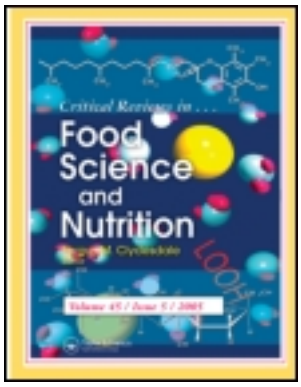

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Iron Nutrition in Adolescence

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Adolescence is an important period of nutritional vulnerability due to increased dietary requirements for growth and development. Iron needs are elevated as a result of intensive growth and muscular development, which implies an increase in blood volume; thus, it is extremely important for the adolescent's iron requirements to be met. Diet, therefore, must provide enough iron and, moreover, nutrients producing adequate iron bioavailability to favor element utilization and thus be sufficient for needs at this stage of life. Currently, many adolescents consume monotonous and unbalanced diets which may limit mineral intake and/or bioavailability, leading to iron deficiency and, consequently, to ferropenic anemia, a nutritional deficit of worldwide prevalence. Iron deficiency, apart from provoking important physiological repercussions, can adversely affect adolescents' cognitive ability and behavior. Accordingly, promoting the consumption of a varied, adjusted, and balanced diet by adolescents will facilitate iron utilization, benefiting their health both at present and in adulthood. This review discusses how physiological changes during adolescence can cause iron requirements to increase. Consequently, it is important that diet should contribute an appropriate amount of this mineral and, moreover, with an adequate bioavailability to satisfy needs during this special period of life.

Keywords Iron, adolescence, growth, anemia

INTRODUCTION

Iron is an essential micronutrient for humans, functioning as a component or cofactor of a number of proteins, including enzymes, which play important roles in physiological functions. This element can exist in different oxidation states, such as ferric (Fe^{3+}) or ferrous (Fe^{2+}). The interconversion of iron oxidation states is a mechanism whereby iron may act as a catalyst in redox reactions, participate in electron transfer, and reversibly bind different ligands. The common biological ligands for iron are oxygen, nitrogen, and sulphur (Beard, 2001). Due to these factors, this mineral plays a fundamental role in important biological reactions.

The total iron content in the body depends on a person's weight, sex, iron-storing capacity, etc. The body of a healthy adult man contains approximately 3.8 g of total iron, and for women the corresponding value is 2.3 g, present as functional, stored or transported iron. Approximately two-thirds of total iron content in an organism is functional iron, which carries out metabolic functions, taking part in enzymatic systems or participating in oxygen transport. Thus, iron is found in the hemoglobin of erythrocytes, which are responsible for transporting oxygen from the pulmonary alveoli to the tissues. Iron is also found in muscle myoglobin, which stores oxygen in the tissue and releases it with the high demands associated with muscle contraction. Stored iron may be found as ferritin or hemosiderin, which constitute body depots, and it is transported bound to transferrin as ferric iron (Bothwell, 1995).

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IRON NEEDS IN ADOLESCENTS: PHYSIOLOGICAL CHANGES

Adolescence is characterized by an accelerated growth and rate of development. These changes require a considerable input of energy and nutrients and, therefore, the diet consumed must meet these requirements. Some nutrients are needed in greater quantities than at any other stage of life. During this period, adolescents acquire 15–25% of their adult size and 40–50% of adult weight. There is an increase in the production of bone and muscle and, consequently, in the expansion of total blood volume, which implies a greater need of iron (Beard, 2000).

During pubertal development, adolescents gain around 10 kg body weight (Finch, 1994), which implies a larger amount of body iron. Iron is required to satisfy the increased hemoglobin demand for the expansion of blood volume, myoglobin for

the higher muscular mass, and enzymes necessary for growth (Muñoz Hoyos and Carballo Molina, 2005). It should be noted that this period is characterized by marked increases in gonadal sex steroidal output. This, in turn, increases growth hormone (GH) and insulin-like growth factor-1 (IGF-1) production (Mauras et al., 1996), parameters that are involved in erythropoiesis regulation (Vihervuori et al., 1996). In the pubertal stage, hemoglobin concentrations increase by 50–100 g/L/year to reach adult levels (Dallman, 1992). Iron is necessary for this expansion and to replace the losses associated with the continual renovation of erythrocytes (Fomon et al., 2003). In boys, moreover, the rapid body growth and the increased muscular mass development and consequently testosterone secretions, involves additional iron requirements of almost 25% to enhance erythropoiesis during the period of maximum growth (Thompsen et al., 1986). In such a situation, the mobilization of iron stores, as reflected by a reduction in serum ferritin, is to be expected. Thereby, absorbed and retained iron is used for hemoglobin production, rather than stored as ferritin, similar to the pattern observed among infants (Lind et al., 2004). In this sense, Hunt and Roughead (2000) reported that incorporation of the absorbed iron into erythrocytes is considerably higher in young males than in adults. Therefore, erythropoiesis is an important regulator of iron absorption during adolescence.

Net iron utilization to increase the hemoglobin mass depends on the rate of increase in blood volume and the rate of change in hemoglobin concentrations. Blood volume is approximately 75 mL/kg in boys and 66 mL/kg in girls, with an iron hemoglobin content of 3.39 mg/g. During the growth spurt period, requirements of dietary iron in boys are 2.9 mg/day due to the higher growth rate than girls, who only need 1.1 mg/day (Institute of Medicine, 2001). However, iron requirements in girls begin to increase after menarquia, once the peak of maximum growth is reached, and this situation is maintained until menopause. It is estimated that 30–40 mL of blood is lost in each menstruation (maximum 80 mL), leading to a loss of around 15–30 mg Fe/cycle. Consequently, menstrual iron losses imply increased iron demands (Hallberg and Rossander-Hulthen, 1991) and female adolescents need around 455 mg Fe/year after the menarche, while males need 350 mg/year (Lanzkowski, 1985). On the contrary, Bergström et al. (1995), in a study carried out on Swedish adolescents, suggest that differences in iron status among boys and girls during adolescence result primarily from physiological differences rather than menstrual bleeding or insufficient iron intake.

Ferritin represents stored iron: it is assumed that 1 μg/L of serum ferritin contains about 8-10 mg of iron. In normal adults, serum ferritin levels are negatively correlated with iron absorption; however, this correlation may disappear in situations of high levels of erythropoiesis (Finch, 1994) or during the intensive growth of the first year of life (Lind et al., 2004). Anttila et al. (1997) demonstrated a strong positive correlation of erythrocyte production with growth speed, height, and weight gain in young males; on the contrary, these anthropometric parameters did not correlate with iron stored. Mesías (2007) did not observe any correlation between ferritin levels and weight or height gain in Spanish male adolescents, whereas weight with erythrocyte production and height with hemoglobin were positively correlated. In these boys, moreover, iron absorption was correlated with height and weight gain during the study, which indicates that micronutrient absorption enhances parallel to corporal development. On the other hand, parameters of growth such as body height and IGF-I have also been related to blood hemoglobin levels (Vihervuori et al., 1996). These authors suggest that the GH-IGF-I axis participates in the regulation of human erythropoiesis. Even if blood hemoglobin concentration remains within the normal range, the GH-IGF-I system may elevate it during growth periods. This increase would appear to guarantee a better oxygen supply to growing tissues. During GH treatment, erythropoiesis and rapid body growth increase iron needs, and serum ferritin concentration decreases (Vihervuori et al., 1994). In early male puberty, the decrease in ferritin prior to accelerated growth may be related to an early increase in GH secretion, and a significant increase in hemoglobin concentration occurs later during pubertal maturation. As mentioned, serum ferritin concentration decreases due to the mobilization of iron stores for blood cellular mass production (Anttila and Siimes, 1996). It has been shown that GH and its main regulator, IGF-I, stimulate erythropoiesis under various in vitro conditions (Boyer et al., 1992; Sanders et al., 2003) and in animal studies (Kurtz et al., 1990). However, in humans, the role of GH in hematopoiesis is unclear, since it has been observed that individuals with GH deficiency are generally not anemic (Vihervuori et al., 1996). Urbano et al. (2002), moreover, suggest that GH mobilizes iron stores, and thereby iron absorption could be favored. On the other hand, transferrin levels may rise during puberty, being related not to iron deficiency but to an increase in the turnover associated with the iron required for erythropoiesis (Romslo et al., 1983; Anttila and Siimes, 1996).

RECOMMENDED IRON INTAKE AND AVERAGE INTAKE IN ADOLESCENTS

Iron intake is needed to replace basal losses derived mainly from the sloughing of epithelial cells, sweat, and menstrual losses, but, as mentioned above, iron is also needed to satisfy growth demands. Recommended iron intakes for adolescents are shown in Table 1. Despite the importance of adequate iron intake during childhood and adolescence, many US and European adolescents, especially girls, fail to achieve recommended levels (Ervin et al., 2004; Elmadfa and Weichselbaum, 2005). Table 2 shows the average iron intake (mg/day) in adolescents from different countries, according to age and sex. These data were obtained from 38 surveys, 4 for males, 2 for females, and 32 for males and females, from 21 different countries. The boys' daily iron intake was always higher than that of the girls, ranging from ∼9.0 mg/day to ∼24.5 mg/day or even more.

Table 1 Iron recommended intakes for adolescent population

Iron recommended intakes (mg/day)								
Spain (Moreiras et al., 2010)			Europe (Scientific Committee for Food, 1993)			United States (Institute of Medicine, 2001)		
Age	Boys	Girls	Age	Boys	Girls	Age	Boys	Girls
10–12	12	18	11–14	10	18–22	9–13	8	8
13–15	15	18	15–17	13	17–21	14–18	11	15
16–19	15	18	18+	9	16–20	19+	8	18

Only slightly insufficient iron consumption is observed among males from the Netherlands, whereas most of them are within the recommended range. Intake of iron is particularly high in adolescents from Greece, Ireland, Spain, and the United States (until ∼ 20 mg/day); the highest data are found in Canada and Estonia (until ∼ 25 mg/day). Unusual values of iron intake have been found among Bolivian adolescents (37 mg/day for boys), calculated using Bolivian Food Composition Tables.

Among girls, intake ranged from ∼ 8.7 mg/day to ∼ 17.2 mg/day, with most of them consuming iron below the recommended levels, in contrast to boys. The lowest consumption of iron among girls was found in Brazil, Denmark, Greece, Hungary, and Norway. Only Spanish and American females reached the recommended iron intake, highlighting again the high iron consumption among Bolivian girls (36 mg/day).

In general, European male adolescents achieve iron recommendations, whereas females largely fail to meet them. On the contrary, both boys and girls in the United States achieve levels of mineral intake that are close to those recommended.

DIETARY SOURCES OF IRON

Iron is found in a limited number of animal and vegetable foods. Among these, the most important are meat, particularly blood, liver and derivatives, followed by legumes, nuts, and certain vegetables, although the latter present lower levels of availability. Although the proportion of heme iron in animal tissues varies, it amounts to an average of 40% of the total iron in all animal tissues. This proceeds from hemoglobin and myoglobin in meats and to a lesser extent, in fish, contributing around 5–10% (Lawson et al., 1998) or even 10–15% of the total iron ingested (1 to 3 mg/day) in diets in developed countries (Spodaryk, 1999). The remaining 60% of iron in animal tissues and the iron in vegetable products is nonheme iron (Herbert, 1987), which constitutes about 85% of the dietary iron intake in Western-type diets (Hallberg and Rossander, 1982). Nevertheless, Carpenter and Mahoney (1992) consider that 45% of the total iron in animal tissues corresponds to heme iron, with nonheme iron constituting the remaining 55% and 100% in the case of vegetable products.

The proportions of iron provided by different food sources reported in the enKid study for the Spanish adolescent population are similar to those observed in studies carried out in different areas of Spain (Román Viñas et al., 2004; Serra-Majem et al., 2007), where cereals are the main source of dietary iron, and meat is the second largest contributor. Fruits and vegetables provide about 10–15% of daily iron, reaching 20% among Catalan adolescents (Serra-Majem et al., 2007). Legumes, foods that are rich in iron, contribute little iron to the total intake, due to their low consumption, whereas dairy products are a minority source of the micronutrient. Among other food sources that contribute to the daily iron intake is cocoa, which is consumed mainly as powdered cocoa or as chocolate in breakfast and afternoon snacks (Mesías, 2007). Mesías et al. (2009a) reported the contribution of the main food sources to daily iron intake among a group of Spanish adolescents (Figure 1). This distribution is similar to that found among other European adolescents (Kersting et al., 2001; Lombardi-Boccia et al., 2003) and that described for American population (Guthrie and Picciano, 1995), where cereals, together with meats, are the main contributors to the daily nutrient intake. Iron fortification of cereals, especially breakfast cereals, makes them a rich source of iron and, therefore, an important contributor to mineral intake among population (Guthrie and Picciano, 1995). According to this distribution, approximately 87–89% of dietary iron is nonheme, with the remaining fraction being heme iron (Lombardi-Boccia et al., 2003).

ABSORPTION AND METABOLISM

Since humans do not possess a mechanism for the active excretion of iron, its retention in the body is determined by the amount absorbed across the proximal small intestine. Consequently, intestinal iron absorption is a highly regulated process (Darshan and Anderson, 2007), although, recent evidence of iron transporter expression in the kidney has been considered (Smith and Thévenod, 2009).

Numerous articles and reviews have been published about intestinal iron absorption and molecular mechanisms involved in this process (Miret et al., 2003; Anderson et al., 2005; Sharp and Srai, 2007; Anderson et al. 2009), some of them specially related to infants and children (Domellöf, 2007; Lönnerdal and Kelleher, 2007). Schematically, dietary iron absorption requires iron to traverse both the apical and basolateral membranes of enterocytes to reach the blood. In this process, different transporters participate depending on the type of iron (Figure 2).

Table 2 Average iron intake (mg/day) in adolescents

Country	Geographic distribution	Fe (mg/day)		Dietary methodology	Sample size	Age (years)	Reference
		Male	Female				
Austria	n.a.	12.0	10.4	7 d WR	n.a.	10–12	(Elmadfa and Weichselbaum, 2005)
		12.6	10.7			13–14	
Bolivia	local	37	36	24 h recall	45	16–19	(Pérez-Cueto et al., 2009)
Brazil	local	10.1	9.4	24 h recall	153	10–14	(Barbosa García et al., 2003)
Canada	local	24.5	20.5	FFQ	395	13–19	(Deegan et al., 2005)
	local	15.2	—	3 d record	180	14–18	(Schenkel et al., 2007)
Denmark	n.a.	11.1	8.9	7 d record	486	11–14	(Elmadfa and Weichselbaum, 2005)
		11.8	9.4			15–18	
England	local	11.2	10	7 d WR	143	11–12	(Nelson et al., 1990)
	local	11.7	11.2	2 × 3 d record	379	11–12	(Adamson et al., 1992)
Estonia	regional	21.0	14.0	48 h recall + FFQ	341	12	(Grünberg et al., 1997)
		21.1	14.2			15	
France	local	11.2	10.5	DH	94	10	(Deheeger et al., 2002)
		12.3	12.1			14	
		13.5	10.9			16	
Germany	national	11.9	10.2	3 d WR	224	10–12	(Kersting et al., 2001)
		13.3	10.5			13–14	
		12.7	10.5			15–18	
	n.a.	11.1	10.1	HBS	3565	10–12	(Elmadfa and Weichselbaum, 2005)
		12.9	10.7			13–14	
Greece	national	11.0	10.0	3 d record	1936	10–11	(Roma-Giannikou et al., 1997)
		11.9	10.1			12–14	
	regional	15.1	12.1	24 h recall + 3 d WR + FFQ	582	11–14	(Hassapidou and Fotiadou, 2001)
	regional	11.4	9.4	3 d WDD	371	11–14	(Hassapidou et al., 2006)
Hungary	n.a.	9.7	9.3	3 × 24 h record	n.a.	12–13	(Elmadfa and Weichselbaum, 2005)
Ireland	regional	10.3	9.0			14–15	
		12.5	10.6	DH	1015	12	
		15.0	10.9			15	(McNulty et al., 1996)
Italy	regional	12	10	3 × 4 d record + FFQ	233	15–19	(Leclercq et al., 2004)
	n.a.	12.5	12.5	n.a.	n.a.	10–14	(Elmadfa and Weichselbaum, 2005)
The Netherlands	regional	9.0	—	24 h recall	123	10–11	(Van Poppel et al., 1991)
Norway	n.a. (13 y)	10.2	8.7	4 d record (13 y)	1009 (13 y)	13	(Elmadfa and Weichselbaum, 2005)
	233 (16–19 y)	13.7	9.8	FFQ (16–19 y)	n.a. (16–19 y)	16–19	
Peru	local	—	10.4	FFQ	355	11–18	(Vila and Quintana, 2008)
Scotland	n.a.	12.9	10.4	FFQ	387	16	(Belton et al., 1997)
	local	10	10	7 d WR	61	12	(McNeill et al., 1991)
Spain	regional	13.0	11.2	2 × 24 h recall	203	10–17	(Serra Majem et al., 2007)
	regional	17.7	16.3	7 d record	n.a.	11	(Estudio Caenpe, 1994)
		17.7	17.2			12	
	national	15.1	12.7	24 h recall + FFQ	1011	10–13	(Serra-Majem et al., 2006)
		16.6	12.5			14–17	
	local	16.8	17.2	7 d record + FFQ	467	12–14	(Fernández Morales et al., 2007)
		20.8	16.6			15–17	
	regional	12.4	11.4	7 d record	n.a.	11–14	(Leis et al., 1999)
	local	16.0	15.0	24 h recall + FFQ	516	11–14	(Casado Górriz et al., 1999)
	local	11.2	9.8	24 h recall	n.a.	11–14	
		12.1	9.3			15–17	(Arija et al., 1996)
	local	17.2	14.9	FFQ	406	13–16	(Durá Trave, 2001)
	national	15.6	12.2	**	2361	13–18	(Serra Majem et al., 2001)
	local	15.4	–	3 d WR	20	11–14	(Mesías et al., 2009a)
Sweden	local	–	11.5	7 d record	28	15–16	(Hoppe et al., 2008a)
Turkey	local	11.7	–	3 d record	1004	12–17	(Turan et al., 2009)
USA	national	18.3	13.4	24 h recall	2208	12–19	(Ervin et al., 2004)
	national	19	17	FFQ	9000	9–14	(Rockett et al., 2001)

WR: weighed food record. n.a.: not available. FFQ: food frequency questionnaire. DH: dietary history. HBS: household budget survey.
WR: weighed food record. FFQ: food frequency questionnaire. WDD: Weighed dietary diary. n.a.: not available. DH: diet history. y: years.
FFQ: food frequency questionnaire. WR: weighed food record. n.a.: not available. **Different methodology depending on region.

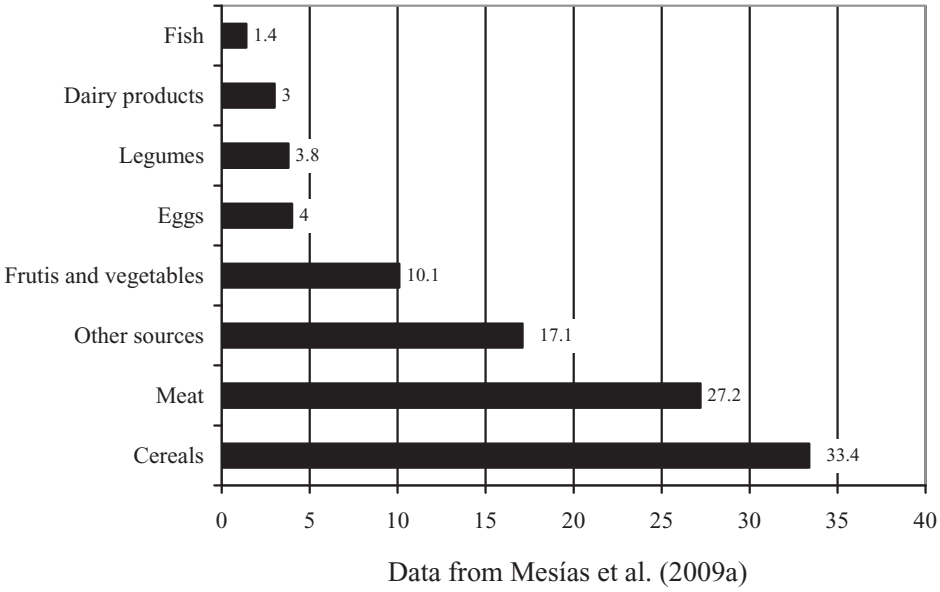

Figure 1 Contributions of the main food sources to the daily iron intake in a group of Spanish adolescents (%).

The transport of nonheme iron across the apical membrane occurs via the divalent metal transporter 1 (DMT1) (Gunshin et al., 1997). Dietary nonheme iron exists mainly in the ferric form and must be reduced to the ferrous form by a reductase duodenal, the duodenal cytochrome *b* (DcytB) prior to transport (Mckie et al., 2001). Heme iron is absorbed bound to a specific protein, heme carrier protein (HCP-1), which may also function as a proton coupled folate transporter, independent from its heme transporting properties (Qiu et al., 2006; Laftah et al., 2009). Once within the enterocyte, inorganic iron is released from the heme group mediated by heme oxygenase. In intestinal enterocytes, iron can either be stored in ferritin or exported into plasma crossing the basolateral membrane by iron exporter ferroportin (Ireg-1). The membrane protein hephaestin and plasma ceruloplasmin are responsible for oxidizing Fe^{2+} to Fe^{3+}, to be incorporated into transferrin (Zhang and Enns, 2009).

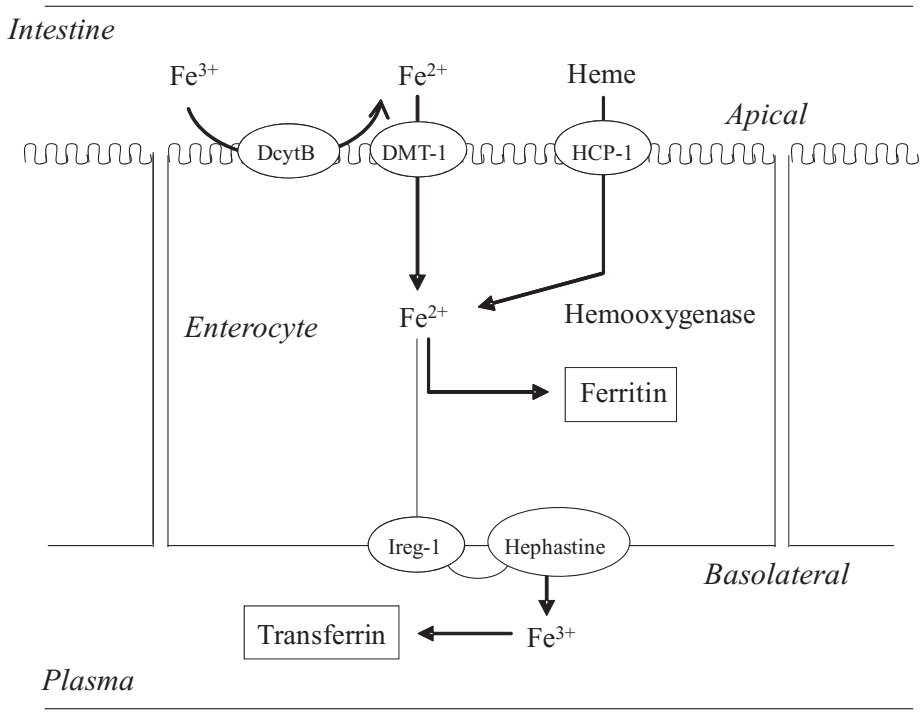

Figure 2 Scheme of iron absorption across the enterocyte.

Physiological Factor Regulators of Iron Absorption In the intestine, iron absorption can be influenced by certain mucosal factors, such as intestinal pH, available mucosal surface, clinical disorders, or intestinal mucosa motility. Since the duodenum and the jejunum are the regions of most efficient iron absorption, factors increasing transit through these areas decrease iron absorption (Conrad, 1993). Moreover, iron transport across enterocytes can be affected by various factors, including the extent of corporal storage, the hematologic stage, the rate of erythropoiesis, and deficiencies or diseases affecting mineral absorption, through the modulation of the expression of DMT-1, DcytB, and Ireg-1 levels. The basolateral transporter Ireg-1 appears to be the main regulator of dietary iron absorption in relation to mineral requirements. Hepcidin, a hormone secreted by hepatocytes, is essential in such regulation; when blood iron levels rise, hepcidin expression is favored, and its binding with Ireg-1 induces protein transporter degradation and, consequently, iron export diminishes. In addition, the increased iron concentration in enterocytes inhibits apical transport (Nemeth and Ganz, 2006). On the contrary, when iron levels are reduced or requirements increase, hepcidin expression is inhibited and iron uptake is enhanced (Darshan and Anderson, 2007). Therefore, it is accepted that an individual's iron status is inversely correlated to the amount of iron absorbed (Reddy et al., 2000).

Though corporal iron stores, hypoxia and inflammation are involved in hepcidin levels, the main regulators are erythropoietic stimuli (Papanikolaou et al., 2005). When erythropoiesis is stimulated, hepcidin expression is suppressed to increase iron levels and, consequently, blood cell production is favored (Frazer et al., 2004). Erythropoietin, a protein involved in erythropoiesis, is responsible for hepcidin expression regulation (Darshan and Anderson, 2007). In intensive growth situations, such as adolescence, erythropoiesis is stimulated and intestinal iron absorption, storage, and mobilization are increased. Thus, hepcidin expression is suppressed despite the iron overload (Zhang and Enns, 2009). In this respect, mineral needs for growth are high, and thus Mesías et al. (2009a) reported increased iron absorption among a group of adolescents after nutritional intervention, despite their normal corporal iron store status.

As mentioned above, iron absorption increases according to body needs. Mineral needs are mainly determined by two factors: repletion status of the stores, which are indicated by serum ferritin, and the hematopoiesis rate, the major regulator of iron absorption. Hematopoietic requirements lead dietary iron to be channeled toward erythropoiesis rather than to storage, especially during the first year of life, intensive growth periods, and accelerated hematopoiesis (Lind et al., 2004; Zhang and Enns, 2009).

Short-term absorption measurements overestimate differences in iron bioavailability since, over time, humans biologically adapt their iron absorption following the initial response (Hunt and Roughead, 2000). Nevertheless, elevated absorption values have been described in intensive anabolic situations which imply an accelerated hematopoiesis. In this sense, iron absorption values of close to 5 mg/day with an efficiency of 10–60% have been observed in blood donors (Hallberg et al., 1997). During pregnancy, iron absorption requirements are 3–4 mg/day (Scholl, 2005) and around 3 mg/day of iron is absorbed among infants aged 6-18 months or adolescents during the growth spurt (Tondeur et al., 2004; Mesías et al., 2009a).

Dietary Factor Regulators of Iron Absorption In addition to the intestinal regulatory process, other factors that modulate the absorption of iron are the amount and type of iron present in the diet and the presence of other dietary compounds that may enhance or inhibit iron absorption. About 0.7, 1.4, or 2.1 mg of available iron is supplied daily depending on whether the diet is of low, intermediate, or high bioavailability, respectively (Bothwell et al., 1989). Heme iron is highly bioavailable as it is absorbed intact within the porphyrin ring and therefore it is not exposed to inhibitory dietary factors (Pizarro et al., 2003). In contrast, nonheme iron is subject to the effects of promoters and inhibitors of iron-binding ligands, which affect its absorption (Hallberg and Hulthen, 2000; Martínez-Navarrete et al., 2002).

The major enhancers of iron absorption are ascorbic acid (Teucher et al., 2004) and animal proteins (meat factor). Meat is not only an excellent source of bioavailable iron, but it also promotes the absorption of both heme and non-heme iron in the diet. In addition, inhibitor effects of phytates and tannins are counteracted in the presence of meat (Layrisse et al., 1984; Hunt, 2003; Hurrell et al., 2006). Vitamin A and β-carotene can enhance non-heme iron absorption and improve hemoglobin levels (García-Casal et al., 1998), although several studies suggest that it is only observed in iron deficient individuals (Walczyk et al., 2003).

A number of factors which limit iron absorption have been identified. These include polyphenols, phytates, and various minerals. Polyphenols, fundamentally the tannins present in tea, coffee, and some vegetables, are thought to interfere with iron absorption by forming insoluble complexes in the gastrointestinal lumen, thereby lowering the availability of iron (Samman et al., 2001). Phytates are reported to negatively correlate with non-heme iron absorption (Vitali et al., 2007). Several minerals, such as zinc, calcium, copper, and manganese, can inhibit iron bioavailability by competing for the same transporter in enterocytes, modifying oxidation status, or interfering with iron metabolism (Rossander-Hulthen et al., 1991; Hallberg, 1998; Sandstrom, 2001; Sharp, 2004).

IRON EXCRETION

Feces are a major route of iron elimination from the body. In normal conditions, urine is only a minority channel, being less important than losses from exfoliated cells (Forellat Barrios et al., 2000). Among male adolescents, a daily iron excretion of around 80 μg/day in urine has been measured (Mesías et al.,

2009a). This value is close to the range described for adults (Bothwell et al., 1979; Powell et al., 1999) and the adolescent population (Greger et al., 1978). As previously mentioned, iron metabolism is regulated mainly at the digestive level. Thus, only the quantity of element required by the organism is absorbed, and this is determined by hematopoietic needs or repletion status of stores. Iron absorption increases when iron stores are low and decreases when stores are adequate, with the result that absorbed iron tends to be conserved and its elimination by routes other than fecal is negligible. Thus, urinary iron is a very small fraction of the amount absorbed, which in turn is close to the value of iron retained. According to some authors, iron *absorption* and *retention* are concepts that are practically synonymous (Fomon et al., 2000).

IRON ABSORPTION IN ADOLESCENCE

Bibliographic data concerning iron balance studies in humans are scarce, especially in adolescents. It is well documented that the amount of absorbed iron changes continuously (Baynes et al., 1987). Moreover, wide interindividual variations in the same diet exist, as has also been observed in infants, with ranges of absorption between 0.7 and 23.1% from the same iron dose (Kastenmayer et al., 1994). Bothwell et al. (1979) estimated values of iron absorption at 0.5 to 2 mg/day, Hallberg et al. (1997) reported 1 mg/day for adult populations and Rossander-Hulthen and Hallberg (1996) reported data of 0.9–2.1 mg/day for adolescents. Lower data, of around 1.05–1.07 and 1.2–1.68 mg/day for young boys and girls, respectively, have also been reported (British Nutrition Foundation, 1995).

During adolescence, iron absorbed should replace habitual losses (around 0.7 mg/day) and, moreover, satisfy demands for growth. The extra iron demands estimated for the growth spurt during this stage of life are 0.55–0.60 mg/day for boys and 0.35–0.55 mg/day for girls (British Nutrition Foundation, 1995). Other studies have reported values of 0.77 mg/day and 0.31 mg/day for males and females, respectively (Fomon et al., 2003). Assuming losses of 0.70 and 0.84 mg Fe/day, a total daily absorption of 1.47 and 1.15 mg Fe during the peak of maximum growth is required for boys and girls, respectively (Fomon et al., 2003). These values are close to the range described by Fairweather-Tait (1996) (1.45–2.03) and lower by Recommended Daily Allowances (RDA) estimations (1.8 mg/day) (National Research Council, 1989). These data are also lower than those reported by authors such as Bothwell et al. (1989) (1.6 mg/day), Tapiero et al. (2001) (2.03 mg/day) and Ilich-Ernst et al. (1998) (3 mg/day) for the same population. As iron intake requirements fundamentally depend on muscle mass, it may be calculated that, in the 50th percentile, male and female adolescents need 42 mg and 31 mg Fe/kg of body weight gain, respectively. The average weight gain during the growth spurt is 15.2 g/day for boys and 6.76 g/day for girls, representing iron demands of around 0.63 mg and 0.20 mg iron/day, respectively (Tojo et al., 1991).

BIOAVAILABILITY

According to Herbert (1987), a diet with medium-high iron availability allows the body to absorb around 23% of heme iron and 8% of nonheme iron when stores are adequate. Balance studies in adults consuming adequate iron availability diets, with normal iron stores, show fractional absorption values of 4.3% (Hunt and Roughead, 2000), whereas the RDA estimated values are around 10% (Monsen et al., 1978). Thus, l iron requirements depend on the source of the mineral, due to wide variations in the availability of different foods. It has been shown that male Spanish adolescents aged 11–14 years, at the peak of the growth spurt and consuming a balanced diet with high bioavailability, may absorb up to 4.9 mg Fe/day (Mesías et al., 2009b). In these boys, the average fractional absorption, after the habitual diet consumption, is similar to the results found in subjects aged 21–40 years (7.4%) also consuming their habitual diet (Cook et al., 1991) or in boys aged 15–18 years (7.5%) on a diet of less available iron. However, these data fall short of the overall value of 8.6% reported by Viglietti and Skinner (1987) for boys consuming diets with different levels of iron availability and also of the 10% estimated by the RDA.

Published results have indicated that adolescents' habitual diet, with a daily iron intake of 15 mg/day, may satisfy the iron needs of almost all boys and girls (Ilich-Ernst et al., 1998; Fomon et al., 2003). Nevertheless, some authors suggest that the average dietary iron availability of 10% assumed for the general population (National Research Council 1989) is too high for the adolescents' habitual diet, mainly due to their frequently inappropriate dietary patterns. Snacks, widely consumed by the adolescent population (Guthrie et al., 2002), contribute little available iron to the diet (3.6-5.9%) (Cook et al. 1991), which could seriously impair total iron absorption. It should be stressed that these considerations are based on the diet of American adolescents, with a high consumption of snacks and fast food (Kelner and Helmuth, 2003), compared with that found in the Mediterranean countries (Amorim, 2000), although current dietary habits of adolescents all over the world are changing towards unhealthy patterns. Both snacks and fast foods include foods rich in proteins, carbohydrates, and /or fat, usually prepared by processes such as frying, roasting, grilling, baking, and even reheating before consumption, such as hamburger, pizza, and fried potatoes, among others. These heat treatments favor the development of processes in which the amino acids of proteins react with carbohydrates or oxidized fat and form complexes called Maillard reaction products (MRP). It has been demonstrated that iron utilization is negatively affected in adolescents consuming diets rich in MRP, as these complexes can bind iron and interfere with its absorption. Thus, Mesías et al. (2009a) observed significant differences in iron absorption after the consumption of a diet rich in MRP (1.84 mg/day) compared with one poor in these products (4.9 mg/day). These significant differences appear both in iron digestibility and bioavailability. These reasons support the conclusion that diets should be varied and balanced, without an excess of snacks or fast foods in

order that adolescents have an adequate nutritional contribution to avoid iron deficits.

Because the composition of the diet has an impact on the amount of iron absorbed, using only iron intake data are inadequate for evaluating whether iron uptake requirements are satisfied (Hoppe et al., 2008a), and it seems more useful to assess the bioavailability of iron in the diet (Zimmermann et al., 2005; Hoppe et al., 2008b).

As mentioned above, iron utilization is strongly influenced by enhancers and inhibitors of mineral absorption present in the same meal. Mesías et al. (2009a) recently reported that iron fractional absorption improved in adolescents consuming a varied and balanced diet based on the Mediterranean patterns, compared with their habitual diet. The Mediterranean diet is characterized by a high intake of fish, legumes, cereals, fruits, and vegetables and olive oil as the main dietary fat. These foods, as a whole, can improve iron utilization. Fish is a relatively good source of available iron (Glahn et al., 1998) and, moreover, it enhances iron absorption from plant foods in a way similar to meat (Layrisse et al., 1974) and can partially counteract the effect of some inhibitors (Navas-Carretero et al., 2008). It has been demonstrated that feeding rats a diet containing fish as the protein source leads to significant increases in dietary iron utilization, compared with control diets (García-Arias et al., 1994; Seiquer et al. 2002). In addition to the above mentioned factors, the role of fat in iron absorption should be considered. It has been suggested that oleic acid promotes iron absorption. Moreover, the interaction between protein and fat digestion products may be involved in enhancing intestinal iron absorption (Kapsokefalou and Miller 1993, 1995), and it has been reported that sardine protein plus olive oil have beneficial effects on dietary iron bioavailability (Seiquer et al., 2002). Higher levels of monounsaturated fatty acids and total fat content can also favor iron absorption (Qian and Eaton, 1991).

Ferritin, an abundant form of nonheme iron in many plant foods, such as legumes, represents a form of iron that is highly bioavailable to humans and its absorption is not influenced by the phytic acid content (Davila-Hicks et al., 2004). Regarding fruits and vegetables, bibliographic data show that the supplementation of certain vitamins or a suitable dietary contribution in the case of deficiency, stimulates intestinal iron absorption or its incorporation into hematopoietic tissue (Fishman, 2000; Walczyk et al., 2003). In this sense, vitamin B_6 deficiency has been associated with alterations in iron utilization (Yu and Cho, 1990). Moreover, an adequate contribution of the vitamin B group may be a metabolic stimulus to enhance hematopoiesis, which, indirectly, would have beneficial effects on iron absorption (Fishman, 2000).

On the other hand, the high intake of cereals, legumes, and vegetables, in general, increases the consumption of phytates, oxalates, and polyphenols, which are inhibitors of iron absorption (Fairweather-Tait, 1996; Hallberg and Hulthen, 2000). However, this negative effect can be counteracted by the presence of vitamin C, as the presence of inhibitors enhances the positive effect of this vitamin on iron absorption. It has been shown that the joint consumption of foods rich in vitamin C, cereals, or legumes compensates for the negative influence of phytates and polyphenols, probably due to the ability of ascorbate to reduce iron, thus preventing the formation of less-soluble ferric complexes (Hallberg and Hulthen, 2000). The habitual diets of adolescents are characterized by a high consumption of fats and animal proteins (meat) and a low consumption of cereals, legumes, fruits, and vegetables, which deviates considerably from the characteristics of the Mediterranean diet. Moreover, as mentioned above, current dietary habits associated with the consumption of highly processed foods such as snacks and fast foods that are rich in MRP can negatively affect iron utilization. Therefore, as most adolescents achieve recommended iron intakes (Table 2), mineral deficiency seems to be associated more with low availability than with low intake. Accordingly, promoting the consumption of varied and balanced diets based on the Mediterranean dietary patterns would improve dietary iron utilization during adolescence (Mesías et al., 2009a) thus preventing ferropenic anemia and associated problems.

IRON DEFICIENCY: FERROPENIC ANEMIA

Iron deficiency is produced by an imbalance between requirements and the quantity that is ingested, absorbed, and used. This deficiency is manifested in consecutive stages: initially, a diminution of iron stores is produced, which takes place when serum ferritin concentration descends below 12 μg/L, but without any fall in hemoglobin levels. A major deficiency will induce erythropoiesis by iron deficiency, decreasing the serum transferrin concentration (below 16% of saturation). At this stage, hemoglobin levels are lower than normal, although they are not yet below the value considered to represent anemia. More severe deficiency originates ferropenic anemia, which is diagnosed when hemoglobin concentration is below 11–13 g/dL, depending on sex and age group (WHO, 2009). Iron deficiency is the most common and widespread nutritional disorder in the world, affecting approximately 66–80% of the population, over 30% of whom are anemic (Haro et al., 2005). Most cases are found in developing countries where sanitary and nutritional conditions are inadequate. Those mainly affected are people with high iron requirements or physiological losses, such as children, adolescents, and fertile age women. The prevalence of iron deficiency in Spanish adolescents is 8.6% and 12.6% in boys and girls, respectively (Durá Travé et al., 2002). These data are surpassed by the rates observed among adolescents in other European countries such as Denmark (16%), United Kingdom (28%), or Ireland (43%). Finland and France, however, present lower values of prevalence of iron deficiency in adolescents (4.7% and 3.1%, respectively) (Hercberg et al., 2001).

Iron plays an important role in the nervous system function, in neurotransmitter synthesis and metabolism and in immune system functionality (Scrimshaw, 1991). Thus, its deficiency during school age years and adolescence can have adverse effects on cognitive functioning (Beard and Connor, 2003), on work

capacity (Haas and Brownlie, 2001), and on motor and mental development. This fact produces behavior alterations and adversely affects learning and scholastic performance (Beard and Connor, 2003; McCann and Ames, 2007; Akramipour et al., 2008). Iron deficiency might also alter the capacity to maintain corporal temperature in cold environments, related to a decrease in the secretion of thyroid-stimulating hormone and thyroid hormone (Beard et al., 1984), reduced resistance to infection (Dallman, 1987), etc. This deficiency appears when quantities of iron intake do not satisfy the needs of organisms, due to insufficient intake, a deficient absorption or large physiological losses associated with hemorrhage, lesions, or disease (Villa Elízaga et al., 1999). In addition, iron can have bone protective effects, and so deficiency of this element can negatively affect bone mass. This fact is especially important during infancy and adolescence (Zofkova and Nemcikova, 2008).

Adolescents are highly susceptible to nutritional iron deficiency, not only because of their considerably increased nutritional requirements, but also due to their poor dietary habits and high consumption of fast foods and snacks, with an elevated energy content but low nutrient density (Durá Travé et al., 2002; Shamah and Villalpando, 2006). Thus, to prevent iron deficiency, adolescents should consume diets with enough available iron to reach recommended intakes (Beard, 2000).

CONCLUSIONS

Iron requirements increase during adolescence due to the intensive growth associated with this period. Adolescents need iron to satisfy the increased demand for hemoglobin due to the expansion of blood volume, myoglobin for the higher muscular mass, and enzymes that increase with growth. An adequate iron intake may prevent iron deficiency and ferropenic anemia, and cognitive ability disorders among adolescents.

The prevention of iron deficiency consists of not only achieving an adequate mineral intake, but also in consuming suitable nutrients, which enhance its absorption and utilization. The diet, therefore, should provide foods that allow meeting iron needs during adolescence, as part of a healthy lifestyle. Promoting the consumption of adjusted and balanced diets by adolescents will improve dietary iron utilization. Due to the enormous physiological importance of this mineral, such a strategy will benefit the individual's health both in adolescence and in future adulthood.

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REFERENCES

Adamson, A., Rugg-Gunn, R., Butler, T., Appleton, D. and Hackett, A. (1992). Nutritional intake, height and weight of 11-12-year-old Northumbrian children in 1990 compared with information obtained in 1980. *Br. J. Nutr.* **68**:543–563.

Akramipour, R., Rezaei, M. and Raimi, Z. (2008). Prevalence of iron deficiency anemia among adolescent schoolgirls from Kermanshah, Western Iran. *Hematology* **13**:352–355.

Amorim, J. A. (2000). Dietary habits and nutritional status in adolescents over Europe-Southern Europe. *Eur. J. Clin. Nutr.* **54**:29S–35S.

Anderson, G. J., Frazer, D. M., McKie, A. T., Vulpe, C. D. and Smith, A. (2005). Mechanisms of haem and non-haem iron absorption: Lessons from inherited disorders of iron metabolism. *Biometals* **18**:339–348.

Anderson, G. J., Frazer, D. M. and McLaren, G. D. (2009). Iron absorption and metabolism. *Curr. Opin. Gastroenterol.* **25**:129–135.

Anttila, R., Cook, J. D. and Siimes, M. A. (1997). Body iron stores in relation to growth and pubertal maturation in healthy boys. *Br. J. Haematol.* **96**: 12–18.

Anttila, R. and Siimes, M. A. (1996). Development of iron status and response to iron medication in puberty boys. *J. Pediatr. Gastroenterol. Nutr.* **22**: 312–317.

Arija, V., Salas, J., Fernández-Ballart, J., Cucó, G. and Martí-Henneberg, C. (1996). Consumo, hábitos alimentarios y estado nutricional de la población de Reus (IX). Evolución del consumo de alimentos, de su participación en la ingesta de energía y nutrientes y de su relación con el nivel socioeconómico y cultural entre 1983 y 1993. Consumption, dietary habits and nutritional status of the population from Reus (IX). Changes in food consumption, their participation in the 650 energy and nutrient intake and its relationship with socioeconomic and cultural level between 1983 and 1993. *Med. Clin.* **106**:174–179.

Barbosa García, G. C., Dianezi Gambardella, A. M. and Petrole Frutuoso, M. F. (2003). Estado nutricional e consumo alimentar de adolescentes de um centro de juventude da cidade de São Paulo. Nutritional status and food consumption of adolescents from a youth center in Sao Paulo. *Rev. Nutr.* **16**: 41–50.

Baynes, R. D., Bothwell, T. H., Bezwoda, W. R., MacPhail, A. P. and Derman, D. P. (1987). Relationship between absorption of inorganic and food iron in field studies. *Ann. Nutr. Metab.* **31**:109–116.

Beard, J. L. (2000). Iron requirements in adolescent females. *J. Nutr.* **130**:440S–442S.

Beard, J. L. (2001). Iron biology in immune function, muscle metabolism and neuronal functioning. *J. Nutr.* **131**:568S–580S.

Beard, J. L. and Connor, J. R. (2003). Iron status and neural functioning. *Annu. Rev. Nutr.* **23**:41–58.

Beard, J., Green, W., Miller, L. and Finch, C. A. (1984). Effect of iron deficiency anemia on hormone levels and thermoregulation during cold exposure. *Am. J. Physiol.* **247**:114–119.

Belton, N. R., Macvean, A. D. L., Richards, N. D., Elton, R. A., Moffat, W. M. U. and Beattie, T. F. (1997). Nutrient intake in Scottish adolescents. *Proc. Nutr. Soc.* **56**:303.

Bergström, E., Hernell, O., Lönnerdal, B. and Persson, L. A. (1995). Sex differences iron stores of adolescents: What is normal? *J. Pediatr. Gastroenterol. Nutr.* **20**:215–224.

Bothwell, T. H. (1995). Overview and mechanisms of iron regulation. *Nutr. Rev.* **53**:237–245.

Bothwell, T. H., Baynes, R. D., MacFarlane, B. J. and MacPhail, A. P. (1989). Nutritional iron requirements and food iron absorption. *J. Intern. Med.* **226**:357–365.

Bothwell, T. H., Charlton, R. W., Cook, J. D. and Finch, C. A. (1979). Iron Metabolism in Man. Blackwell Scientific, Oxford, UK.

Boyer, S. H., Bishop, T. R., Rogers, O. C., Noyes, A. N., Freilin, L. P. and Hobbs, S. (1992). Roles of erythropoietin, insulin-like growth factor 1, and unidentified serum factors in promoting maturation of purified murine erythroid colony-forming units. *Blood* **80**:2503–2512.

British Nutrition Foundation. (1995). Iron: Nutritional and Physiological Significance. British Nutrition Foundation, UK.

Carpenter, C. E. and Mahoney, A. W. (1992). Contribution of heme and nonheme iron to human nutrition. *Crit. Rev. Food Sci. Nutr.* **31**:333–367.

Casado Górriz, M. R., Casado Górriz, I. and Díaz Grávalos, G. J. (1999). La alimentación de los escolares de trece años del municipio de Zaragoza. The feeding schoolchildren of thirteen from the region of Zaragoza. *Rev. Esp. Salud Pública.* **73**:501–510.

Conrad, M. E. (1993). Regulation of iron absorption. **In:** Essential and Toxic Trace Elements in Human Disease: An Update, pp. 203–219. Prasad, A. S., Ed., Wiley-Liss, New York.

Cook, J. D., Dassenko, S. A. and Lynch, S. R. (1991). Assessment of the role of nonheme-iron availability in iron balance. *Am. J. Clin. Nutr.* **54**:717–722.

Dallman, P. R. (1987). Iron deficiency and the immune response. *Am. J. Clin. Nutr.* **46**:329–334.

Dallman, P. R. (1992). Changing iron needs from birth through adolescence. **In:** Nutritional Anemias, pp. 29–38. Fomon, S. J. and Zlotkin, S., Eds., Raven Press, New York.

Darshan, D. and Anderson, G. J. (2007). Liver-gut axis in the regulation of iron homeostasis. *World J. Gastroenterol.* **13**:4737–4745.

Davila-Hicks, P., Theil, E. C. and Lönnerdal, B. (2004). Iron in ferritin or in salts (ferrous sulfate) is equally bioavailable in nonanenic women. *Am. J. Clin. Nutr.* **80**:936–940.

Deegan, H., Bates, H. M. and McCargar, L. J. (2005). Assessment of iron status in adolescents: Dietary, biochemical and lifestyle determinants. *J. Adolesc. Health* **37**:15–21.

Deheeger, M., Bellisle, F. and Rolland-Cachera, M. F. (2002). The French longitudinal study of growth and nutrition: Data in adolescent males and females. *J. Hum. Nutr. Diet.* **14**:429–438.

Dietary Reference Intakes. (2001). Recommendations to Prevent and Control Iron Deficiency in the United States. Morbid Mortal Nuk MMWR 1998. Institute of Medicine, National Academy Press, Washington, DC.

Dömellof, M. (2007). Iron requirements, absorption and metabolism in infancy and childhood. *Curr. Opin. Clin. Metab. Care* **10**:329–335.

Durá Travé, T. (2001). Ingesta de energía y nutrientes en los alumnos de educación secundaria obligatoria. Intake of energy and nutrients in secondary education students. *An. Esp. Pediatr.* **54**:547–554.

Durá Travé, T., Aguirre Abad, P., Mauleón Rosquil, C., Oteiza Flores, M. S. and Díaz Velaz, L. (2002). Iron deficiency in adolescents 10–14 years of age. *Aten. Primaria* **29**:72–78.

Elmadfa, I. and Weichselbaum, E. (2005). Energy and nutrient intake in the European Union. *European Nutrition and Health Report 2004. Forum Nutr.* **58**:19–46.

Ervin, R. B., Wang, C. Y., Wright, J. D. and Kennedy-Stephenson, J. (2004). Dietary intake of selected minerals for the United States population: 1999–2000. *Adv. Data* **27**:1–5.

Estudio Caenpe: Consumo de alimentos y estado nutricional de la población escolar de la Comunidad de Madrid. Grupo CAENPE. Food consumption and nutritional status of the population Community School from Madrid. (1994). Ministerio de Sanidad y Consumo. Secretaría General Técnica, Madrid, Spain.

Fairweather-Tait, S. (1996). Iron requirement and prevalence of iron deficiency in adolescents. An overview. **In:** Iron Nutrition in Health and Diseases, pp. 137–148. Hallberg, L., Asp, N. G., Eds., John Libbey and Co, Eastleigh, UK.

Fernández-Morales, I., Aguilar Vilas, M. V., Mateos Veja, C. J. and Martínez Para, M. C. (2007). Ingesta de nutrientes en una población juvenil. Prevalencia de sobrepeso y obesidad. Nutrient intake in a young population. prevalence overweight and obesity. *Nutr. Clin. Diet. Hosp.* **3**:18–29.

Finch, C. (1994). Regulators of iron balance in humans. *Blood* **84**:1697–1702.

Fishman, S. M. (2000). The role of vitamins in the prevention and control of anemia. *Public Health Nutr.* **3**:125–150.

Fomon, S. J., Drulis, J. M., Nelson, S. E., Serfass, R. E., Woodhead, J. C. and Ziegler, E. E. (2003). Inevitable iron loss by human adolescents, with calculations of the requirement for absorbed iron. *J. Nutr.* **133**: 167–172.

Fomon, S. J., Nelson, S. E. and Ziegler, E. E. (2000). Retention of iron by infants. *Annu. Rev. Nutr.* **1**:273–290.

Forellat Barrios, M., Gautier du Défaix Gómez, H. and Fernández Delgado, N. (2000). Metabolismo del hierro. Iron metabolism. *Rev. Cubana Hematol. Inmunol. Hemoter.* **16**:146–160.

Frazer, D. M., Inglis, H. R., Wilkins, S. J., Millard, K. N., Steele, T. M., McLaren, G. D., McKie, A. T., Vulpe, C. D. and Anderson, G. J. (2004). Delayed hepcidin response explains the lag period in iron absorption following a stimulus to increase erythropoiesis. *Gut* **53**:1509–1515.

García-Arias, M. T., Castrillón, A. M. and Navarro, M. P. (1994). Protein, calcium and phosphorus utilisation in diets containing raw, cooked or canned tuna. Influence of 1 and 3 years'storage time. *Int. J. Food Sci. Nutr.* **45**:237–247.

García-Casal, M. N., Layrisse, M., Solano, L. and Barón, A. (1998). Vitamin A and β-carotene can improve nonheme iron absorption from rice, wheat and corn by humans. *J. Nutr.* **128**:646–650.

Glahn, R. P., Lee, O. A., Yeung, A., Goldman, M. I. and Miller, D. D. (1998). Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an in vitro digestion/Caco-2 cell culture model. *J. Nutr.* **128**:1555–1561.

Greger, J. L., Zaikis, S. C., Abernathy, R. P., Bennett, O. A. and Huffman, J. (1978). Zinc, nitrogen, copper, iron and manganese balance in adolescent females fed two levels of zinc. *J. Nutr.* **108**:1449–1456.

Grünberg, H., Mitt, K. and Thetloff, M. (1997). Food habits and dietary intake of school children in Estonia. *Scand. J. Nutr.* **41**:18–22.

Gunshin, H., Mackenzie, B., Berger, U. V., Gunshin, Y., Romero, M. F., Boron, W. F., Nussberger, S., Gollan, J. L. and Hediger, M. A. (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* **388**:482–488.

Guthrie, B. and Picciano, M. F. (1995). Human Nutrition. Mosby-Year Book, St. Louis, USA.

Guthrie, J. F., Lin, B. H. and Frazao, E. (2002). Role of food prepared away from home in the American Diet, 1977–1978 versus 1994–1996: Changes and consequences. *J. Nutr. Educ. Behav.* **34**:140–150.

Haas, J. D. and Brownlie, T. (2001). Iron deficiency and reduced work capacity: A critical review of the research to determine a causal relationship. *J. Nutr.* **131**:676–690.

Hallberg, L. (1998). Does calcium interfere with iron absorption? *Am. J. Clin. Nutr.* **68**:3–4.

Hallberg, L. and Hulthen, L. (2000). Prediction of dietary iron absorption: An algorithm for calculating absorption and bioavailability of dietary iron. *Am. J. Clin. Nutr.* **71**:1147–1160.

Hallberg, L. and Rossander, L. (1982). Effect of different drinks on the absorption of non heme iron from composite meals. *Hum. Nutr. Appl. Nutr.* **36**:116–123.

Hallberg, L. and Rossander-Hulthen, L. (1991). Iron requirements in menstruating women. *Am. J. Clin. Nutr.* **54**:1047–1058.

Hallberg, L., Hulthen, L. and Gramatkovski, E. (1997). Iron absorption from the whole diet in men: How effective is the regulation of iron absorption?, *Am. J. Clin. Nutr.* **66**:347–356.

Haro, J. F., Martínez Graciá, C., Periago, M. J. and Ros, G. (2005). Prevención de la deficiencia de hierro mediante el enriquecimiento de los alimentos. Prevention of iron deficiency through food fortification. *An. Vet.* **21**:7–21.

Hassapidou, M. N. and Fotiadou, E. (2001). Dietary intakes and food habits of adolescents in Northern Greece. *Int. J. Food Sci. Nutr.* **52**:109–116.

Hassapidou, M., Fotiadou, E., Maglara, E. and Papadopoulou, S. K. (2006). Energy intake, diet composition, energy expenditure, and body fatness of adolescents in Northern Greece. *Obesity* **14**:855–862.

Herbert, V. (1987). Recommended dietary intakes (RDI) of iron in humans. *Am. J. Clin. Nutr.* **45**:679–686.

Hercberg, S., Preziosi, P. and Galan, P. (2001). Iron deficiency in Europe. *Public Health Nutr.* **4**:537–545.

Hoppe, M., Hulthén, L. and Hallberg, L. (2008b). The importance of bioavailability of dietary iron in relation to the expected effect from iron fortification. *Eur. J. Clin. Nutr.* **62**:761–769.

Hoppe, M., Sjöberg, A., Hallberg, L. and Hulthén, L. (2008a). Iron status in Swedish teenage girls: Impact of low dietary iron bioavailability. *Nutrition* **24**:638–645.

Hunt, J. R. (2003). Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. *Am. J. Clin. Nutr.* **78**:633–639.

Hunt, J. R. and Roughead, Z. K. (2000). Adaptation of iron absorption in men consuming diets with high or low iron bioavailability. *Am. J. Clin. Nutr.* **71**:94–102.

Hurrell, R. F., Reddy, M. B., Juillerat, M. and Cook, J. D. (2006). Meat protein fractions enhance nonheme iron absorption in humans. *J. Nutr.* **136**:2808–2812.

Ilich-Ernst, J. Z., Mckenna, A. A., Badenhop, N. E., Clairmont, A. C., Andon, M. B. and Nahhas, R. W. (1998). Iron status, menarche, and calcium supplementation in adolescent girls. *Am. J. Clin. Nutr.* **68**:880–887.

Institute of Medicine. (2001). Iron. **In:** Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc, pp. 290–393. National Academy Press, Washington, DC.

Kapsokefalou, M. and Miller, D. D. (1993). Lean beef and beef fat interact to enhance nonheme iron absorption in rats. *J. Nutr.* **123**:1429–1434.

Kapsokefalou, M. and Miller, D. D. (1995). Iron speciation in intestinal contents of rats fed meals composed of meat and nonmeat sources of protein and fat. *Food Chem.* **52**:330–342.

Kastenmayer, P., Davidsson, L., Galan, P., Cherouvrier, F., Hercberg, S. and Hurrell, R. F. (1994). A double stable isotope technique for measuring iron absorption in infants. *Br. J. Nutr.* **71**:411–424.

Kelner, K. and Helmuth, L. (2003). Obesity. What is to be done? *Science* **299**:845–849.

Kersting, M., Alexy, U. and Sichert-Hellert, W. (2001). Dietary intake and food sources of minerals in 1 to 18 year old German children and adolescents. *Nutr. Res.* **21**:607–616.

Kurtz, A., Matter, R., Eckardt, K. U. and Zapf, J. (1990). Erythropoiesis, serum erythropoietin, and serum IGF-I in rats during accelerated growth. *Acta Endocrinol. (Copenh)* **122**:323–328.

Laftah, A. H., Latunde-Dada, G. O., Fakih, S., Hider, R. C., Simpson, R. J. and McKie, A. T. (2009). Haem and folate transport by proton-coupled folate transporter/haem carrier protein 1 (SLC46A1). *Br. J. Nutr.* **101**: 1150–1156.

Lanzkowski, P. (1985). Metabolismo del hierro y anemia ferripriva. **In:** Hematología Pediátrica, Iron metabolism and iron deficiency anemia. In "Pediatric Hematology" pp. 121–193. Neiler, D. R., Pearson, H. A., and Balkner, R. L., Eds., Editorial Científico Técnica, La Habana.

Lawson, M. S., Thomas, M. and Hardiman, A. (1998). Iron status of Asian children aged 2 years living in England. *Arch. Dis. Child.* **78**:420–426.

Layrisse, M., Martínez-Torres, C. and González, M. (1974). Measurement of the total dietary absorption by the extrinsic tag model. *Am. J. Clin. Nutr.* **27**:152–162.

Layrisse, M., Martinez-Torres, C., Leets, I., Taylor, P. and Ramírez, J. (1984). Effect of histidine, cysteine, glutathione or beef on iron absorption in humans. *J. Nutr.* **114**:217–223.

Leclercq, C., Piccinelli, R., Arcella, D. and Le Donne, C. (2004). Food consumption and nutrient intake in a sample of Italian secondary school students: Results from the INRAN-RM-2001 food survey. *Int. J. Food Sci. Nutr.* **55**:265–277.

Leis, R., Pavón, P., Queiro, T., Recarey, D. and Tojo, R. (1999). Atherogenic diet and blood lipid profile in children and adolescent from Galicia, NW Spain. The Galinut study. *Acta Pediatr.* **88**:19–23.

Lind, T., Hernell, O., Lönnerdal, B., Stenlund, H., Domellöf, M. and Persson, L. A. (2004). Dietary iron intake is positively associated with haemoglobin concentration during infancy but not during the second year of life. *J. Nutr.* **134**:1064–1070.

Lombardi-Boccia, G., Aguzzi, A., Cappelloni, M., Di Lullo, G. and Lucarini, M. (2003). Total-diet study: Dietary intakes of macro elements and trace elements in Italy. *Br. J. Nutr.* **90**:1117–1121.

Lönnerdal, B. and Kelleher, S. L. (2007). Iron metabolism in infants and children. *Food Nutr. Bull.* **28**:S491–499.

Martínez-Navarrete, N., Camacho, M. M., Martínez-Lahuerta, J., Martínez-Monzó, J. and Fito, P. (2002). Iron deficiency and iron fortified foods–a review. *Food Res. Int.* **35**:225–231.

Mauras, N., Rogol, A. D., Haymond, M. W. and Veldhuis, J. D. (1996). Sex steroids, growth hormone, insulin-like growth factor-1: Neuroendocrine and metabolic regulation in puberty. *Horm. Res.* **45**:1–2.

McCann, J. C. and Ames, B. N. (2007). An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am. J. Clin. Nutr.* **85**:931–945.

McKie, A. T., Barrow, D., Latunde-Dada, G. O., Rolfs, A., Sager, G., Mudaly, E., Mudaly, M., Richardson, C., Barlow, D., Bomford, A., Peters, T. J., Raja, K. B., Shirali, S., Hediger, M. A., Farzaneh, F. and Simpson, R. J. (2001). An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* **291**:1755–1759.

McNeill, G., Davidson, L., Morrison, D. C., Crombie, I. K., Keighran, J. and Todman, J. (1991). Nutrient intake in school children: Some practical considerations. *Proc. Nutr. Soc.* **50**:37–43.

McNulty, H., Eaton-Evans, J., Cran, G, Woulahan, G., Boreham, C., Savage, J. M., Fletcher, R. and Strain, J. J. (1996). Nutrient intakes and impact of fortified breakfast cereals in school children. *Arch. Dis. Child.* **75**:474–481.

Mesías, M. (2007). Importancia de la dieta en la digestibilidad y metabolismo de hierro y calcio en la adolescencia. Influencia del consumo de productos de la reacción de Maillard. Importance of diet on digestibility and metabolism of iron and calcium in adolescence. Influence of the consumption of Maillard reaction products. The Maillard reaction. Doctoral Thesis. University of Granada, Spain.

Mesías, M., Seiquer, I., Delgado-Andrade, C., Galdó, G. and Navarro, M. P. (2009b). Intake of Maillard reaction products reduces iron bioavailability in male adolescents. *Mol. Nutr. Food Res.* **53**:1551–1560.

Mesías, M., Seiquer, I., Muñoz-Hoyos, A., Galdó, G. and Navarro, M. P. (2009a). The beneficial effect of Mediterranean dietary patterns on dietary iron utilization in male adolescents aged 11–14 years. *Int. J. Food Sci. Nutr.* **60**:355–368.

Miret, S., Simpson, R. J. and McKie, A. T. (2003). Physiology and molecular biology of dietary iron absorption. *Annu. Rev. Nutr.* **23**:283–301.

Monsen, E. R., Hallberg, L., Layrisse, M., Hegsted, D. M., Cook, J. D., Mertz, W. and Finch, C. A. (1978). Estimation of available dietary iron. *Am. J. Clin. Nutr.* **31**:134–141.

Moreiras, O., Carbajal, A., Cabrera, L. and Cuadrado, C. (2010). Ingestas recomendadas de energía y nutrientes para la población española (revisadas y ampliadas 2006). **In:** Tablas de Composición de Alimentos, Recommended intakes of energy and nutrients for the Spanish population (revised and expanded 2006). In: Tables of Food Composition pp. 227–243. Departamento de Nutrición, Universidad complutense de Madrid, Ed., Ediciones Pirámide, Madrid, Spain.

Muñoz Hoyos, A. and Molina Carballo, A. (2005). Hierro. (Iron. In: Treaty on Nutrition.). **In:** Tratado de Nutrición, pp. 927–972. Gil Hernández, A., Ed., Acción Médica, Madrid, Spain.

National Research Council. Recommended Dietary Allowances. (1989). Report of the Subcommittee on the Tenth Edition of the RDA. Food and Nutrition Board and the Commission on Life Sciences. National Academy Press, Washington, DC.

Navas-Carretero, S., Pérez-Granados, A. M., Sarriá, B., Carbajal, A., Pedrosa, M. M., Roe, M. A., Fairweather-Tait, S. J. and Vaquero, M. P. (2008). Oily fish increases iron bioavailability of a phytate rich meal in young iron deficient women. *J. Am. Coll. Nutr.* **27**:96–101.

Nelson, M., Naismith, D. J., Burley, V., Gatenby, S. and Geddes, N. (1990). Nutrient intakes, vitamin-mineral supplementation, and intelligence in British school children. *Br. J. Nutr.* **64**:13–22.

Nemeth, E. and Ganz, T. (2006). Regulation of iron metabolism by hepcidin. *Ann. Rev. Nutr.* **26**:323–342.

Papanikolaou, G., Tzilianos, M., Christakis, J. I., Bogdanos, D., Tsimirika, K., MacFarlane, J., Goldberg, Y. P., Sakellaropoulos, N., Ganz, T. and Nemeth, E. (2005). Hepcidin in iron overload disorders. *Blood* **105**:4103–4105.

Pérez-Cueto, F. J. A., Almanza-López, M. J., Pérez-Cueto, J. D. and Eulert, M. E. (2009). Estado nutricional y características de la dieta de un grupo de adolescentes de la localidad rural de Calama, Bolivia. Nutritional status and dietary characteristics of a group of teenagers from the rural town of Calama, Bolivia. *Nutr. Hosp.* **24**:46–50.

Pizarro, F., Olivares, M., Hertrampf, E., Mazariegos, D. I. and Arredondo, M. (2003). Heme-iron absorption is saturable by heme-iron dose in women. *J. Nutr.* **133**:2214–2217.

Powell, J. J., Burden, T. J., Greenfield, S. M., Taylor, P. D. and Thompson, R. P. H. (1999). Urinary excretion of essential metals following intravenous calcium disodium edetate: An estimate of free zinc and zinc status in man. *J. Inorg. Biochem.* **75**:159–165.

Qian, M. and Eaton, J. W. (1991). Iron translocation by free fatty acids. *Am. J. Pathol.* **139**:1425–1434.

Qiu, A., Jansen, M., Sakaris, A., Min, S. H., Chattopadhyay, S., Tsai, E., Sandoval, C., Zhao, R., Akabas, M. H. and Goldman, I. D. (2006). Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell* **127**:917–928.

Reddy, M. B., Hurell, R. F. and Cook, J. D. (2000). Estimation of nonheme-iron bioavailability from meal composition. *Am. J. Clin. Nutr.* **71**:937–943.

Rockett, H. R. H., Berkey, C. S., Field, A. E. and Colditz, G. A. (2001). Cross-sectional measurement of nutrient intake among adolescents in 1996. *Prev. Med.* **33**:27–37.

Roma-Giannikou, E., Adamidis, D., Gianniou, M., Nikolara, R. and Matsaniotis, N. (1997). Nutritional survey in Greek children: Nutrient intake. *Eur. J. Clin. Nutr.* **51**:273–285.

Román Viñas, B., Serra Majem, L., Ribas Barba, L., Pérez Rodrigo, C. and Aranceta Bartrina, J. (2004). Fuentes alimentarias de energía, macronutrientes y micronutrientes en la población española infantil y juvenil. **In:** Nutrición Infantil y Juvenil, Dietary sources of energy, macronutrients and micronutrients in children and young Spanish population. In: Infant and child Nutrition. pp. 73–101. Serra Majem, L. and Aranceta Bartrina, J., Eds., Masson, Barcelona, Spain.

Romslo, I., Haram, K., Sagen, N. and Augensen, K. (1983). Iron requirement in normal pregnancy as assessed by serum ferritin, serum transferrin saturation and erythrocyte protoporphyrin determinations. *Br. J. Obstet. Gynecol.* **90**:101–107.

Rossander-Hulthen, L., Brune, M., Sandstrom, B., Lonnerdal, B. and Hallberg, L. (1991). Competitive inhibition of iron absorption by manganese and zinc in humans. *Am. J. Clin. Nutr.* **54**:152–156.

Rossander-Hulthen, L. and Hallberg, L. (1996). Prevalence of iron deficiency in adolescents. **In:** Iron Nutrition in Health and Disease, pp. 149–156. Hallberg, L. and Asp, N. G., Eds., John Libbery and Co, London, UK.

Samman, S., Sandstrom, B., Toft, M. B., Bukhave, K., Jensen, M., Sørensen, S. S. and Hansen, M. (2001). Green tea or rosemary extract added to foods reduces nonheme iron absorption. *Am. J. Clin. Nutr.* **73**:607–612.

Sanders, M., Sorba, S. and Dainiak, N. (1993). Insulin-like growth factors stimulate erythropoiesis in serum-substituted umbilical cord blood cultures. *Exp. Hematol.* **21**:25–30.

Sandstrom, B. (2001). Micronutrient interactions: Effects on absorption and bioavailability. *Br. J. Nutr.* **85**:181–185.

Schenkel, T. C., Stockman, N. K. A., Brown, J. N. and Duncan, A. M. (2007). Evaluation of energy, nutrient and dietary fiber intakes of adolescent males. *J. Am. Coll. Nutr.* **26**:264–271.

Scholl, T. O. (2005). Iron status during pregnancy: Setting the stage for mother and infant. *Am. J. Clin. Nutr.* **81**:1218–1222.

Scientific Committee for Food. 1993. Nutrient and Energy Intakes for the European Community. Opinion Adopted by the Scientific Committee on Food on 11 December 1992. Reports of the Scientific Committee for Food, Thirty-First Series. European Commission, Luxembourg. Available at: http://ec.europa.eu/food/fs/sc/scf/reports_en.html (accessed September 18, 2010)

Scrimshaw, N. (1991). Iron deficiency. *Sci. Am.* **265**:46–52.

Seiquer, I., Aspe, T, Pérez-Granados, A. M. and Navarro, M. P. (2002). Consumption of raw and fried sardine (Clupea pilchardus) as protein source of diets: Effects on iron metabolism in rats. *J. Sci. Food Agric.* **82**:1497–1503.

Serra Majem, L., Ribas, L., Ngo, J., Aranceta, J., Garaulet, M., Carazo, E., Mataix, J., Pérez-Rodrigo, C., Quemada, M., Tojo, R. and Vázquez, C. (2001). Risk of inadequate intake for vitamin A, B1, B6, C, E, folate, iron and calcium in Spanish population aged 4 to 18. *Int. J. Nutr. Res.* **71**:325–331.

Serra-Majem, L., Ribas-Barba, L., Pérez-Rodrigo, C. and Aranceta Bartrina, J. (2006). Nutrient adequacy in Spanish children and adolescents. *Br. J. Nutr.* **96**:49–57.

Serra-Majem, L., Ribas-Barba, L., Salvador, G., Jover, L., Raidó, B., Ngo, J. and Plasencia, A. (2007). Trends in energy and nutrient intake and risk of inadequate intakes in Catalonia, Spain (1992–2003). *Public Health Nutr.* **10**:1354–1367.

Shamah, T. and Villalpando, S. (2006). The role of enriched foods in infant and child nutrition. *Br. J. Nutr.* **96**:73–77.

Sharp, P. (2004). The molecular basis of copper and iron interactions. *Proc. Nutr. Soc.* **63**:563–569.

Sharp, P. and Srai, S. K. (2007). Molecular mechanisms involved in intestinal iron absorption. *World J. Gastroenterol.* **13**:4716–4724.

Smith, C. P. and Thévenod, F. (2009). Iron transport and the kidney. *Biochim. Biophys. Acta* **1790**:724–730.

Spodaryk, K. (1999). Disparity between dietary iron intake and iron status of children aged 10–12 years. *Arch. Physiol. Biochem.* **107**:361–366.

Tapiero, H., Gate, L. and Tew, K. D. (2001). Iron: Deficiencies and requirements. *Biomed. Pharmacother.* **55**:324–332.

Teucher, B., Olivares, M. and Cori, H. (2004). Enhancers of iron absorption: Ascorbic acid and other organic acids. *Int. J. Vitam. Nutr. Res.* **74**:403–419.

Thompsen, K., Riis, B., Krabbe, S. and Christiansen, C. (1986). Testosterone regulates the haemoglobin concentration in male puberty. *Acta Paediatr. Scand.* **75**:793–796.

Tojo, R., Leis, R. and Queiro, T. (1991). Nutrición en el adolescente. Factores de riesgo biopsicosociales. Nutrition in the adolescent. Biopsychosocial risk factors. *An. Esp. Pediatr.* **35**:74–83.

Tondeur, M. C., Schauer, C. S., Christofides, A. L., Asante, K. P., Newton, S., Serfass, R. E. and Zlotkin, S. H. (2004). Determination of iron absorption from intrinsically labeled microencapsulated ferrous fumarate (sprinkles) in infants with different iron isotope method. *Am. J. Clin. Nutr.* **80**:1436–1444.

Turan, C., Öner, N., Garipagaoglu, M., Küçükugurluoglu, Y., Tokuç, B. and Acunas, B. (2009). Evaluation of the nutritional status of male adolescents. *Trakya Univ. Tip. Fak. Derg.* **26**:59–67.

Urbano, M., Vitalle, M., Juliano, Y. and Amancio, O. (2002). Iron, copper and zinc in adolescents during puberty growth spurt. *J. Pediatr.* **78**:327–334.

Van Poppel, G., Schneijder, P., Löwik, M. R. H., Schrijver, J. and Kok, F. J. (1991). Nutritional status and food consumption in 10–11 year old Dutch boys (Dutch Nutrition Surveillance System). *Br. J. Nutr.* **66**:161–169.

Viglietti, G. C. and Skinner, J. D. (1987). Estimation of iron bioavailability in adolescents' meals and snacks. *J. Am. Diet. Assoc.* **87**:903–907.

Vihervuori, E., Sipilä, I. and Siimes, M. A. (1994). Increases in hemoglobin concentration and iron needs in response to growth hormone treatment. *J. Pediatr.* **125**:242–245.

Vihervuori, E., Virtanen, M., Koistinen, H., Koistinen, R., Seppälä, M. and Siimes, M. A. (1996). Hemoglobin level is linked to growth hormone-dependent proteins in short children. *Blood* **87**:2075–2081.

Vila, M. and Quintana, M. (2008). Ingesta de hierro dietario en mujeres adolescentes de instituciones educativas. Dietary iron intake in adolescent girls from educational institutions. *An. Fac. Med.* **69**:172–175.

Villa Elízaga, I., Navarro Blasco, I. and Martín Pérez, A. (1999). Elementos trazas. **In:** Tratado de Nutrición, Trace elements. In: Treaty of Nutrition. pp. 229–247. Hernández, M. and Sastre, A., Eds., Ediciones Díaz de Santos, Madrid, Spain.

Vitali, D., Vedrina Dragojevic, I., Sebecic, B. and Vujic, L. (2007). Impact of modifying tea biscuit composition on phytate levels and iron content and availability. *Food Chem.* **102**:82–89.

Walczyk, T., Davidsson, L., Rossander-Hulthen, L., Hallberg, L. and Hurrell, R. F. (2003). No enhancing effect of vitamin A on iron absorption in humans. *Am. J. Clin. Nutr.* **77**:144–149.

World Health Organization (WHO). (2009). Available at: http://www.who.int/es/. (Accessed July 1, 2010).

Yu, J. Y. and Cho, Y. O. (1990). Tissue iron content in riboflavin and pyridoxine deficient rats. *J. Nutr. Biochem.* **1**:310–314.

Zhang, A. S. and Enns, C. A. (2009). Molecular mechanisms of normal iron homeostasis. *Hematology* 207–214.

Zimmermann, M. B., Chaouki, N. and Hurrell, R. F. (2005). Iron deficiency due to consumption of a habitual diet low in bioavailable iron: A longitudinal cohort study in Moroccan children. *Am. J. Clin. Nutr.* **81**:115–121.

Zofkova, I. and Nemcikova, P. (2008). Trace elements and their relationship to bone metabolism. *Diabetol. Metab. Endokr. Vyz.* **11**:171–176.