on sports, post menopausal women, children.

1.4. Dosage and administration

The dosage of Lactiferon® plus is one gastro-resistant tablet per day. Lactiferon® plus should be taken immediately (e.g. within 15min) after the main meal of the day with the intent to increase the absorption of the iron from food along with the iron contained in the supplement (Ferrochel®).

1.5. Do not use

Lactiferon® plus contains lactoferrin, a protein isolated from cow's milk. Lactiferon® plus should not be used in case of cow's milk allergy or intolerance.

1.6. Caution

Lactiferon® plus should be used in association with a correct and properly balanced diet. Do not exceed the recommended daily intake. Keep out of reach of children. Store Lactiferon® plus in a cool and dry place. Do not use the product beyond the expiration date printed on the outer package. If you are pregnant or breastfeeding consult your doctor about dietary supplements before taking Lactiferon®plus. If you are taking prescription or nonprescription medicines consult your doctor before taking Lactiferon® plus. This product is not intended for prevention, treatment or cure of a disease. Dietary supplements should not be used as a substitute for a varied diet.

1.7. Packaging

Carton box containing 30 gastro-resistant tablets in 2 blisters and a patient information leaflet.

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Manufacturer: Pharcoterm S.r.l. Via Merli 1, 20095 Cusano Milanino, Italy

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