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Title: Evaluation of the Iron Status of a Population of Adults in Morocco: Influence of Dietary Intake

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James E. Leklem

The diets of rural Moroccans are monotonous and cereal-based. Iron supplied from foods is mostly non-heme iron. The consumption of flesh foods (meat, poultry and fish), that provide readily available iron, is low. This study was undertaken to determine the iron status of a rural population of adults in Morocco, and the influence of the diet on iron status. Food consumption was determined in 28 families, using a seven-days weighed-intake method. Caloric, protein and iron intakes were determined using Food Composition Tables. Blood samples were drawn from 47 adult males and 50 adult females. The prevalence of iron deficiency in men and women was evaluated using a Tri-index model (mean corpuscular hemoglobin concentration (MCHC), serum transferrin saturation (STS), and serum ferritin (SF)) and mixed distribution analysis of hemoglobin.

The availability of dietary iron was estimated to be 6%. Using hemoglobin concentration as an indicator of iron status, six (12%) females were anemic (Hb<120g/L). A similar figure was found using mixed distribution analysis of hemoglobin. The use of STS indicated that 10 (21%) males and 34 (68%) females were iron deficient (second stage). Only 2 (4%) males and 24 (48%) females had depleted iron stores (SF < 12 μg/L) (first stage of iron deficiency). These results confirmed that iron deficiency could not be correctly identified using a single biochemical test such as hemoglobin and that a minimum of two independent biochemical measures should be used. With the Tri-index model (two and three abnormal values), 19 (38%) females were iron deficient.

The high prevalence of iron deficiency, particularly among females, may be partially attributed to the low intake of readily available dietary iron. The lack of a strong correlation between dietary intake and biochemical indices of iron may be attributed to the fact that the latter may vary considerably with non-dietary factors, such as the presence of parasites. The results of this study suggest that other studies considering different age groups should be conducted in Morocco in order to better understand the etiology of iron deficiency anemia, prior to considering any intervention trial toward the eradication of iron deficiency.

Evaluation of the Iron Status of a Population of Adults in Morocco: Influence of Dietary Intake

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CHAPTER 1

INTRODUCTION

Nutritional anemia is a worldwide problem. Iron deficiency is the most common cause of anemia and the most prevalent nutritional deficiency affecting humans (World Health Organization [WHO], 1975). Cook and Lynch (1986) reported that about 30% of the estimated world population (4.5 billion) are anemic, and at least half of these, 500 to 600 million people, are believed to have iron deficiency anemia. In situations where there is adequate food, iron deficiency is most likely caused by low bioavailability of dietary iron rather than low intake per se. Rice, maize and wheat, forming the major staple foods of poor populations of the world, appear to contain sufficient iron, however, only 1%, 3% and 5%, respectively of the iron in rice, maize and wheat have been found to be absorbed (Gillooly et al., 1984). There can be no question that cereal iron is poorly bioavailable because cereals contain substances that inhibit iron absorption, mainly phytates, polyphenols and fiber. While cereal products limit the availability of iron, iron in meats appears to be highly available.

Absorption of iron from plant foods ranges from 1 to 7% , while it is from 11 to 22% in foods of animal origin (Hazell, 1985).

The staple food in Morocco is bread made from cereals (mainly wheat) of varying degrees of refinement. Cereals are also consumed as couscous (a Moroccan pasta), soup, pasta, vermicelli and noodles. Vegetables and fruits (mainly oranges) are also consumed in relatively higher amounts than foods of animal origin. Tea consumption is high, particularly in rural areas, where tea constitutes the everyday beverage. Hallberg reported that the consumption of tea at breakfast meals reduces the iron availability, while the consumption of orange juice (containing ascorbic acid) enhance it (Hallberg, 1981). The effect of tea on iron availability was attributed to its content of tannins. Iron absorption was higher in anemic subjects consuming a sorghum-based diet with low levels of tannins than those consuming similar diets with high levels of tannins (Radhakrishnan and Siva Prasad, 1980).

In a previous study, we found that in four rural areas of Morocco, the total dietary iron supply ranged from 14.5 to 22.5 mg/person/day, and that more than 80% of this supply was non-heme iron from cereals and vegetables. Cereals contributed greater than 68% of the total iron supply (Alaoui and Leklem, 1987). From these observations, it was concluded that the availability of iron from such diets is

low and might affect iron status of the populations, particularly those living in rural areas. In addition, iron supplementation has been one of the programs considered for improvement of nutrition in Morocco (Waslien, 1981). As in many African countries, most surveys conducted in Morocco have mainly involved either clinical patients or specific groups such as children, pregnant and lactating women, and do not reflect the true prevalence of iron deficiency anemia in the population. In addition, those surveys have been based on either only one or a few laboratory parameters, usually hemoglobin, hematocrit (Ministère de la santé publique, S.C. PSME-Nutrition, 1985) and serum iron in some clinical cases. The usual approach, of separating normal from iron deficient subjects on the basis of only one criterion, involves errors in the diagnosis of both subjects (Hercberg et al., 1988). The present work was designed for the following objectives:

- To assess the iron status of a randomized sample of a population living in a rural area of Morocco.
- To determine to what extent the consumption of a typical cereal based diet may lead to iron deficiency as a result of low bioavailability of iron.
- To establish the prevalence of iron deficiency anemia in a rural population of adults.
- To suggest possible means to correct any deficiency detected.

The area of the study is in the Province of Chefchaounen (see Figs. 1.1 & 1.2). This region is characterized by its isolation from the commercial "circuit" and the high consumption of locally produced foods. Bioavailability of iron from meals was determined using the Monsen Model (Monsen, 1980), based upon the total dietary iron intake and the intake of ascorbic acid and meat, poulty and fish as enhancers of iron absorption.

The determination of iron status was based on a Tri-index model (Bindra and Gibson, 1986) using a combination of three iron indices, namely, mean corpuscular hemoglobin concentration (MCHC), serum transferrin saturation (STS), and serum ferritin (SF). MCHC is the concentration of Hb per unit volume of erythrocytes. Low values of MCHC are indicative of the final stage of iron deficiency, hypochronic, microcytic anemia. STS is an index of iron transport in the blood, and serum ferritin is an index of iron stores (Cook et al., 1976). These measures were chosen based on their accuracy in determining iron status and on the availability of the equipment needed to do the assays.

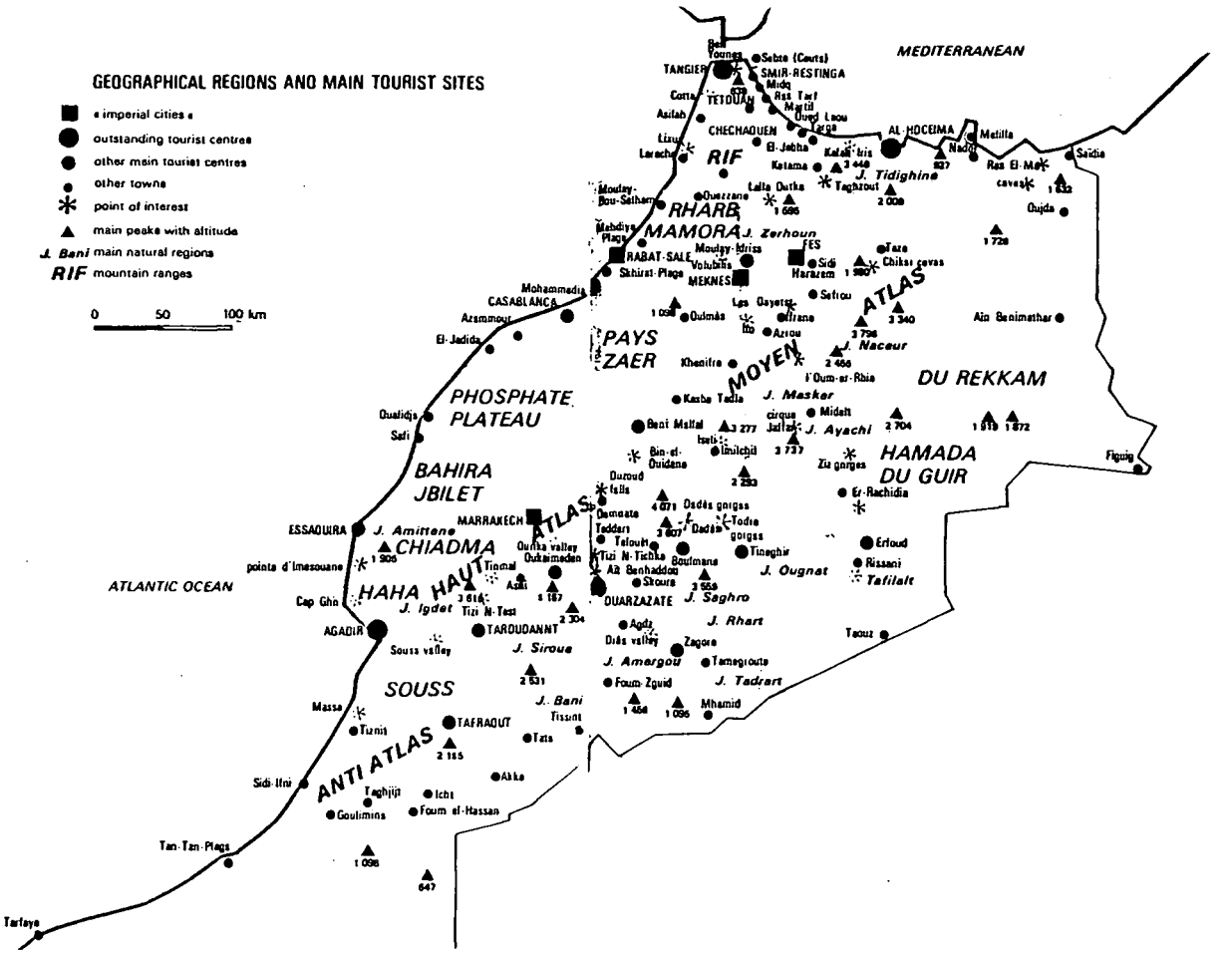

Figure 1.1. Map of Morocco.

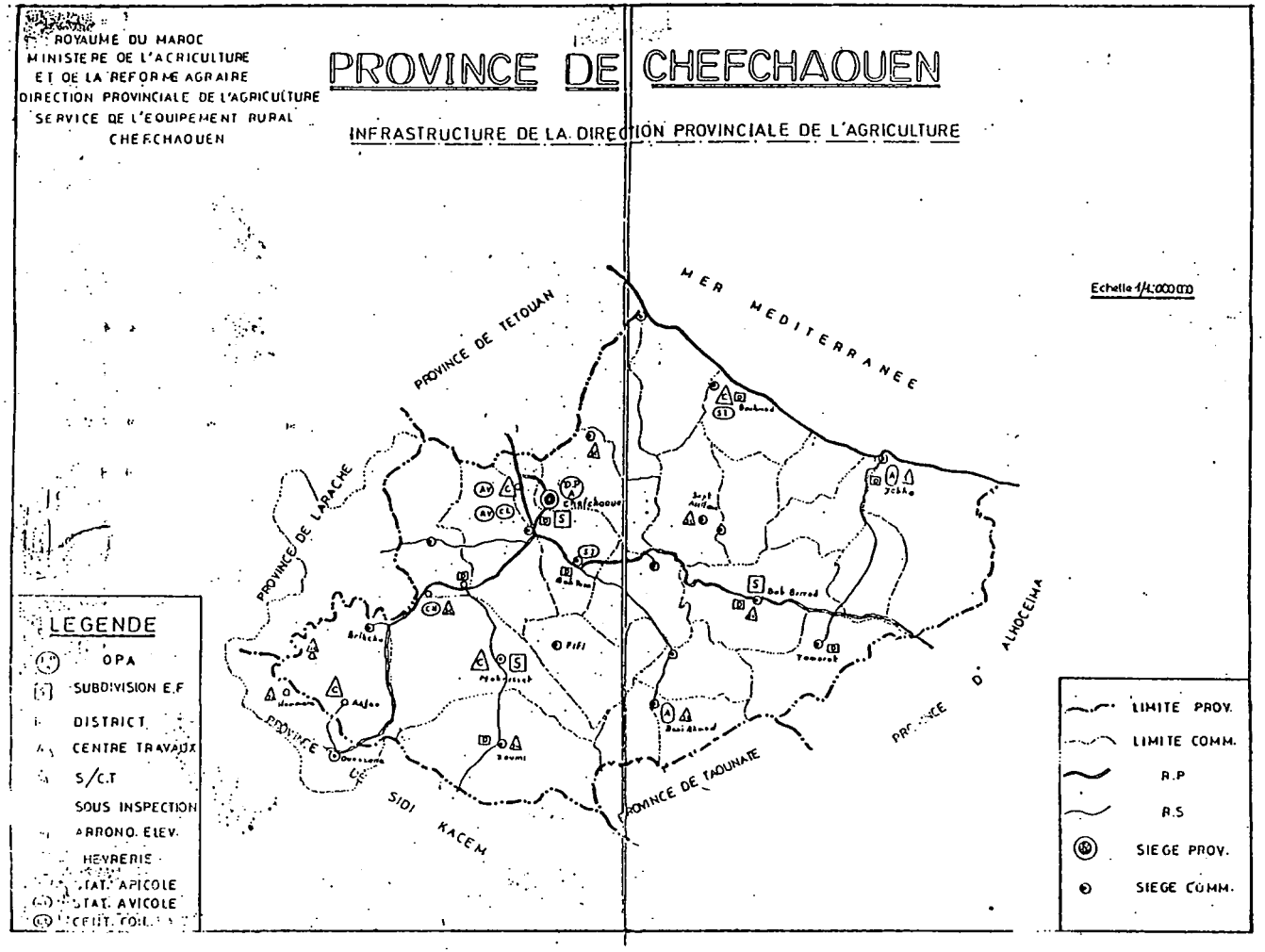

Figure 1.2. Map of the Province of Chefchaouen.

CHAPTER 2

REVIEW OF LITERATURE

2.1 **IRON METABOLISM**

The total iron content of the human body varies with age, sex, nutrition, and the state of health. For a normal adult man, the iron content is approximately 60 mg/kg body weight or a total of 4 grams. Most of the iron in the body is involved in oxygen transport, oxygen storage, and in a variety of enzymatic processes (Brittenham et al., 1981). Body iron may be divided into essential iron and iron reserves. Essential iron is mainly concentrated in hemoglobin and iron enzymes. Storage iron is in ferritin and hemosiderin and is useful in times of iron needs (Finch and Huebers, 1982). Hemoglobin represent about 2/3 of the iron in the body (Table 2.1) and contains 0.34% iron (Zittoun, 1987).

Table 2.1. Iron distribution in the human body (mean values in adults).

	grams of iron	% of total
Hemoglobin	2.4	65
Ferritin and hemosiderin	1	30
Myoglobin	0.15	3.5
Hemic enzymes and flavoproteins	0.02	0.5
Fe bound to transferrin	0.004	0.1

While iron in ferritin is easily mobilized when needed, the hemosiderin iron is in an insoluble iron-protein complex not easily mobilized. Iron content of hemosiderin is about 25 to 35% of the complex. Iron bound to transferrin represents only 0.1% of the total iron in the body, but constitutes the most dynamic site, and is in continuous exchange with the other body iron compartments.

2.1.1 Iron Absorption

Iron absorption takes place mainly in the duodenum and to some extent in the jejunum. In the rat, iron is absorbed more efficiently from the duodenum than the jejunum in both sexes, with duodenal absorption of iron more efficient in females than in males (Richter and Rochester, 1982). Solubility of iron in the intraluminal milieu is required for its absorption. Iron is absorbed most effectively in the ferrous (Fe^{+2}) form and in the intestinal cell it is converted to the ferric Fe (Fe^{+3}) form. This form is then transported across the cell wall and goes into the blood, where it binds with transferrin. Fig. 2.1 summarizes the three phases of iron absorption from the gut (Narasinga Rao, 1981). In the first phase of iron absorption (intraluminal phase), food is digested and iron is released in a soluble form. The second phase of iron absorption is the mucosal phase, where iron is taken up by the mucosal cell. Finally, in the third phase (corporeal

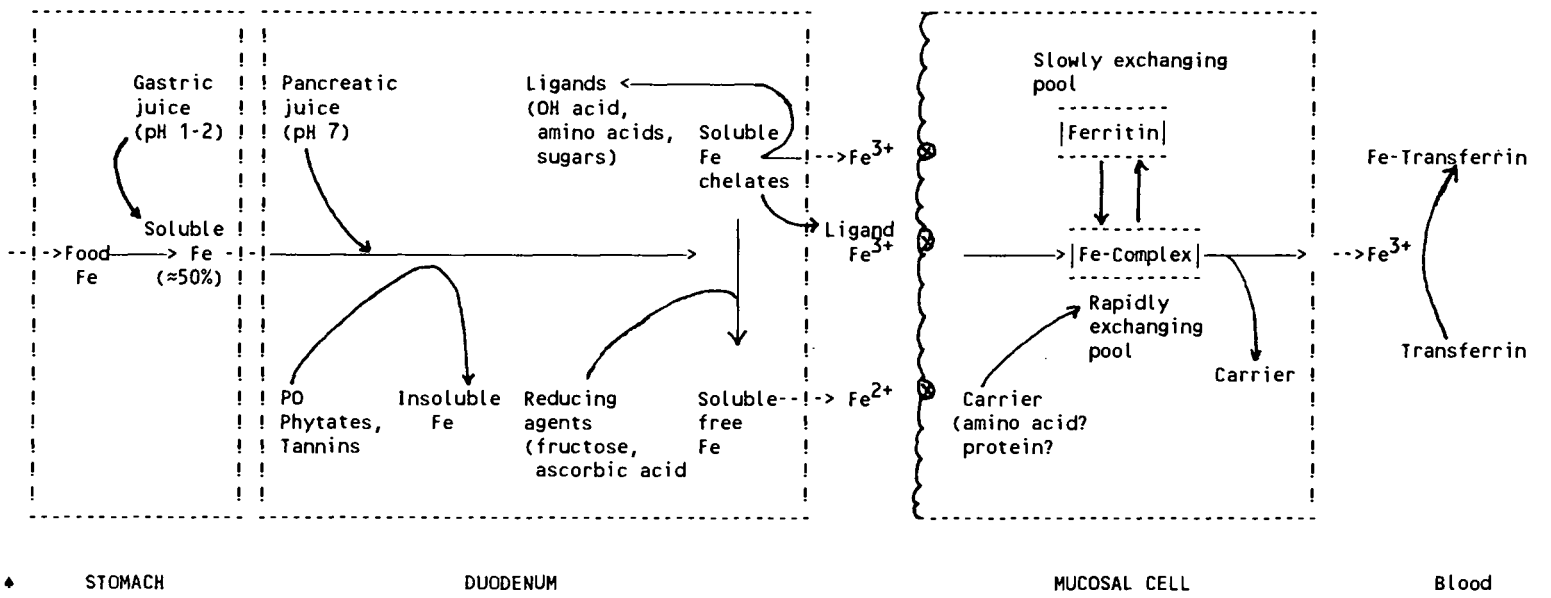

Fig. 2.1. Schematic representation of iron absorption from the gut (from Narasinga Rao, 1981).

phase), iron is taken up by transferrin in plasma on the secosal side of the mucosal cell and transported to tissues for further metabolism. Narasinga reported that once taken up by the mucosal cell iron is bound to specific carriers, probably transferrin-like specific proteins and amino acids, and than transferred to the serosal side (Narasinga Rao, 1981). The exact mechanism of this transfer is not clear. Within the mucosal cell, two proteins were identified: ferritin and a transferrin-like protein which seems to be the true iron transporter. The control of the quantity of iron that enters the blood takes place at the brush border of the mucosal cell, and iron excretion is relatively constant (Zittoun, 1987).

Iron balance is controlled by regulation of its absorption, even though the ability of the human gastrointestinal tract to increase its dietary iron absorption is limited (Hazell, 1985). Humans can adapt to iron requirements and intakes by modifying the rate of iron absorption according to body iron needs (Cook, 1990). Depending on the concentration of iron within the intestinal lumen, it seems that two mechanisms of absorption of iron are involved (Hercberg, 1988). At physiological doses (as found in a normal diet), iron is taken up by membrane receptors of the enterocytes by an active process with limited capacity. However, at high doses, a passive mechanism predominates. Within the mucosal cell, non-heme iron is

partially related to specific transporters. This results in the rapid transfer of iron from the cell to the blood (serum). Excess iron within the cell is trapped within an apoferritin and stored as ferritin. Absorption of heme iron is not influenced by pH and is little affected by gastric secretions. Morley and coworkers (1983) showed that iron is taken up by the rat hepatocyte from serum transferrin by a process not requiring energy or movement of serum transferrin into the cell interior, and that intracellular transferrin is involved in acquiring iron from serum transferrin at the cell surface, with the iron then being transferred to the ferritin

Several factors influence iron absorption. This aspect will be examined later when addressing bioavailability.

2.1.2 Iron Transport

When iron is absorbed from the small intestine, it immediately combines with a beta globulin, transferrin, with which it is transported in the blood plasma. The main function of transferrin is in the transport of iron. Transferrin is synthesized mainly in the liver (Pike and Brown, 1975). Transferrin can be separated into four protein bands, namely apotransferrin, di-ferric transferrin and two forms of mono-ferric transferrin (one of which bear its single iron atom at the site near the C-terminal of the protein and the other bears it at the site near the N-

terminal). In the process of iron uptake by cells, the di-ferric transferrin releases its iron from the acid-labile site at the N-domain first before the other iron from the acid-stable site is released (Loh, 1983). The di-ferric transferrin has a seven-fold capacity for delivering iron to reticulocytes, and the binding of iron to the transferrin sites is strong but reversible and depends on the form of iron. The plasma transferrin mediates iron exchange between body tissues. Iron is delivered to tissues by the interaction of the transferrin-iron complex with specific membrane receptors (Finch and Huebers, 1982). Each molecule of transferrin can carry 2 atoms of ferric iron. The total amount of transferrin-bound iron averages 4 mg, and normally 20 to 50% of transferrin is saturated with iron (Arthur and Isbister, 1987).

In addition, iron can be complexed with histidine, fructose, oxalate, glutamate, or tricine (N-trihydroxymethylglycine), forming low-molecular-weight complexes that circulate in plasma. High levels of this non-transferrin bound iron in plasma may be responsible for hepatic iron loading in iron overload states (Brissot et al., 1985). On the other hand, others found that metallothionein failed to mobilize iron from ghosts of rabbit reticulocytes and thus is unlikely to act as an intracellular iron-transport agent (Kojima et al., 1982). Morley and coworkers (1983) concluded that in the rat, iron is taken up by the liver cell

from serum transferrin by a process not requiring energy for the movement of the transferrin into the cell, and that an intracellular transferrin is involved in acquiring iron from the serum transferrin. The iron is than transferred to the small molecular weight iron pool and subsequently to ferritin.

2.1.3 Iron Storage

The primary form of iron storage in the body is ferritin. This is a soluble protein that can be degraded to an insoluble derivative, hemosiderin (Worwood, 1986). In addition to iron, the principal component of ferritin is a globular protein called apoferritin. Iron is taken up within the interior apoferritin shell, and may accumulate as a crystalline lattice structure of ferric oxyhydroxide (Fe OOH). Each ferritin molecule may incorporate as much as 4500 atoms as the ferric oxide hydrate (Macara et al., 1972). Therefore, the major function of ferritin is to provide a store of iron which may be used when required, mainly for heme synthesis. Iron in excess is stored intracellularly as ferritin and hemosiderin mainly in the reticuloendothelial system of the liver, spleen, bone marrow and other organs. In healthy adult men and women in the United States with predicted iron stores of 900 and 300 mg iron, median serum ferritin values of 90 and 30 ng/ml, respectively, were observed. This indicates that a concentration of 10 ng/ml of serum ferritin was equivalent to

about 100 mg of storage iron (Cook, Finch, and Smith, 1976). Cavill and coworkers (1986) reported that the cause of a low serum ferritin concentration is a reduction in the level of iron stores. Ferritin is predominantly intracellular, and it has been quantitated in human serum and used as an appropriate index for the assessment of iron status (Cook et al., 1974).

2.1.4 Iron Function in the Body

The main functions of iron in the body are to transport oxygen via hemoglobin, for cellular oxidative phosphorylation, and to serve in a variety of enzymatic processes (Table 2.2). In the body, iron serves a role as a mediator of oxidative processes.

Table 2.2. Iron-dependent enzymes and their functions (Galan et al., 1984, Arthur and Isbister, 1987).

Enzymes	Functions
Mitochondrial cytochromes	Electron transfer in oxidative phosphorylation leading to ATP (energy) production.
Cytochrone P_{450} (endoplasmic reticulum)	Hydroxylation of steroids, oxidation of foreign compounds, drug detoxification
Ribonucleotide reductase (cytosol, nucleus)	DNA synthesis
Catalase and lactic peroxidase (peroxisomes)	Peroxide breakdown
Tryptophan pyrrolase (cytosol)	L- tryptophan -----> formyl kynurenine
Proline hydroxylase	Amino acid metabolism
Monoamine oxidase	Catecholamine metabolism.
Glucose-6P and 6-phosphogluconate dehydrogenase	Pentose phosphate cycle; NADPH and pentose synthesis

Heme compounds carry oxygen to tissue cells and transport hydrogen to molecular oxygen as a part of the cellular electron transport system. Fig. 2.2 illustrates the basic chemical steps in the synthesis of heme (Pike and Brown, 1975). Hemoglobin is synthesized from heme and globin (Guyton, 1981).

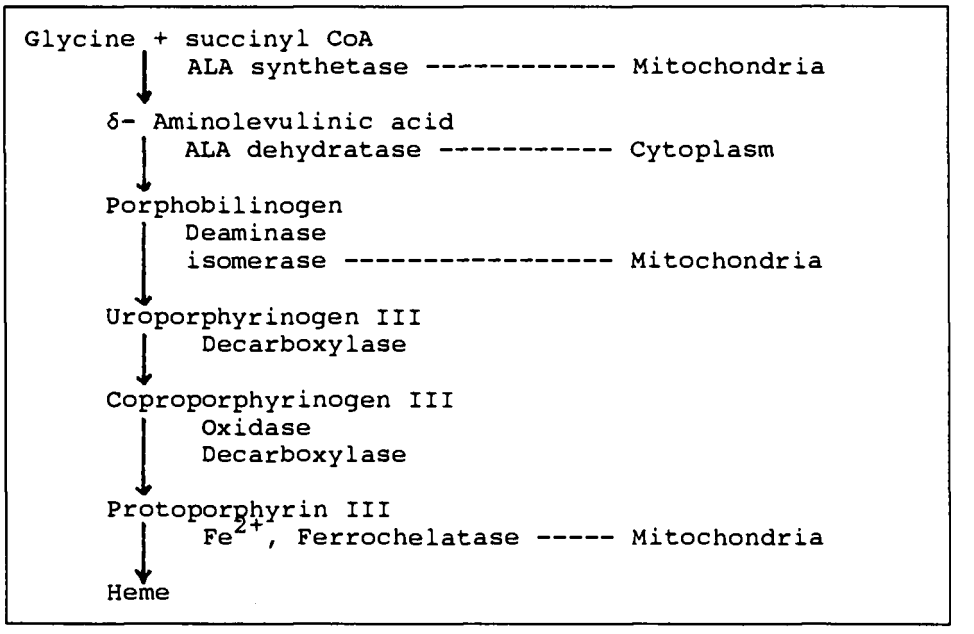

Figure 2.2. Biosynthesis of heme.

There are a number of enzymes in which iron is a cofactor. Iron is required for the hydroxylation of proline and lysine, compounds involved in the synthesis of collagen. It acts as a cofactor for proline and for lysine hydroxylase, which could explain why collagen synthesis is impaired in iron deficiency (Jacobs, 1977).

Iron is involved in other metabolic processes, the mechanisms of which are less well understood. Galan and

coworkers (1984) reported that iron is involved in DNA synthesis, lipid metabolism, body temperature regulation and in host defense to infection. The latter effects of iron were observed by different authors in iron deficient man and animals.

2.1.5 Iron Requirements

The requirements for iron are those needed to assure normal tissue growth, hemoglobin formation, and to replace iron losses in the feces, urine and sweat. Additional iron is needed in the female related to menstruation, gestation and lactation (Underwood, 1971). Estimated iron requirements for humans are summarized in Table 2.3 (WHO, 1970 and 1972).

Table 2.3. Requirement for iron.

Age groups	Requirements (mg/day)*
Infants 5 to 12 months	0.7
Children, 1 to 12 years	1.0
Boys 13 to 16 years	1.8
Girls 13 to 16 years	2.4
Men	0.9
Women: Menstruating	2.8
Pregnant, first half	0.8
Pregnant, second half	3.0
Lactating	2.4

* = Amount that must be absorbed to maintain homeostatis.

In the adult male, iron losses are about 1 mg iron/day, while in the adult menstruating female, this loss amounts to about 1.4 mg/day. Additional losses occur dur-

ing pregnancy. Iron losses decrease in iron deficiency and increase in iron overload; therefore, intestinal absorption is the main mechanism of regulation (Finch, 1977).

Additional iron losses may occur with hookworm infection leading to intestinal bleeding (Baker and Demaeyer, 1979). Hookworm is considered to be an important cause of iron imbalance (Hercberg et al., 1987).

2.2 **FORMS OF IRON IN THE DIET**

Dietary iron exists in two major forms: heme iron and non-heme iron. These two forms of dietary iron are absorbed in different manners and at different rates. This results in the formation of a pool of heme iron and another pool of non-heme iron (Monsen, 1980). Heme iron consists of iron which comes from hemoglobin and myoglobin present in meat, poultry and fish. It is initially taken-up as an intact porphyrin molecule with its iron being freed within the intestinal mucosal wall.

Non-heme (ferric iron) iron comes from other foods such as vegetables, cereals, dry legumes, fruits and dietary products as well as from the non-heme iron from meats, poultry and fish (Monsen et al., 1978). Hazell reported that iron compounds belonging to the same pool show the same percentage absorption when they are administered together (Hazell, 1985).

Some of the iron present in the diet may be in a chemical form that is either poorly or not at all absorbable. Examples are, ironically, iron compounds that have been used for fortification of foods such as most forms of reduced iron as ferric orthophosphate (Hallberg, 1981). Iron supplements are numerous and include soluble iron compounds such as ferrous sulfate, ferrous citrate, ferrous fumarate, ferrous gluconate, ferrous lactate and ferrous tartrate. Among these, ferrous sulfate is recommended for use in public health programs (INACG, 1977). These soluble iron compounds are equally well absorbed.

2.3 **IRON BIOAVAILABILITY**

The main cause of nutritional iron deficiency is the poor assimilation of dietary iron (Cook et al., 1981). Several factors that influence iron absorption have been identified as shown in Table 2.4.

Table 2.4. Factors influencing iron absorption.

Enhancers	Potency
Heme*	+ + +
Ascorbic Acid	+ + +
Amino Acids (Cysteine)	+

Inhibitors	Potency
Tannins	+ + +
Ca/PO_4	+ +
Protein	+
Phytate	+
Fiber	+
Ferritin*	+

* intrinsic availability

Methods of determination of iron bioavailability from foods have been discussed (Hallberg, 1981). These methods include mainly the chemical balance technique, the introduction of radioisotopes, and the extrinsic tag method. In the latter method, when single foods were biosynthetically labeled with radioiron (intrinsic tracer) were carefully mixed with a trace amount of iron salt labeled with another radioiron isotope (extrinsic tracer), the absorption of the two tracers, from such doubly labeled foods, was almost identical (Cook et al., 1972). On the basis of recent studies of food iron absorption employing this latter technique, Monsen and coworkers proposed a model for the estimation of the percent absorption of dietary iron (Monsen et al., 1978). This model takes into account the total iron intake, iron stores and the quantities of meat, poultry and fish (MPF) and ascorbic acid in the diet. A simplified method for calculation of available iron has been developed and computerized (Monsen, 1980; Monsen and Balintfy, 1982).

Miller and coworkers have described an in vitro method for the estimation of iron availability that simulates human digestion and absorption of dietary iron from complex meals (Miller et al., 1981).

By using animal models, other methods have been developed to study dietary bioavailability of iron. These methods include the hemoglobin repletion assay in rats and the iron absorption test in rats. Total iron absorption can be

estimated from assessment of the gain in carcass iron or in hemoglobin iron (analysis of hemoglobin during the regeneration period). The ease of measuring blood hemoglobin relative to that of carcass iron makes the hemoglobin iron gain the response of choice for assessing iron bioavailability (Miller, 1982). Using this technique, Nelson and Patter found that protein-bound ferrous iron was as available biologically as the standard ferrous sulfate. The protein-bound iron is released upon digestion by acid and proteolytic enzymes (Nelson and Patter, 1980). These findings indicate that protein-bound iron should be readily freed for absorption within the gastrointestinal tract. Using an in vitro technique, Hurrell et al. (1989) showed that casein and whey protein reduced the iron dialyzable fraction from 3.32% (found with egg white) to 0.19-0.56% and 0.86-1.6% , respectively. The mean absorption values fell from 6.67 to 3.65% and from 2.53 to 0.98% respectively. The in vitro technique was considered a promising screening technique, while the AOAC method (using the hemoglobin repletion test) serves as the most reliable prediction of iron bioavailability in the human (Forbes et al., 1989). The effects of the major dietary constituents (proteins, carbohydrates and fat) on iron absorption have been studied by Monsen and Cook (1979). Dietary constituents were administered to human subjects as egg albumin, dextromaltose and corn oil, respectively, in a semisynthetic

meal, and radioiron absorption tests were performed in each subject (Monsen and Cook, 1979). They found that carbohydrates and fat had little influence, whereas the presence of protein in the diet had a significant inhibitory effect (40% decrease) on the iron absorption of non-heme iron.

There can be no question that cereal iron is poorly bioavailable because cereals contain substances that inhibit iron absorption, mainly phytates, polyphenols and fiber (Gillooly et al., 1984). Wheat bran binds iron at the pH range found in human gastric juice, depending on the time of contact and the concentration of iron: with a short time of exposure, more ferric iron (non-heme iron) is bound to the bran than ferrous iron, but at longer times, these forms are equally bound (Dintzis and Watson, 1984).

Removing the outer layers of sorghum, by pearling, reduced the polyphenol and phytate content by 96 and 92%, respectively. This treatment led to an increase in iron absorption from 0.017% to 0.035% (geometric mean). On the other hand, addition of sodium phytate to a highly iron-available broccoli meal reduced iron absorption in humans from 0.185% to 0.037% , and the addition of wheat bran decreased iron absorption from white flour from 0.116% to 0.043% (Gillooly et al., 1984). Using an in vitro model, Sandberg and coworkers found that degradation of inositol hexa and penta-phosphates significantly reduced the inhibiting effect on iron availability (Sandberg et al.,

1989). Less iron was bound to raw wheat bran (RWB), alfalfa meal (AM), and cellulose when the iron was added as iron ascorbate chelate than as ferric chloride. Raw wheat bran and alfalfa meal caused dissociation of the chelate and bound only iron dissociated from the chelate (Mackler and Herbert, 1985). Thus, processing may affect iron availability from cereals. Ascorbic acid and citric acid in juices enhance iron availability from breakfast cereals. This enhancement is more pronounced with citric acid than ascorbic acid (Carlson and Miller, 1983). Citrate, rather than ascorbate, appeared to be the major enhancer of iron diffusibility from many fruits and vegetables (Hazell and Johnson, 1987). Carlson and Miller (1983) found that addition of citric acid to a 25% US RDA iron-fortified wheat cereal resulted in a 1.5-fold increase in iron availability over ascorbic, malic or tartaric acid addition.

The effects of tannins on iron availability have been studied. At the levels of tannins (20-136 mg/100g) present in sorghum, they had minor effect on iron availability (Radhakrishnan and Sivaprasad 1980). However, Layrisse reported that the strong inhibitory effect of tea, coffee and legumes was attributed to the presence of polyphenol and tannins in these foods (Layrisse, 1985). Similar findings were presented by Hallberg who reported that tea consumption at breakfast reduces iron absorption (2-2 1/2 times lower than coffee) as compared with other drinks (Hallberg,

1981). Hercberg reported that one cup of tea taken with a meal may reduce iron absorption from 11% to 2.5%. Absorption of iron from ferric chloride can be reduced from 22% to 6% when the iron supplement is taken with tea. Furthermore, iron absorption from a western type breakfast can be reduced by 60% with tea consumption (Hercberg, 1988). Tea without tannins had no effect on iron absorption. The effect of tannins on iron absorption can be explained by the formation of insoluble precipitates.

While cereal products and tea limit the availability of iron, iron in meats appears to be highly available. In addition, ascorbic acid exhibits important interactions with iron. Of these, the best characterized is the enhancement of iron absorption (Pirzio-Biroli et al., 1958; Moore, 1955; Cook and Monsen, 1977). Ascorbic acid promotes iron absorption by maintaining iron in the reduced state (ferrous iron). In an in vitro system, Conrad and Schade (1968) demonstrated that the addition of ascorbic acid to iron at an acid pH led to a displacement of hydrogen iron from the ascorbate to form ascorbic acid-iron chelates of variable molecular size. These chelates kept iron in solution over a range in pH from 2-11. This reaction occurred only at an acid pH. The role of ascorbic acid in the postabsorptive metabolism of iron is not well defined, however, it has been suggested that ascorbic acid affects the interorgan and intracellular distribution of

iron. Lipschitz and coworkers found that ascorbic acid deprivation in guinea pig increased the total non-heme iron concentration in the spleen and reduced it in the liver, and that repletion restored the iron in the spleen to control levels, but had no significant effect on the hepatic iron (Lipschitz et al., 1971). They also suggested that ascorbic acid deficiency appeared to impair the release of iron from the cells of the reticulo-endothelial system while promoting its release from hepatic parenchymal cells. Others suggested that ascorbic acid inhibited lysosomal degradation of ferritin to hemosiderin (Bridges and Hoffman, 1986)

2.4 **EVALUATION OF IRON STATUS**

Laboratory assessment makes a valuable adjunct to the nutritional evaluation. For assessing iron status, biochemical investigation should be directed to determining the status of the main iron-containing compartments, possibly as a prelude to measuring the pathophysiology of iron loss and flow rates within the body.

Several indicators of iron status have been described (Hercberg and Galan, 1985). The indices discussed below include: hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte counts, red blood cell distri-

bution width, erythrocyte protoporphyrin, serum iron and total iron binding capacity, and serum ferritin.

2.4.1 Hemoglobin (Hb)

2.4.1.1 Background

Hemoglobin is the oxygen carrying protein in the blood that contains iron. The determination of blood hemoglobin is one of the most common clinical analyses in use today. Hemoglobin normally accounts for over 60% of the body's iron. About 35 mg of iron is incorporated daily into developing erythroblasts for this purpose. No assessment of iron status can be made without reference to the hemoglobin concentration. Erythropoiesis represents the main demand for plasma iron. Red cell output will normally be maintained until the supply of iron from recycled hemoglobin and from other iron stores is exhausted. A fall in hemoglobin concentration is thus a late stage in the development of iron deficiency and is a sign that storage iron is exhausted (Cavill et al., 1986). Thus, hemoglobin concentration may be the easiest and most effective way of gauging iron status in populations where blood loss and iron deficiency are a common problem. Some investigators regard an increase in hemoglobin concentration following a trial of iron supplementation as the best test for evaluating iron deficiency. Such a test is not feasible in a cross-sectional survey (Klassing and Pilch, 1985). When erythropoiesis is suppressed as a result of chronic dis-

ease, iron supply remains adequate but poorly utilized and, indeed, there will be a net transfer of iron from the red cells to the storage pool (Cavill et al., 1986).

2.4.1.2 Normal values

Low hemoglobin concentration is associated with more severe degree of iron deficiency (compared to low values of the other iron indices), but no cutoff value can delineate a clear separation between normal and iron deficient individuals because of the substantial overlap of normal and anemic values. The suggested normal values for hemoglobin determination are shown in Table 2.5 (Simko and Cowell, 1984). In adult males and females (> 15 years), the cut-off values for hemoglobin are > 120 g/L and > 110 g/L, respectively (International Nutritional Anemia Consultative Group [INACG], 1985).

Table 2.5. Hemoglobin values (g/dl).[1]

	Criteria of Status		
Age (Yrs)	Deficient	Marginal	Acceptable
6 - 23 months	< 9.0	9.0 - 9.9	10.0+
2 - 5	< 10.0	10.0 - 10.9	11.0+
6 - 12	< 10.0	10.0 - 11.4	11.5+
13 - 15 (M)	< 12.0	12.0 - 12.9	13.0+
13 - 15 (F)	< 10.0	10.0 - 11.4	11.5+
16+ (M)	< 12.0	12.0 - 13.9	14.0+
16+ (F)	< 10.0	10.0 - 11.9	12.0+
Pregnant			
trimester 2	< 9.5	9.5 - 10.9	11.0+
trimester 3	< 9.0	9.0 - 10.5	10.5+

[1]Taken from Simko and Cowell (1984).

2.4.1.3 Factors that can influence hemoglobin values

Interpretation of hemoglobin concentration is complicated by differences in hemoglobin distribution in blacks and whites. Kenny and coworkers reported that hemoglobin values are higher in white than in black adolescent females (Kenny et al., 1985). In addition, low hemoglobin concentration can also result from infection, inflammation, thalassemia, folate and vitamin B_{12} deficiency, hemoglobinopathies, pregnancy and other physiological states in which there is overhydration or acute plasma volume expansion (Makarem, 1974). Also, slight but nonsystematic hemoglobin differences between lean, normal and obese 1- to 17-year old subjects were reported (obese greater than non obese). Those differences tended to vary considerably with respect to age and sex (Scheer, 1982). Systematic differences in hemoglobin levels between obese and lean white participants in NHANES I were reported (Garn and Ryan, 1982). The use of hormonal contraceptives was not associated with any improvement in hemoglobin status. A long-term use of IUD didn't have a deleterious effect on hemoglobin status (Prema, 1979). Interpretation of hemoglobin values depends on altitude. For instance,anemic individuals at high altitude had adequate hemoglobin values relative to low altitude standards (Tufts et al., 1985). Thus, functional anemia is population specific and depends on the normal

hemoglobin distribution of the population and not a function of some absolute hemoglobin concentration.

2.4.2 Hematocrit (Hct)

2.4.2.1 Background

Hematocrit is the packed red cell volume; that is, the percentage of the total blood volume as red cell volume (Simko and Cowell 1984). Hct alone is not entirely conclusive in the detection of iron deficiency, although it is useful in the overall diagnosis (Sauberlich et al., 1980).

2.4.2.2 Hematocrit values

Hematocrit values for evaluation of iron status are presented in Table 2.6 (Simko and Cowell, 1984). Iron deficiency is considered in adult males (>15 years) with hematocrit values of <40% , and in adult females (>15 years) with hematocrit values of <36% (International Nutritional Anemia Consultative Group, 1985).

Table 2.6. Hematocrit values (%).[1]

	Criteria of Status		
Age (Yrs)	Deficient	Marginal	Acceptable
up to 2	< 28	28 - 30	31+
2 - 5	< 30	30 - 33	34+
6 - 12	< 30	30 - 35	36+
13 - 16 (M)	< 37	37 - 39	40+
13 - 16 (F)	< 31	31 - 35	36+
16+ (M)	< 37	37 - 43	44+
16+ (F)	< 31	31 - 37	38+
Pregnant			
Trimester 2	< 30	30 - 35	35+
Trimester 3	< 30	30 - 33	33+

[1]Taken from Simko and Cowell (1984).

2.4.2.3 Influencing factors

Hct can increase in hemoconcentration and decrease in excessive administration of fluids in conditions associated with hydremia. Hct also varies with altitude. Adult men and women presented higher values of Hct at high altitude (Sauberlich et al., 1980).

2.4.2.4 Sensitivity in detecting anemia

Graintcer and coworkers showed that Hct at presumed equivalent levels identify different prevalences of anemia in the same population (Graintcer et al., 1981). Hb and Hct screening tests are not comparable in detecting anemia in the same population. Hct remains a reliable and inexpensive screening test even though it is difficult to reproduce.

2.4.3 Hb and/or Hct-Dependent Parameters

In addition to Hb and Hct, measurement of the red cells (corpuscles) themselves, which may be characterized as to size and the amount of Hb in them, is also conducted. The three calculated values and their derivation are as follows (Simko and cowell, 1984).

2.4.3.1 Mean corpuscular volume (MCV)

$$MCV = \frac{\text{Hematocrit in ml/1000 ml}}{\text{Cells in millions/mm}^3}$$

In NHANES II, the MCV was calculated from the formula (Klaasing and Pilch, 1985):

$$\text{MCV (femtoliters)} = \frac{\text{Hct (\%)}}{\text{RBC per liter}} \times 100$$

A subnormal value for MCV is observed when iron deficiency becomes severe. A low MCV is a fairly specific indicator of iron deficiency once thalassemia traits and the effects of inflammation have been excluded (Klaasing & Pilch, 1985). The normal range is 80-90 cuμ or μm3 (Simko and Cowell, 1984).

2.4.3.2 Mean corpuscular Hb (MCH)

$$\text{MCH} = \frac{\text{1000 ml in g/1000 ml}}{\text{RBC in millions/mm}^3}$$

MCH as well as MCV are low in the microcytic anemia of iron deficiency. The normal range is 27-32pg (Thiele, 1980)

2.4.3.3 Mean corpuscular hemoglobin concentration (MCHC)

The MCHC is the concentration of Hb per unit volume of erythrocytes expressed as a percentage.

$$\text{MCHC} = \frac{\text{Hb in g/100 ml}}{\text{Hct in ml/100 ml}}$$

In Hb deficiency the MCHC is less than 32%. The normal range is 33% to 38% (Thiele, 1980; Simko and Cowell, 1984). In the literature, the value of MCHC associated with iron deficiency in adults is MCHC < 32% (Bindra and Gibson, 1986; Thiele, 1980). The calculated "normal" value of the MCHC from the hemoglobin and hematocrit normal values in adult men (INACG, 1985) is:

$$MCHC = \frac{13\ g/dl}{40\%} \times 100 = 32.5\%$$

This value agreed with the cutoff limit for MCHC of <32%.

2.4.4 Reticulocyte Counts

Reticulocytes are immature red blood cells that represent a stage of development between a normoblast and an adult cell. Assessing the reticulocyte count can indicate whether a period of rapid erythropoiesis is occurring and perhaps aid in the interpretation of iron status in children and adolescents. However, a single count has limited value unless it is markedly abnormal (Klaasing & Pilch, 1985).

2.4.5 Red Blood Cell Distribution Width (RDW)

Measurement of the RDW, usually reported by automated blood cell counters as the coefficient of variation of red cell size, is considered an indicator of very early iron deficiency (McClure et al., 1985). Elevated RDW values may be useful in distinguishing between thalassemia and iron deficiency as the cause of low MCV, however, its use is impractical particularly in field studies because it requires a sophisticated cell counter to perform this test (Klaasing and Pilch, 1985). The normal value is 13.2% ± 1.6%.

2.4.6 Erythrocyte Protoporphyrin (ER)

2.4.6.1 Background

Erythrocyte protoporphyrin is the immediate precursor of heme. EP levels become elevated in RBC when heme synthesis is disturbed, as in iron deficiency. Their levels (i.e., porphyrins) can also be elevated in other conditions, such as anemias, infection, protoporphyria, and lead poisoning. EP test for screening for lead poisoning has been established. It was found that an elevated EP level, by itself, represents inadequate iron supply for hematopoiesis and signals iron deficiency (Yip et al., 1983). The lack of iron in the developing red cell limits heme synthesis and results in the accumulation of protoporphyrin IX in the RBC. The blood levels of EP are physiologically stable and respond mainly to changes in iron supply (Klaasing and Pilch, 1985).

2.4.6.2 Uses and limitations

The measurement of EP levels as an indicator of iron deficiency has particular advantages in pediatric hematology and in large scale surveys where the small sample size and simplicity of the test are important. However, in the general clinical laboratory it provides less information about iron storage levels in anemic patients, and provides no help in the diagnosis of iron overload (Cavill et al., 1986). This is because the main form of iron storage is ferritin.

2.4.6.3 Normal values

Several values for normal levels of EP have been reported in the literature: The normal protoporphyrin content of RBC in children and adults is about 15-100 μg/100 ml RBC. Other values varying from about 46 to 155 μg/100 ml RBC have been reported (Henry et al., 1974). Iron status was considered to be abnormal at EP levels above 100 μg/100 ml packed cells (Cook et al., 1976) and values of EP above 70 μg/100 ml RBC were considered a criterion of iron deficiency (Cook et al., 1986). In 4,000 specimens assayed by Heller and coworkers, porphyrin concentrations ranged from < 40 to > 900 μg/100 ml of RBC, and the mean value was 86 μg/100 ml of RBC (Heller et al., 1971).

In children, 6 months to 12 years old, the optimal cutoff limit for the EP test appears to be 35 μg/dl of whole blood, when using a serum ferritin value of < 15 μg/l as the criterion of iron deficiency (Yip et al., 1983). The serum ferritin will be addressed later in this report. Based on the above information, the interpretation of EP values might be confusing, since there is no cutoff value for normal conditions.

2.4.7 Serum Iron/TIBC/Transferrin Saturation

2.4.7.1 Background

Iron is normally bound to transferrin at neutral pH and can be released by acidification. Free iron can be maintained in solution only by complexing with suitable

ligands or at acid pH. Transferrin iron represents only 0.1% of the total body iron. Rapid changes in the plasma iron levels can be brought about by minute differences in supply and demand (Cavill et al., 1986). A 5% change in iron balance will cause a change in serum iron concentration within one day. Transferrin saturation is used more frequently than serum iron concentration or TIBC as an indicator of iron status (Klaasing and Pilch, 1985). Transferrin saturation is calculated by the formula:

$$\text{Transferrin saturation (\%)} = \frac{\text{Serum } \mu\text{g/dl}}{\text{TIBC } \mu\text{g/dl}} \times 100\%$$

2.4.7.2 Uses and limitations

For a long period, serum iron concentration formed the basis of much clinical investigation of iron metabolism. It is now seen to be an entirely inadequate index of storage iron status. The main limitation in the diagnostic usefulness of the serum iron concentration is its instability (changes of more than 20% can be seen within 10 minutes). In addition, low serum iron concentration may result from a number of factors (colds, rheumatoid arthritis, malignant disease) and high serum iron levels may also result from certain disease states (red cell destruction) (Cavill et al., 1986). Ascorbic acid deficiency was reported to reduce both serum iron and ferritin concentration in man and animal (Worwood, 1986).

Transferrin saturation can be as variable as serum iron. Low levels of transferrin saturation are associated with iron deficiency but also with pregnancy and chronic disease. There seems little point in measuring TIBC to assess the level of iron stores (Cavill. 1986). Transferrin saturation of less than 16% in adults is widely regarded as indicative of iron deficient erythropoiesis (Klaasing & Pilch, 1985). The marrow receives a normal amount of iron even when the transferrin saturation falls below 16% . It was concluded that measurement of serum iron and TIBC in population survey may not be a reliable index of iron deficiency when multiple deficiencies are present (Shapcott et al., 1980). Iron deficiency can be detected by using serum iron and TIBC when it is already relatively advanced, i.e, when the iron stores of the body are already significantly depleted or even exhausted (Frank and Wang, 1981).

2.4.7.3 Normal values

The normal range for serum iron is 65-175 μg/100 ml (Henry et al., 1974). For TIBC the range is 250-410 μg Fe/100 ml. The determination of transferrin by immunologic techniques has given results of 220-372 mg transferrin/100 ml (equivalent to 265-465 μg Fe/100 ml) (Henry et al., 1974). In other reports, the normal range for plasma iron is 45-150 μg/dl and for transferrin saturation is 35 ± 15 percent (Simko & Cowell, 1984).

Zeman (1983) reported values for serum iron of 60-200 μg/dl and for iron binding capacity of 250-450μg/dl. The cut off values for serum iron and total iron binding capacity are < 60 μg/dl and > 400 μg/dl, respectively, for individuals > 15 years old. Transferrin saturation < 16% was considered abnormal for adults, 15-74 years old (Expert Sci. Working Group, 1985) or for adults, > 10 years old (INACG, 1985.)

2.4.8 Serum Ferritin

2.4.8.1 Background

Ferritin is a soluble protein but is degraded to an insoluble derivative, hemosiderin, which accumulates in lysosomes and is the "stainable iron" referred to by pathologists and hematologists. The major function of ferritin is clearly that of providing a store of iron which may be used for heme synthesis when required (Worwood, 1986). The only cause of a low serum ferritin concentration is a reduction in the level of iron stores; therefore, serum ferritin concentration can be used to assess whether or not the iron stores are exhausted (Cavill et al., 1986).

2.4.8.2 Uses and limitations

Serum ferritin estimation is thought to be the most useful index of iron status (Cavill & Jacobs, 1986). In normal adults, the concentration of serum ferritin parallels the total amount of storage iron in the tissues (Klaasing & Pilch, 1985). A significant correlation be-

tween serum ferritin and iron stores was reported by Fairbanks and Klee (1985). These authors reported a negative correlation with iron absorption, since the rate of iron absorption is inversely correlated with the size of iron stores; and a variable correlation (i.e. not consistent) with bone marrow hemosiderin. They also reported a correlation of ferritin with liver iron stores, no correlation with serum iron in normal adults, and a negative correlation with TIBC in patients with inflammatory disease (Fairbanks & Klee, 1985). A low plasma ferritin level has a high predictive value for the diagnosis of uncomplicated iron deficiency anemia. However, it is of less value in anemia associated with infection, chronic inflammatory disorders, liver disease and malignant hematologic diseases. Plasma ferritin measurement is also useful for the detection of iron overload (Valberg, 1980).

2.4.8.3 Normal values

The ferritin concentrations in normal subjects were reported to be between 15-300μg/l serum (Worwood, 1986). In other reports, serum ferritin values of less than 12μg/l serum were considered abnormal (Cook et al., 1986, Cook et al., 1976). The widely accepted normal range for serum ferritin is 12 to 300 ng/ml (Lipschitz et al., 1974, Cook et al., 1974). The cutoff point for serum ferritin in adults (>15 years) is < 12 μg/l (INACG, 1985).

2.4.9 Newer Methods in Iron Status Evaluation

New approaches to the assessment of iron nutriture involve the measurement of plasma transferrin receptors (Cook and Skikne, 1989; Huebers et al., 1989). Serum transferrin receptor is a reliable index of cellular iron need and may provide the most reliable estimate of mild deficits in functional iron. Elevated values are seen in patients with either increased erythropoiesis or iron deficiency (Cook and Skikne, 1989).

In addition, erythrocyte ferritin has been used as an indicator of iron status in newborns (Diallo et al 1989); and in infants and adults (Guillemin et al., 1989). A rapid and simple method for the determination of erythrocyte ferritin has been developed (Van Der Weyden et al., 1983). This assay utilizes a hepatic ferritin radioimmunoassay.

From the above biochemical indices, several indices were selected for the present study. The choice of indices depend on the feasibility of the test, their costs and on the quality of the tests in detecting iron deficiency. The iron indices representing the different stages in the development of iron deficiency anemia were proposed by the INACG (1985). Those indices include: serum ferritin, which identifies depletion of storage iron (early stage); serum-transferrin saturation, an index of reduced transport-iron supply (second stage), and hemoglobin/

hematocrit, values of which fall in iron deficiency anemia (final stage). These indices were grouped as a Tri-index model (Bindra and Gibson, 1986). This model includes the determination of serum ferritin, transferrin saturation and MCHC. MCHC measures the concentration of hemoglobin in an average red corpuscle. Low values of MCHC (<32.0%) are indicative of the final stage of iron deficiency, hypochromic, microcytic anemia. This test seems to be reasonable and suitable for the determination of iron status of small numbers of individuals.

2.5 **IRON DEFICIENCY**

Iron deficiency can be defined as a deficit in essential body iron (Beard et al., 1981). Iron deficiency is most likely to be caused by either blood loss or from inadequate iron absorption (Finch and Cook, 1984). Deleterious effects of iron deficiency were discussed extensively (Beard et al., 1981, Scrimshaw, 1983). Iron deficiency can result in clinical anemia which in turn affects work performance. In addition work performance is reduced in iron deficiency because of the associated decrease in the activity of iron-containing enzymes. Iron deficiency affects infant growth and development, cognitive performance and behavior, and reduces the resistance to infection. Iron deficiency can occur in all strata of society, it is a result of postnatal feeding practices rather than congenital

deficiency of iron, and it can be prevented by appropriate diets (Oski, 1985). In the early stage, iron deficiency there may be a decrease in intellectual performance and in the ability to perform physical activity. There may also be alterations of temperature regulation (i.e. inability to maintain body temperature) (Galan et al., 1984).

2.6 **CONTROL OF ANEMIA IN DEVELOPING COUNTRIES**

Iron deficiency anemia is considered a major public health problem in many developing countries. Industrialized countries are not exempt from this problem, but in the latter case this problem is relatively minor compared to the situation in developing countries. Young children and pregnant women are especially vulnerable because of their higher iron requirements (Demaeyer, 1981). The prevalence of iron deficiency in different countries has been reported. Dhur and Hercberg (1989) reported that the prevalence of iron deficiency in France, Spain and Italy for the main groups of individuals at risk (i.e. women of childbearing age, pregnant women and growing children) is high. For example, in 476 menstruating, young French women (17-42 years old), the anemia was present in 1.3% of the population (hemoglobin level below 12 g/dl); but 16% had exhausted iron stores (serum ferritin below 12 μg/l). In a second group of 203 menstruating women (16-53 years old), 2.9% were anemic and 21% had depleted iron stores.

In South Benin, iron status were assessed in a representative sample of 2,968 subjects. Iron deficiency, defined by two or more abnormal values of four indicators of iron status (transferrin saturation, erythrocyte protoporphyrin, serum ferritin and MCV) was present in 31% of subjects, with higher prevalence of iron deficiency anemia in children and menstruating women (Hercberg et al., 1988). Available information on the prevalence of iron deficiency in Africa was reported by Hercberg and coworkers (1987). These authors reported that the estimated prevalence of anemia by age and sex categories for Africa in 1980 is as follows: children, 0-4 years (56%); children, 5-12 years (49%); adult men, 15-59 years (20%); adult women, 15-49 years (44%); and pregnant women (63%). In Algeria, Assami and coworkers (1987) evaluated the nutritional status of 302 menstruating women. Iron deficiency (serum ferritin $\leq 12 \mu g/l$ and transferrin saturation <15%) was observed in 29, 27 and 38% of the subjects living in urban (102 women), suburban (100 women) and rural (100 women) areas, respectively. In a sample of 222 pregnant Algerian women, iron deficiency defined by the presence of abnormalities of two or more indicators of iron status (transferrin saturation <16% , MCV < 80 fl, serum ferritin < 12 $\mu g/l$) was present in 33.8% of the women (Assami et al., 1988). In Morocco, anemia (hemoglobin < 110 g/l) was present in 20% of a total

of 737 pregnant women (Ministère de la Santé publique, 1985).

Iron deficiency can be alleviated in two ways: Supplementation with iron as in pregnancy and fortification of the diet with small quantities of iron, aimed at improving iron nutrition on a long term basis (Bothwell, 1985). Guidelines have been prepared for the proper conduct of supplementation and fortification trials (INACG, 1977). A number of iron fortification programs in developing countries have been reviewed (Viteri et al., 1981). In Chile, high-fat (26%) powdered milk was fortified with 15 mg/dl iron as ferrous sulfate and 100 mg of ascorbic acid per 100 g of powder and destined for infants (3 months old) (INACG, 1986). At nine and 15 months of age, there was a highly significant difference ($p<0.001$) in all laboratory parameters indicative of iron nutrition (Hb, Hct, serum iron, total iron binding capacity (TIBC), free erythrocyte protoporphyrin and serum ferritin). At 9 months, anemia determined by Hb<110g/dl was present in 7.5% of the infants in the treatment group versus 34.7% in the control group, receiving unfortified milk. At 15 months, the percentages were 1.6% and 27.8% in the two groups, respectively.

Also, hemoglobin-enriched cookies and cereal products for children and infants are being studied (INACG, 1986; Calvo et al., 1981). In a pilot field study, one group of children received three 10-g fortified cookies (containing

1.8 g bovine hemoglobin concentrate) and another group received the unfortified cookies for about 180 days per year. At the end of the trial, there was a small, but statistically significant increase in hemoglobin concentration in the group consuming fortified cookies. Anemia (hemoglobin < 120 g/dl) existed in 3.1% of the control group and 0.6% in the fortified group. In the subgroup of females over 10 years of age, at 18 months, ferritin was higher in the fortified group (27.22 μg/l vs 17.28 μg/l). When the cutoff point for ferritin is set at < 10 μg/l, 6% of girls in the fortified group were iron deficient, compared to 11% in the control group. In another field study, a new infant weaning cereal (hemoglobin-rice cereal) was designed to be introduced at four months of age. The geometric mean absorption of iron from this product was 9%, and 36% for the standard dose (Calvo et al., 1981). A total of 198 infants, 94 in the fortified group and 104 in the control group, were followed to 12 months of age. The fortified group received 1.5 kg of the fortified cereal per month, providing 14 mg of iron/100 g of cereal. The mean value for Hb in the fortified group was 123 g/l, and 118 g/l in the control group. Significant differences were also observed in mean values for MCV, TIBC, transferrin saturation, serum ferritin, and erythrocyte protopophyrin (INACG, 1986).

It was reported that in Jamaica, anemia is a serious public health problem for pregnant women (61.6% had Hb < 110 g/l), lactating woman (58.7% had Hb < 120 g/l), and preschool aged children (69.1% had Hb < 110 g/l). The Jamaican government has implemented programs for the control of anemia (Simmons and Gurney, 1982). Those programs included: fortification of foodstuffs with iron, distribution of iron and iron/folate; and sanitation/education programs. The outcome of these programs was not presented. The most common cause of nutritional anemia in those populations was iron deficiency. This is probably due to an inadequate iron intake with a low absorption.

The fortification of refined sugar was suggested in areas where consumption is high as in North African countries including Morocco (Hercberg et al., 1987). A number of iron supplementation trials in developing countries were reviewed (Hercberg et al., 1987). In those programs, elemental iron (from 20 to 240 mg/day) was given to women, resulting in general in a beneficial effect (i.e., prevention of iron deficiency by increasing Hb levels).

2.7 **PROCEDURES FOR EVALUATION OF DIETARY INTAKE**

One of the purposes of dietary surveys is to obtain data that can be useful in planning and monitoring therapeutic and educational programs. A number of methods for collection of dietary data have been described.

2.7.1 Twenty-Four Hour Recall

This method can be done either by interview or by a self-administered questionnaire. In this case, food models, measuring cups and other devices are helpful. This approach has the advantage of requiring only a limited and short period of time for the investigation; direct observation and evaluation of intake is possible and practical. The likelihood that the subjects will modify their food habits is minimized. However, the limitations of this method include the fact that the recall is not necessarily representative of the usual intake in terms of quality and quantity. In addition, the days being recalled may differ greatly within a season and from one season to another. It was noted that a single 24-hour recall is not an appropriate tool for assessing the usual diet of an individual (Block, 1982). The 24-hour recall can be used, mainly to provide descriptive information on dietary patterns for group comparisons (Christakis, 1972).

2.7.2 Food Frequencies

In this technique, clients are asked about their usual intake of forty to eighty of the most frequently used types of food per day, week or month. The value of the information obtained is limited because of its ambiguous nature (Simko et al., 1984). Questionnaires which ask only the frequency with which specified foods were eaten in a given

period of time, lend themselves to large scale epidemiologic research (Block, 1982).

2.7.3 Dietary History

Dietary history includes a cross-check in the form of a detailed list of foods with questions about their use, likes and dislikes. This method has insufficient quantitative value (Simko et al., 1984). It was concluded that the history method is reflecting some reasonably stable marker which is similarly revealed by different methods and on different occasions, and which bears some relationship to clinical criteria (Block, 1982).

2.7.4 Food Records

This method is intended to describe an individual's current intake. Weighing methods are necessary for some studies. A period of seven days is considered optimal. This method is recommended, especially if the data are to be compared with biochemical assessment. It was found that it is important to include weekend days in a dietary survey period, and that assessment of the significance of correlations between dietary trace element intakes and biochemical parameters should take into account the intra-individual variation associated with both variables (Gibson et al., 1985). Sources of error and variability in dietary assessment methods have been reviewed (Gibson, 1987). Inter and intra-subject variations were discussed as well. Measure-

ment errors can occur during any stage in the process of measuring food or nutrient intakes. The sources of measurement error in dietary survey methods include: Respondent biases, interview biases, respondent memory lapses, incorrect estimations of portion size, flat slope syndrome (tendency to overestimate low intakes and underestimate high intakes), coding and computation errors (when converting a food portion to grams for example), errors in the compilation of nutrient composition data, and errors during the nutrient analysis of food items. When comparing protein and energy intakes recorded by weighing to those recorded in household measures, there was less variability in the records obtained by weighing intake, even though there was no difference in the mean protein and energy intake in both approaches. Furthermore, regardless of which method was used, a one-day record gave a meaningless estimate of an individual's usual diet (Todd et al., 1983). Elsewhere it was noted that individual dietary recall data were of limited value in predicting laboratory indices (Kerr et al., 1982).

The selection of an appropriate method depends on the objectives of the study, as well as the economic situation, since some methods are more costly and/or time consuming than others. For small surveys and individual cases, a seven-day intake information, covering the period before physical and biochemical examination is recommended. This

approach is the one that appears to rest on firmer ground; however, the chief objection is that it is impractical for clinical or epidemiologic studies since it demands a high degree of cooperation on the part of the subjects (Block, 1982). The analysis of the data collected should be used with reference to appropriate food composition tables and the interpretation of the data by reference to standards.

2.8 **INTAKES OF Fe, FIBER AND PHYTATES IN MOROCCO**

Cereals constitute the staple food in Morocco and occupy an important place in Moroccan agriculture. Cereals are consumed as bread and semolina, used to make soup, couscous, pasta, or vermicelli. The average consumption level of cereals is 241.89 kg/person/year in the rural areas, 169.14 kg/person/year in the urban area, and 210.44 kg/person/year at the national level. These numbers were obtained from a national survey in 1984-1985 (Direction de la Statistique, 1988). Cereals represent a good source of dietary fiber. In addition they contain high levels of phytic acid. Fiber and phytic acid content of Moroccan foods, including cereals, have been determined (Alaoui & Essatara, 1985). The mean dietary fiber contents of whole wheat flour, soft wheat flour and barley flour were 16.2, 11.8 and 21.6 g/100 g dry matter (DM) respectively. Their phytic acid contents were 700.0, 530.0 and 753.5 mg/100 g DM respectively. Intakes of crude fiber and phytic acid

per person per day have been calculated at the national, urban and rural levels, as well as in different areas in Morocco (unpublished data). It was found that Moroccan diets are characterized by high levels of fiber and phytates, mainly in rural areas. The total supply of crude fiber in grams/person/day was 20.1, 25.6 and 12.5 at the national, the rural and the urban milieus respectively. The phytic acid intake was 2867, 3495 and 2153 mg/person/day in the 3 cases, respectively. Mineral deficiencies in some countries were attributed partially to the high consumption of breads containing fiber and phytates (Prasad et al., 1963). On the other hand, it was previously found that in four rural areas of Morocco, the total dietary iron supply ranges from 14.5 to 22.5 mg/person/day, and that more than 80% of this supply was non-heme iron from cereals and vegetables. Cereals contributed greater than 68% of the total iron supply. From this one can conclude that the availability of iron from such diets would be low and might affect iron status (Alaoui and Leklem, 1987). However, there are no biochemical data to support such a hypothesis. A study that relates the composition of the diets traditionally consumed to the iron status of a rural population in Morocco, is needed in order to determine the iron status, and to better understand the influence of the diet on the iron status.

CHAPTER 3

HYPOTHESIS

My hypothesis is that the consumption of a vegetable-based diet in a rural milieu influences iron status and results in iron deficiency.

CHAPTER 4

MATERIALS AND METHODS

4.1 **LOCATION: GEOGRAPHICAL SETTING**

The area of study is located in the Province of Chefchaouen (250 km, NE from Rabat). The area was chosen based on several criteria, including: accessibility, acceptance of the study by local authorities and health professionals, and willingness of participating families or individuals to contribute to the study. This region was also chosen because it is isolated from the commercial routes, and therefore the consumption of locally produced foods is more likely to be high. Also within a season the variations in food consumption are minor. Thus there is a monotonous type of diet. This area is located in the occidental "Rif"; therefore, there are mountains and hills which has made the introduction of mechanization into the agriculture system very difficult. The region is characterized by an abundance of small farms. More than ninety percent of the population is rural. The main foods grown annually for human consumption are cereals (hard wheat, soft wheat, barley and corn); and dry legumes (fava, chick-peas, and lentils). Cereal plantings occupy more than 46 percent of the area suitable for agriculture production, and dry legume

plantings occupy more than 15% (Direction Provinciale de l'Agriculture de Chefchaouen, 1989). Livestock in this region is mainly goats destined for milk and meat production.

4.2 **POPULATION AND SUBJECTS**

Necessary approvals to conduct the study were obtained from the Ministry of Interior and the Ministry of Health in Rabat. Following the approval of the Ministry of Health, the objectives of the study were fully explained to the medical professionals in Rabat and in Chefchaouen. A commission was formed in Chefchaouen and was charged with contacting the villages and explaining the objectives and the interest of the study at the family level. Four villages were selected in the Circle of Bab Taza.

The villages were Gharouzim, Dar Akouba, Lachaïch and Akarat. There were twenty eight families with a total of 236 family members. The number of families was chosen based on available funds, time to do the research, and feasibility of the various tests, as well as the available technical personnel. these families agreed to participate in the dietary survey.

From this population, 47 adult men and 50 adult women, excluding pregnant or lactating females, were registered and agreed to participate in the study. Socio-economic status of each subject was obtained at the time the blood

sample was drawn. A questionnaire was prepared and used for this purpose. Ten subjects refused to have blood drawn, and were replaced by ten others; however, we were not able to obtain dietary information from these latter subjects.

4.3 **FOOD SURVEY AND DIETARY ASSESSMENT**

The dietary survey took place from May 13 to 19, 1989. The survey was carried out using a seven-day weighed-intake method. The seven-day intake information was obtained just prior to the physical and biochemical examination. For each family, daily food consumption was recorded for seven days by trained personnel. The families were asked to maintain their usual food habits in terms of food purchased, quality and quantity and their manner of cooking and serving foods within the family. The dietary survey was carried out by females, in order to facilitate the contacts with the individuals in the households who prepared the meals, and to have access to the kitchens. One or two of these personnel lived in the respective village for seven days and visited each family for each meal. At this time, all foods and ingredients were weighed by this person. Diet scales and diet record books were used. Tea leaves and coffee grounds were weighed before preparation. The caloric, protein, and iron intakes were determined by using a national food composition table (Table de composi-

tion des aliments à l'usage du Maroc, M.A.R.A, DIV.A.E, 1977) and when necessary a Food and Agriculture Organisation (FAO) food composition table (Table de composition des aliments a l'usage de l'Afrique, FAO, 1968).

The contribution of different foods to the total daily iron intake was determined. For each family, we compared the total daily iron intake to the daily iron recommendations, and the results were expressed as a percent of this recommendation. The total iron, ascorbic acid and the total weight of meat, poultry, and fish were determined per meal or snack for each day on the seven days and for each family. The results were expressed (as mean ± SD) in mg/person/day. This information was used to calculate available iron using the monsen model (Monsen, 1980) (Table 4.1). Heme/non-heme iron was calculated knowing that heme iron represents 40% of the total iron from meat, poultry and fish (Monsen, 1980). The meals were served in one or two plates depending on the size of the family; therefore, it was not possible to determine individual food consumption. The individual food consumption was estimated using the Unit of Consumption (UC) formula as follows (IAVH II, 1979):

- For each male $\geq$ 14 years, the UC = 1.0,
- For each female $\geq$ 10 years, the UC = 0.8, and
- For each male $<$ 14 years and each female $<$ 10 years, UC = 0.3 + 0.05 $\times$ age (years).

Table 4.1. Estimation of available dietary iron, assuming that the level of iron stores for adults is about 500 mg (Monsen, 1980).

Meal	% availability		
	Non-Heme Fe	Heme Fe	Combined factor for MPF*
Low availability < 30g MPF < 25mg ascorbic acid	3	23	11
Medium availability 30-90g MPF or 25-75mg ascorbic acid	5	23	12
High availability > 90g MPF or > 75 mg ascorbic acid or 30-90g MPF 25-75mg ascorbic acid	8	23	14

* Assumptions: MPF; meat, poultry, and fish: 40% of the Fe from MPF is heme Fe; 60% of the Fe from MPF is non-heme Fe.

4.4 BLOOD COLLECTION

For the blood collection and health evaluation, all the necessary equipment and supplies were taken to the hospital in the Province of Chefchaouen (250km from Rabat). The blood sampling process took place for eight days over a two-week period of time. Ten to fourteen persons were sampled each day. On arrival at the hospital, each individual adult subject responded to a questionnaire. One person conducted the interviews. Weights and heights were determined using a clinical scale. Body temperature was determined by placing a thermometer under the subject's arm.

Blood pressure was determined by using a sphyngomanometer. Peripheral venous blood samples (20ml) were drawn from each subject in the morning between 7 and 10 a.m. after an overnight fast,by using non-heparinized and heparinized Vacutainer tubes and Vacutainer-brand blood collection needles (Becton Dickinson, Rutherford, New Jersey, U.S.A). Immediately following blood collection, whole anticoagulated blood was used to determine hemoglobin and hematocrit.

Serum was obtained by centrifugation for 15 min at 1200 × g (3000 rpm) and separated using polyethylene transfer pipets (VWR Company, San Francisco, U.S.A). The serum obtained was divided in three parts as described in Fig. 4.1 and frozen in either glass vials at -20°C for subsequent analysis of serum iron and total iron-binding capacity (TIBC), or in Nalgene Cryovials (NALGENE, Rochester N.Y., U.S.A) and immediately frozen in a liquid nitrogen container to facilitate transportation to the laboratory in Rabat for subsequent analysis of serum ferritin. Plasma samples were also obtained to determine vitamin B_6 status (not as a part of this thesis).

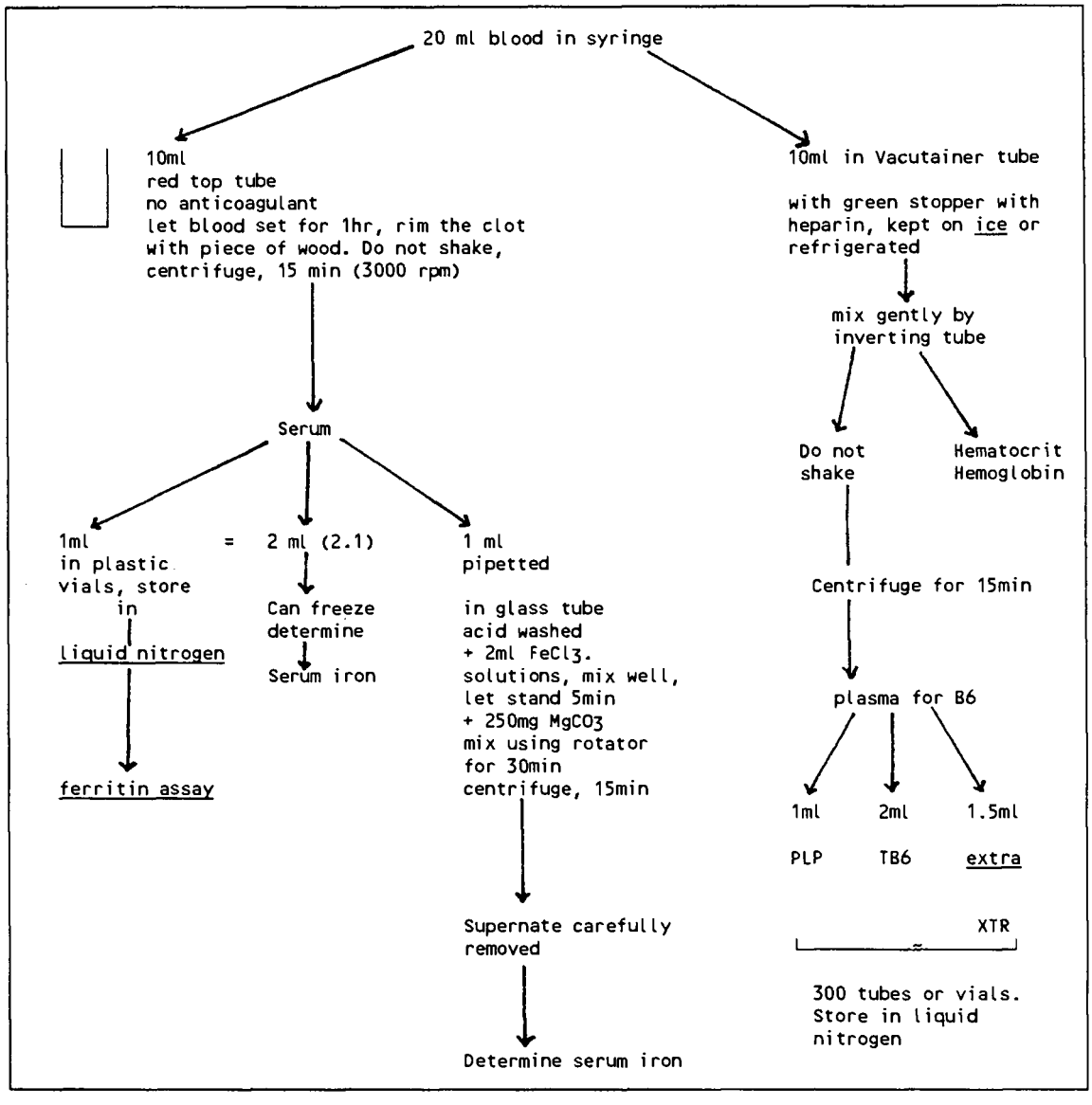

Figure 4.1 Blood drawing and uses.

4.5 **BIOCHEMICAL ANALYSIS: IRON INDICES**

The biochemical-iron indices used to determine iron status of the subjects were based on a Tri-index model (Bindra and Gibson, 1986). This model was based on the three independent tests of iron status, representing the three different stages in the development of iron deficiency anemia. Tests used were serum ferritin, which identifies depletion of iron stores; serum transferrin saturation, a derived index which indicates reduction in iron transport, and mean corpuscular hemoglobin concentration (MCHC), which indicates the final stage of iron deficiency. Hemoglobin, hematocrit, serum iron, total iron binding capacity and serum ferritin were determined as described below:

a) Hemoglobin (Hb):

Hemoglobin levels were determined by the cyanomethemoglobin method (Makarem, 1974). Total Hb of blood is converted to cyanomethemoglobin and measured spectrophotometrically at 540 nm. A combination of potassium ferricyanide (converts iron in the hemoglobin from the ferrous to the ferric state to form methemoglobin) and potassium cyanide (converts methemoglobin to stable cyanomethemoglobin) were used. The Fe (+2) of heme in hemoglobin, oxyhemoglobin and carboxyhemoglobin are oxidized to the ferric state by ferricyanide to form methemoglobin.

b) Hematocrit (Hct):

Hematocrit levels were determined as follows (Richterich, 1968): A capillary tube was filled with anti-coagulated blood, sealed at one end and centrifuged (3000 rpm) for 5 min. Percent red cell volume was calculated with a calibrated reader (DAMON/IEC Division, MASS).

The mean corpuscular hemoglobin concentration (MCHC) was calculated by the following formula:

MCHC (%) = (hemoglobin (g/100 ml)/Hematocrit) × 100%

c) Serum iron/Total Iron Binding Capacity (TIBC)/Transferrin saturation:

Serum iron and TIBC were determined by a spectrophotometric procedure, using bathophenanthroline (Henry et al., 1974). Proteins were precipitated with hot 20% trichloroacetic acid (TCA) and the iron was freed simultaneously. The iron in the supernate was reduced by hydrazine. The Fe^{2+} was quantitated photometrically at 535 nm after reaction with sulfonated bathophenanthroline (0.05%). Correction for background absorption was made by reading the absorbance prior to color development. This method has been tested in the laboratory and is useful when the automated Technicon autoanalyzer is not available. All chemicals were purchased from SIGMA-Chimie in France.

The TIBC was measured after saturating the serum transferrin with iron and removing the excess iron. Excess iron (2 ml $FeCl_3$) was added to saturate the transferrin. Unbound iron was then removed by using $MgCO_3$ (250 mg). The

bound iron remaining in the supernate is the Fe binding capacity and it is determined by the same technique used for serum iron. There were four controls, and their serum iron levels were determined three different times. This gave a coefficient of variation from 0.6 to 8.2% . Transferrin saturation (TS) was calculated from the formula:

$$\text{Transferrin saturation (\%)} = \frac{\text{Serum iron } (\mu g/dl)}{\text{TIBC } (\mu g/dl)} \times 100$$

d) Serum ferritin:

Serum ferritin was assayed using a radioimmunoassay (RIA) procedure based on the procedure described by Addison et al. (1972) (ferritin RIA kit IM.1051, Amersham, 1986). This method depends upon the competition for the binding sites of a specific antibody between ferritin I^{125} and ferritin in serum or plasma. The proportion of ferritin I^{125} bound to the antibody is inversely related to the concentration of serum or plasma ferritin. The antibody-bound ferritin is then reacted with a second antibody reagent to precipitate the double antibody complex. The precipitate is settled by centrifugation (>1500 × g) and after discarding the supernatant containing free ferritin, the proportion of ferritin I^{125} bound to the first antibody is measured by assaying the I^{125} in the precipitate. Thus, the concentration of ferritin in unknown samples can be interpolated from a standard curve derived by plotting the proportion of ferritin I^{125} bound in the presence of a ref-

erence standard solution containing varying concentrations of ferritin. Polystyrene tubes were used (VWR Scientific Inc). RIA kits were obtained from Amersham France, les Ulis Cedex (France). All samples were run in duplicate. A gamma scintillation counter (model Packard 35412, Multidetector RIA system) was used. There were four controls with mean values of 10, 10, 34 and 120 μg/l for two assays, with between-assay variation of <1μg/l for each value.

Iron status was also determined in four adult males and six adult females living in Rabat, using the iron indices previously described.

4.6 **DIAGNOSIS OF IRON DEFICIENCY ANEMIA**

Anemia is usually considered to be present when the hemoglobin level has decreased below a specific cut-off point defined according to sex, age and other physiological states. The normal levels for hemoglobin most frequently used are those given by the World Health Organization (WHO, 1968). The cutoff points used in the present study were: for adult males: 130 g/l; for adult females: 120 g/l. The normal values for hematocrit were: 40% for males and 36% for females (INACG, 1985). The cutoff values for the Tri-index model indices were selected from the literature and were: serum ferritin < 12 μg/l (Cook et al., 1986; INACG, 1985); transferrin saturation (TS) < 16% (Expert Scientific

Working Group, 1985; INACG, 1985); MCHC < 32% (Thiele, 1980; Bindra and Gibson, 1986; INACG, 1985).

Relative prevalence of iron deficiency anemia was determined either by using abnormal values for the Tri-index model indices (Bindra and Gibson, 1986) or by using mixed distribution analysis of hemoglobin (Hercberg et al., 1988). In the latter model, the frequency distribution for hemoglobin concentration shifted towards higher values after excluding subjects who have serum ferritin values < 12 µg/l and transferrin saturation values < 16%.

4.7 **RELATIONSHIPS BETWEEN DIETARY IRON AND IRON INDICES**

For different age and sex groups, Person's correlation coefficients were determined between each of the dietary variables of total iron intake, heme iron, non-heme iron and available iron (mg/person/day) and each of the blood iron indices (hemoglobin, hematocrit, serum iron, TIBC, TS and serum ferritin).

4.8 **EFFECT OF TEA CONSUMPTION AND SMOKING ON IRON STATUS**

The effect of tea consumption was studied in different age and sex groups. In fact we noted high tea consumption in the region as in most rural areas of Morocco. Cigarette smoking by the adult men was noted. We evaluated the correlation between amount of tea consumed or the number of cigarettes smoked per day and the iron indices.

4.9 **STATISTICAL ANALYSIS**

Descriptive statistics were applied. For each iron index the mean ± standard deviation was calculated for different age and sex groups. Data were compared to values selected from the literature. The comparison of the means of the groups was carried out using analysis of variance. Correlation coefficients between dietary iron and iron indices were determined. Also correlations were determined between iron indices. Correlation coefficients of Pearson were used (Muller et al., 1977; Snedecor, 1967).

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 GENERAL INFORMATION ON THE SUBJECTS: ANTHROPOMETRIC VALUES, BLOOD PRESSURE AND BODY TEMPERATURE

The subjects were 47 adult males aged 17-65 years and 50 adult females aged 16-55 years from four villages (Dar Akouba, Lachaïch, Akarat, and Gharouzim). The respective male and female subjects were divided in two different age groups (Table 5.1). This is because of the possible variations of the studied parameters from one age group to the other. Results are expressed as mean ± standard deviation of the mean.

Table 5.1. Anthropometric measurements, blood pressure, and body temperature of the different groups.

Groups	Young males (17-30 yrs)	Older males (31-64 yrs)	Young females (16-30 yrs)	Older females (31-55 yrs)
n	21	26	30	20
Ages (years)	24 ± 4*	43 ± 8	22 ± 4	43 ± 6
Weight (Kg; W)	63.2 ± 7.20[a]	60.9 ± 7.00[a]	54.6 ± 5.60[b]	57.3 ± 7.50[b]
Height (M; Ht)	1.70 ± 0.07[c]	1.66 ± 0.06[c]	1.59 ± 0.06[d]	1.58 ± 0.04[d]
Body mass index (W / Ht^2)	22.1 ± 2.3[e]	22.1 ± 2.6[e]	21.4 ± 2.0[e]	22.9 ± 2.7[e]
Blood pressure (mm Hg)				
Diastolic	62 ± 8[f]	66 ± 10[f]	62 ± 4[f]	62 ± 10[f]
Systolic	117 ± 7[g]	120 ± 10[g]	116 ± 12[g]	113 ± 13[g]
Body temperature (°C)	37.0 ± 0.3	37.0 ± 0.4	37.6 ± 2.0	37.2 ± 0.2

* Mean ± SD

a, b,...,g: For the same measurement, different subscripts revealed highly significant differences(at 0.001 significance level). The males are heavier and taller than the females.

The results in Table 5.1 reflect that the population was healthy, based on the finding that the blood pressure and the temperature were in the normal range; none were being treated for any medical condition. In the present study, body mass index (BMI) values were similar to those reported for premenopausal women consuming self-selected diets (Worthington-Roberts et al., 1988). Lower values of BMI (<18 kg/m^2) indicate that the subjects are very thin, values between 18 and 20 kg/m^2 indicate that the subjects are thin, and values of BMI > 25 kg/m^2 are indicative of overweight (Assami et al., 1987). The values found in the present study (Table 5.1) indicate that the subjects had normal weight for height. Pregnant or lactating females and individuals known to have an infection were excluded from the study. We did not do blood smears or collect stools to identify parasites or the presence of infection. This constitutes a limitation of this study.

5.2 **SOCIOECONOMIC STATUS**

The average size of the 28 families studied was 9 people. The occupation of the heads of the families, their education level and their estimated monthly income are presented in Table 5.2. The average income corresponds to that of the labor class in Morocco. Most of the men were small farmers and all of the females were housewives and not employed outside the home.

Table 5.2. Socioeconomic status of the families.

Village	Family code	Family size	number of UC	number of participants in Fe status assessment		Head of the family		
						Occupation	Education*	Income
				M	F			(DH/mo)**
Gharouzim	1	7	3.95	1	2	Worker		300
	2	6	2.85	1	1	Worker		850
	3	5	3.65		1	Soldier (retired)		800
	4	9	4.60	2	1	Farmer		300
	5	8	5.85			Soldier		1000
	6	9	4.2		2	Farmer		300
	7	9	7.15	1		Worker (France)		3000
	8	7	5.90	1	1	Farmer		850
	mean	8	4.77					937
Dar Akouba	1	8	5.45	1		Farmer		300
	2	16	10.45	2	2	Farmer	primary	500
	3	5	3.44	1	1	Farmer		300
	4	4	2.25	2	1	Truck-driver		1000
	5	5	2.95	1	1	Farmer		300
	6	8	5.61	1	1	Farmer	primary	500
	7	7	5.80	1	2	Farmer		200
	8	8	5.20	1	2	Farmer		300
	9	9	5.75	1	2	Farmer		300
	mean	8	5.21					411
Lachaich	1	7	6.20		3	Farmer		400
	2	7	6.05	2	3	Farmer		400
	3	10	8.30	1	5	Farmer		400
	4	17	11.62	2	3	Farmer		400
	5	12	9.55	3	3	Farmer		400
	mean	11	8.34					400
Akarat	1	11	8.25	2	2	Employee		400
	2	12	8.06	2	1	Employee		300
	3	13	9.60	2	1	Farmer	primary	700
	4	6	5.05	2	2	Farmer		400
	5	8	6.75	2	2	Farmer		700
	6	12	11.25	2	2	Farmer	primary	300
	mean	10	8.16					466

* Level of education; a blank indicates no formal education.

** DH = Moroccan currency, 100 dirhams ≈ $12.

5.3 **DIETARY ASSESSMENT**

5.3.1 Structure of the Diets and Food Habits

In the region studied three main meals are served daily; breakfast, lunch and dinner. Lunch is qualitatively and quantitatively the most important meal of the day. The composition of the meals was very similar in all the families. Breakfast is usually composed of bread, tea or coffee, milk butter, olives and rarely eggs. Lunch is com-

posed mainly of bread and a typical Moroccan dish named "Tagin" composed of vegetables with or without meat. Often "Couscous" is served at lunch with a mixture of vegetables, legumes and meat. The main vegetables and legumes consumed are: tomatoes, onions, turnips, potatoes, peas, beans and lentils. Sardines are often consumed mainly on the day of "Souk". The Souk occurs on two different days each week. On the day of the Souk, people buy and sell goods. Mint tea is usually served with each meal. The composition of dinner is similar qualitatively to that of lunch with the absence of meat at dinner. The food making up a meal is served as one or two dishes, depending on the family size. This practice therefore influenced the process used to determine food intake.

5.3.2 Food Intake

The calculated mean daily intakes of each of the different food groups for each family in the four villages are shown in Table 5.3. This table illustrates the pattern of daily food consumption, assessed for seven days. The results are expressed in grams/person/day (g/p/d). Eight food groups were identified. When the intakes of foods are considered, it is clear that as in most African countries, cereals constitute the staple food for all families. This typical diet based on cereals with small quantities of meat, or fish often contains inhibitors of iron absorption.

Table 5.3. Mean daily intakes of foods for the different families in the four villages (g/p/d).

Village	Cereals	Dry legumes	Vegetables	Fruits	Meat, poultry, fish & eggs	Milk, & milk products	Fat and oil	Sugar
Gharouzim	432 ± 82.7*	64.5 ± 48.4	137 ± 72.4	36.0 ± 56.9	100 ± 68.3	120 ± 52.0	50.8 ± 13.2	36.3 ± 9.2
Darakouba	366 ± 60.1	61.1 ± 38.1	189 ± 54.8	15.9 ± 14.2	58.7 ± 21.1	116 ± 68.1	40.1 ± 9.7	39.7 ± 13.7
Lachaich	421 ± 87.6	56.7 ± 22.8	214 ± 58.3	18.4 ± 18.2	102 ± 27.6	143 ± 36.6	65.52 ± 20.0	38.9 ± 6.0
Akarat	427 ± 154	123 ± 76.9	181 ± 61.4	34.7 ± 30.6	48.3 ± 23.2	105 ± 48.2	67.0 ± 22.0	45.9 ± 15.2

* Mean ± SD

Cereals are consumed as bread, couscous or fancy pastas such as spaghetti and noodles. Bread is still the dominating form of cereal consumption and is consumed at all the meals. In all the families studied, cereal consumption averaged about 400 g/p/d. Expressed as grains, this value is close to that reported at the national level in rural areas (241.89 kg/person/year; Direction de la Statistique, 1988). The dry legumes consumed were fava, beans, beans and lentils.

The dry legume consumed most frequently was fava, which was served in the form of fava puree made with olive oil, locally known as "Bissara". Consumption of dry legumes was variable within the families as reflected in the data in Table 5.3. Vegetables were consumed individually as potatoes or mixed, forming the "Tagin" that may or may not contain meat. The levels of vegetables consumed were variable for the families studied. Fruit consumption (mainly oranges) was low, probably because of their low availability in the region.

Meat consumption (mostly goat meat) was low. When consumed, sardines are often consumed at lunch either grilled or in the form of "Tagine" with tomatoes. Milk (cow's or goat's milk) is usually consumed at breakfast heated alone or with the addition of coffee. Sugar is consumed whenever tea or coffee is served. Pure sugar con-

sumption was similar in the four villages and ranged from 36 to 46 g/p/d.

Tea and coffee consumption is shown in Table 5.4. Mint tea can be served at all meals and mainly at lunch and dinner. The average consumption of tea was 2-4 g/p/d. This is equivalent to 3-5 glasses of tea; and the consumption of coffee is in the same range. Coffee is mostly served at breakfast either alone or mixed with milk.

Table 5.4. Tea and coffee consumption in the four villages (g/p/d).

Village	Number of families	Tea leaves	Coffee grounds
Gharouzim	8	2.93 ± 1.19 *	3.56 ± 2.09
Dar Abouba	9	4.35 ± 2.19	2.48 ± 2.21
Lachaich	5	2.55 ± 0.65	4.36 ± 1.36
Akarat	6	2.01 ± 1.13	1.62 ± 1.01

* Mean ± SD

The consumption of tea and/or coffee may have a detrimental effect on iron status. Consumption of tea or coffee at breakfast meals has been reported to reduce iron absorption from those meals (Hallberg, 1981). The effect of tea and coffee consumption on iron status will be discussed later in the present work.

5.3.3 Dietary Iron Intake

The mean dietary-iron intake (Table 5.5) were determined in each village for the families studied. Results are expressed as mean ± standard deviation of the average

intake for seven days. The calculated mean daily iron intake was 15 to 17 mg/p/d. The values of iron intake are similar to those reported in other regions of Morocco (Alaoui and Leklem, 1987). When compared to dietary iron intakes in other countries, our findings are within the range of values reported in adult men in TOGO (from 11.5 to 18 mg/p/d) in Cameroun (10.0 mg/p/d), in Kenya (16.8 mg/p/d); or in the population of Algeria (12.3 mg/p/d) (Hercberg et al., 1987). In dietary surveys in Africa, extremes range from 8 to 400 mg/p/d. Analysis of food balance sheets show that total iron per capita in the African diet varies from 14 to 21 mg/p/d (Hercberg et al., 1987).

Table 5.5. Total iron intake, heme iron and non-heme iron for the families studied (mg/p/d).

Village	Iron intake	Heme iron	Non-heme iron
Gharouzim	16.6 ± 4.0*	0.6 ± 0.5	16.0 ± 4.0
Dar Akouba	15.3 ± 2.4	0.4 ± 0.2	14.9 ± 2.3
Lachaich	17.0 ± 2.0	0.6 ± 0.2	16.4 ± 2.0
Akarat	17.7 ± 8.0	0.2 ± 0.1	17.4 ± 8.1

* Mean ± SD

Non-heme iron represented from 96 to 98 percent of the total dietary iron intake in the villages. These values are higher than those reported for other countries. Non-heme iron has been reported to be 85 to 89 percent of total iron intake in France, UK, in Sweden and the U.S.A (Galan et al., 1985). Also, the mean dietary iron intake of pre-

dominantly lacto-ovo-vegetarian adult Indian immigrants to Canada was 18.7 mg/p/d for males and 14.4 mg/p/d for females (Bindra and Gibson, 1986). The non-heme iron in their diets represented 17.84 mg/p/d and 14.01 mg/p/d for males and females, respectively (that is 95.4 and 97.3 percent). These values are similar to those found in the present study.

5.3.4 Sources of Iron

Table 5.6 shows that cereals constitute the main source of iron for the population studied. Cereal contribution to the total dietary iron supply was from 52 to 56 percent in the four villages studied. These values are lower than those found in other regions of Morocco (about 75%) (Alaoui and Leklem, 1987). Meat, poultry and fish contributed less than 2% of the total iron supply. There were similarities in the MPF consumed in the four villages. The other foods (mainly dry legumes and vegetables) contributed from 34 to 45% of the total intake of the iron. Fig. 5.1 illustrates the proportions of heme and non-heme iron intakes in the four villages studied, and their main dietary sources. These data are consistent with other reports which show that most of the dietary iron in African countries is supplied by foods of vegetable origin (Hercberg et al., 1987). In contrast, Soustre and coworkers (1986) found that the sources of iron in the meals of two hundred

Table 5.6. Dietary iron sources, iron supply and percent of total supply (mg/p/d)

Village	Total dietary iron intake	Iron sources					
		Cereals		Meat, poultry, fish		Other sources	
		Q*	%	Q*	%	Q*	%
Gharouzim	16.6 ± 4.0†	9.3 ± 1.6	57.5 ± 9.5	1.6 ± 1.3	9.5 ± 7.5	5.5 ± 2.5	33.0 ± 11.0
Dar Akouba	15.3 ± 2.4	8.2 ± 1.4	54.5 ± 13.1	1.1 ± 0.5	6.5 ± 3.3	6.1 ± 2.2	38.5 ± 13.2
Lachaich	17.0 ± 2.0	9.5 ± 1.4	56.2 ± 7.4	1.5 ± 0.5	9.0 ± 3.0	6.0 ± 1.5	35.0 ± 6.5
Akarat	17.7 ± 8.0	9.3 ± 3.5	55.4 ± 13.6	0.5 ± 0.2	3.4 ± 2.7	8.0 ± 5.7	41.1 ± 14.5

* Q represents the quantity of iron supplied by the food

† Mean ± SD

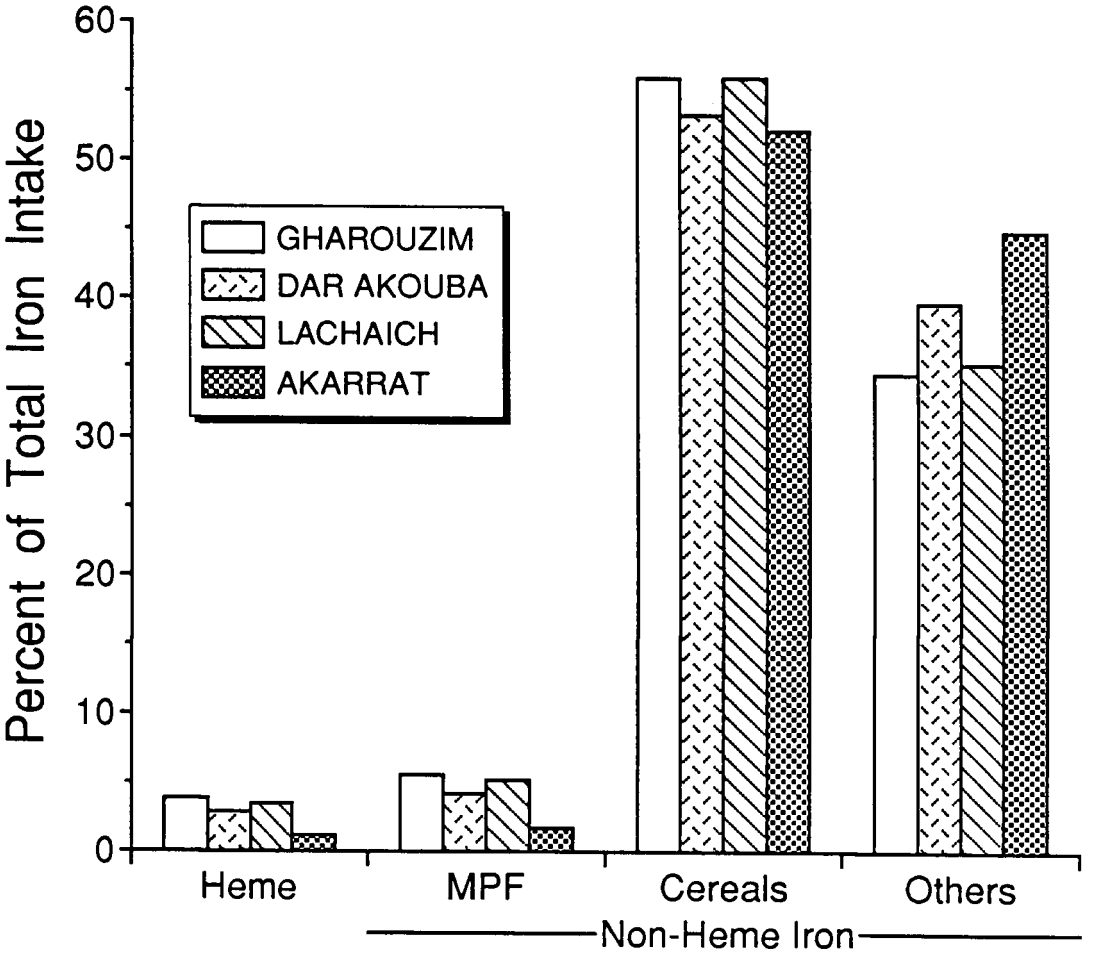

Figure 5.1. Sources of dietary iron in the four villages.

menstruating Parisian women were 50% of animal origin and 50% of vegetable origin. In the United Kingdom, the contribution of cereals, including iron fortified products was found to be 39%, that of meat 24%, and that of green vegetables 11% (Hazell, 1985). This information emphasizes the difference in the composition of the diets between African and European countries. Takkuen observed that the diets of iron deficient subjects were higher in cereals and dairy products and lower in meat than those of the non-deficient subjects (Takkuen, 1976).

5.3.5 Estimation of Available Iron

Mean daily intakes of meat, poultry and fish (MPF in g/p/d), ascorbic acid, iron, and the subsequent calculated available iron are given in Table 5.7. Except for one of the families studied, the calculated available iron is less than 2 mg/p/d and represents from 4.5 to 8.5% of the total iron intake. These findings are similar to those of Bindra and Gibson (1986) who found that, using the assumption of the Monsen model, the mean daily calculated available iron intake was 1.27 mg (6.8%) and 1.08 mg (7.5%) for men and women, respectively. Their subjects were predominantly, lacto-ovo-vegetarian east Indian immigrants to Canada. These levels are lower than the estimated average iron absorption (10%) used by the US Food and Nutrition Board RDA for iron (RDA, U.S.A., 1980). Hercberg and coworkers (1987) reported that most African people consumed a diet

based on cereals or roots and tubers with no or very small quantities of meat, fish or foods rich in ascorbic acid. The iron bioavailability was about 5% or even less of the total iron intake in a very monotonous cereal based diet consumed in some rural areas. This is in agreement with the present study. The low iron availability from cereal diets, that appear to contain sufficient iron, is related to their content of substances that inhibit iron absorption, namely, phytates, polyphenols and fiber (Gillooly et al., 1984; Hazell, 1985).

Table 5.7. Mean daily intakes of meat, poultry and fish (MPF), ascorbic acid, iron and available iron in the different villages.

Village	MPF (g/p/d)	Ascorbic acid (mg/p/d)	Iron intake (mg/p/d)	Available Fe (mg/p/d)	Fe availability (%)
Gharouzim	92.0 ± 67.6*	41.1 ± 29.5	16.6 ± 4.0	1.1 ± 0.3	6.7 ± 1.0
Dar Akouba	50.0 ± 17.9	45.8 ± 18.1	15.3 ± 2.4	1.1 ± 0.5	5.7 ± 1.0
Lachaich	79.2 ± 25.7	41.7 ± 16.2	17.0 ± 1.2	1.1 ± 0.1	6.6 ± 0.5
Akarat	44.0 ± 22.3	50.7 ± 18.7	17.7 ± 8.1	1.1 ± 0.5	6.4 ± 0.6

* Mean ± SD

In addition, the tannins present in tea or coffee may contribute to the reduction of iron availability from foods. Hallberg reported that tea and coffee consumption reduced the absorption of non-heme iron from a hamburger meal by 61% and 33% respectively (Hallberg, 1981).

Using an in vitro technique for estimation of iron availability, Hazell and Johnson (1987) showed that among other foods, cereals and legumes displayed iron diffusibil-

ity values below 6%. This was attributed mainly to their phytate content. Brune and coworkers found that the inhibitory effect of bran (source of phytates) on iron absorption was similar in vegetarians and omnivorous subjects. Their finding suggests that no intestinal adaptation to a high phytate intake occurs in populations consuming a vegetarian diet for a long period of time (Brune et al., 1989). This implies that in most developing countries where the diets consumed are monotonous and high in phytates, the inhibitory effect of phytate should be considered. The consumption of such diets can be satisfactory in terms of iron nutriture if the diet contains sufficient amounts of ascorbic acid and or citric acid that counteract the inhibitory effect of phytates. In addition, some components of dietary fiber (hemicellulose and lignin) may contribute to the low availability of iron from cereals (Gillooly et al., 1984). This low availability of dietary iron is related to the source of the iron. In the present study cereals and vegetable products were the major source of iron in the diet of the population. In addition, the actual values of available iron may be even lower, since the Monsen model used (Monsen, 1980) did not directly take into account the inhibitory effects of phytates, dietary fiber, and tannins on iron absorption. The phytate and fiber content of Moroccan foods have been determined (Alaoui and Essatara, 1985). Crude fiber intake in Morocco was

20.08, 25.58 and 12.42 g/p/d at the national, the rural and the urban milieu, respectively. The daily intake of phytates was 2867, 3495 and 2153 mg/p at the national, the rural and the urban milieu, respectively (unpublished data).

5.3.6 Iron Intake and Recommended Intake

Total daily recommended iron intake (DRII) was determined for each family based on the subject's age and sex using the recommendations of WHO/FAO, when diets are poor in proteins of animal origin, (WHO/FAO, 1974). These recommended intakes of iron were compared to daily dietary iron intake for each family (Table 5.8). Average dietary iron intakes for the families ranged from 104 to 111 percent of the recommended nutrient intake for iron (WHO/FAO, 1976). In each village at least one of the families had an average daily iron intake of less than 100 percent of the DRII.

Table 5.8. Daily iron intake, as a percent of the daily recommended iron intake for the families studied (mg/d/family).

Village	Family code	Iron intake (1)	Recommended iron intake (2)	(1)/(2) x 100%
Gharouzim	1	96.0	85	113
	2	68.2	85	80
	3	114.2	67	170
	4	97.0	87	112
	5	81.3	112	73
	6	95.2	81	118
	7	154.2	151	102
	8	119.9	118	103
	mean			109 ± 29.5*
Dar Akouba	1	98.3	97	101
	2	264.0	250	106
	3	74.0	67	110
	4	58.2	47	124
	5	84.5	77	110
	6	98.2	68	144
	7	119.6	111	108
	8	118.9	111	107
	9	115.0	147	78
	mean			110 ± 17.6
Lachaich	1	218.0	169	129
	2	133.2	117	114
	3	122.8	136	90
	4	143.0	188	76
	5	268.6	234	115
	mean			105 ± 21.2
Akarat	1	108.1	172	63
	2	148.0	199	74
	3	166.0	218	76
	4	179.4	84	214
	5	200.2	132	151
	6	210.7	240	88
	mean			111 ± 59.2

* Mean ± SD

5.4. **IRON STATUS OF THE ADULT MEN AND WOMEN**

5.4.1 Hemoglobin, Hematocrit and Mean Corpuscular Hemoglobin Concentration (MCHC) Values

The mean hemoglobin, hematocrit and MCHC values of adult men and women for different age and sex groups, are shown in Table 5.9. The results indicate highly signifi-

Table 5.9. Hemoglobin, hematocrit and MCHC values (Mean ± SD).

Groups	n	Hemoglobin (g/l)	Hematocrit (1)	MCHC (g/L)
Young males (17-30 yrs)	21	167 ± 8.0[a]	0.48 ± 0.02[c]	350 ± 10.0[e]
Older males (31-64 yrs)	26	166 ± 10.0[a]	0.48 ± 0.03[c]	350 ± 10.0[e]
All males	47	166 ± 10.0[a]	0.48 ± 0.03[c]	350 ± 10.0[e]
Young females (16-30 yrs)	30	139 ± 17.0[b]	0.41 ± 0.04[d]	340 ± 20.0[f]
Older females (31-55 yrs)	20	138 ± 14.0[b]	0.41 ± 0.02[d]	330 ± 30.0[f]
All females	50	139 ± 16.0[b]	0.41 ± 0.04[d]	340 ± 30.0[f]

a,b,...,f: For the same indicator of iron status, different subscripts revealed highly significant differences (at 0.001 significance level). The males have higher values than the females.

cant differences in hemoglobin, hematocrit and MCHC between males and females. However, there were no significant differences between young males and older males, or between young females and older females. Consistent with other studies (Cook et al., 1976; Bindra and Gibson, 1986; Sauberlich et al., 1980) hemoglobin, hematocrit and MCHC levels were lower in women than men. The mean hemoglobin values for both men and women are greater than the cutoff points (130 g/l for adult men and 120 g/l for adult women) determined by the WHO (WHO, 1968). However, there were individual females with lower values of hemoglobin. The mean hematocrit values for both men and women are higher than the cutoff levels (< 40% (0.40) in adult males and < 36% (0.36) in adult females) determined by the International Nutritional Anemia Consultative Group (INACG, 1985). For the MCHC, the mean values for both sexes are higher than

the cutoff point (<32% or 320 g/l) calculated from the normal values for hemoglobin and hematocrit, considered by the INACG (INACG, 1985) and found in the literature (Thiele, 1980; Bindra and Gibson, 1986).

5.4.2 Serum Iron, Total Iron Binding Capacity (TIBC) and Serum Transferrin Saturation

Table 5.10 shows a two-way analysis of variance (ANOVA) test comparing the mean serum iron concentration, TIBC and STS values for the different age and sex groups of males and females. The results indicate highly significant differences in serum iron and STS between young males and older males; young females and older females; and between males and females. For both sexes the young individuals have higher values, of serum iron and STS, than the older ones. In both age groups, the males have higher values, of serum iron and STS, than the females. There were no differences in TIBC between males or between females; however, the males have lower values of TIBC than the females. In males, the mean values for serum iron were higher than the cutoff point (<60 μg/dl or 11μmol/l) determined by the INACG (1985). In contrast, the serum iron values for females were lower than the cutoff point. Compared with the cutoff point of > 400 μg/dl (72μmol/l) determined by the INACG, the mean values of TIBC for males are normal and those for females are abnormal.

Table 5.10. Serum iron, TIBC and STS values (mean ± SD).

Groups	n	Serum iron (μmol/L)	TIBC (μmol/L)	STS (%)
Young males (17-30 yrs)	21	19 ± 5.0[a]	67 ± 8.0[e]	28 ± 7.0[g]
Older males (31-64 yrs)	26	13 ± 4.0[b]	64 ± 8.0[e]	20 ± 7.0[h]
All males	47	16 ± 5.0[c]	65 ± 8.0[e]	24 ± 8.0[i]
Young females (16-30 yrs)	30	11 ± 6.0[d]	74 ± 11[f]	16 ± 9.0[j]
Older females (31-55 yrs)	20	8.0 ± 3.0[e]	71 ± 12[f]	12 ± 5.0[k]
All females	50	10 ± 5.0[d]	73 ± 11[f]	14 ± 8.0[j]

a,b,...,k: For the same indicator of iron status, different subscripts revealed highly significant differences (at 0.001 significance level for serum iron and STS; and at 0.005 significance level for TIBC).

The mean values for STS are lower than the cutoff point of < 16% or < 0.16 (INACG, 1985) in the group of older females and in the combined group of young and older females. These findings emphasize the differences in iron status between males and females, with the latter group being of more concern relative to the risk of developing iron deficiency. Similar findings were reported by Bindra and Gibson (1986) in a population of adults consuming a vegetarian type of diets.

5.4.3 The Tri-index Model Indices

The MCHC, STS and serum ferritin (SF) represent the Tri-index model indices. These values are presented in Table 5.11. The results indicate that the mean serum ferritin concentration values are significantly higher (2-2.5

times) in males than females. In both sexes, those mean values are higher than the cutoff point (<12μg/l; INACG, 1985). However, the range is wide and for 24 females the level of serum ferritin was less than 12μg/l. These females have depleted iron stores. In the two groups of men, only two subjects had serum ferritin concentration values below the cutoff point. These data provide further support for the premise that iron deficiency is more prevalent among females than males. This is in agreement with other studies (Cook et al., 1976; Cook et al., 1986; Bindra and Gibson, 1986).

Table 5.11. Values for the Tri-index model indices (mean ± SD).

Groups	n	MCHC (g/l)	STS (%)	SF (μg/L)
Young males (17-30 yrs)	21	350 ± 10.0	28 ± 7.0	39 ± 25[a] (10,130)*
Older males (31-64 yrs)	26	350 ± 10.0	20 ± 7.0	50 ± 29[a] (10,130)
All males	47	350 ± 10.0	24 ± 8.0	45 ± 28[a] (10,130)
Young females (16-30 yrs)	30	340 ± 20.0	16 ± 9.0	15 ± 16[b] (2,67)
Older females (31-55 yrs)	20	330 ± 30.0	12 ± 5.0	22 ± 22[b] (3,88)
All females	50	340 ± 30.0	14 ± 8.0	18 ± 19[b] (2,88)

* figures in parentheses refer to range.

a,b: For values of SF, different subscripts revealed highly significant differences (at 0.001 significance level).

For both males and females, the young individuals had slightly lower mean values for serum ferritin concentration (Table 5.11), even though the differences were not statis-

tically significant. The lower values in the young subjects may be explained by the possibility that young individuals of both sexes have higher physical activity; and thus, iron is removed from the storage protein (ferritin) in order to be incorporated into hemoglobin, myoglobin and other iron-containing enzymes. Myoglobin can store oxygen and deliver it when needed in activated skeletal muscle (Astrand, 1981). It was assumed that serum iron is a valid monitor of the adequacy of the availability of iron to the tissues that are directly associated with work performance (Edgerton and Ohira, 1981). Also, the latter authors reported that hemoglobin concentration is closely related to work capacity. The need for iron during work and physical activity may provide some explanation to the mobilization of iron from its storage form as ferritin to the form(s) where it is functional (i.e., hemoglobin, myoglobin and enzymes). In the present study, the young active subjects of both sexes had lower values for SF and slightly higher values for hemoglobin, and serum iron than the older individuals (Tables 5.9-5.11), whose physical activity presumably slows down with age. This findings confirms our previous suggestion that the lower iron stores in the young subjects may be attributed to their higher physical activity. Iron is mobilized from ferritin to the circulating pool to be transported to tissue, including muscle. Clin-

ical studies demonstrated the reduction of work performance as a result of iron deficit (Galan et al., 1984).

The major significant correlations observed between the different indicators of iron status of males and females were for hemoglobin concentration and hematocrit ($r=0.83$, 0.94, 0.85 and 0.65 in the groups of males and females, respectively, $p<0.01$); hemoglobin and MCHC ($r=0.50$, 0.43, 0.65 and 0.85 in the four groups, respectively, $p<0.05$); and between serum iron and STS ($r=0.88$, 0.89, 0.96 and 0.94 in the four groups respectively, $p<0.01$). In older females there was a positive correlation between serum ferritin concentration and hemoglobin ($r=0.57$, $p < 0.01$) and SF and MCHC ($r=0.64$, $p < 0.01$). A significant negative correlation was found between STS and TIBC ($r=-0.46$, $p<0.05$) and TIBC and SF ($r=-0.62$, $p<0.01$) in the older females. For the other three groups, there were nonsignificant negative correlations for these comparisons.

The strongest correlation was observed between hemoglobin concentration and hematocrit. This finding is consistent with that reported in pregnant Algerian women (Assami et al., 1988), in individuals of both sexes living in the rural district of Toric Bossito in South Benin (Hercberg et al., 1986), and in lacto-ovo-vegetarian East Indian adult men and women, immigrants to Canada (Bindra and Gibson, 1986).

Iron status was also determined in individuals living in the city of Rabat. The subjects were four males aged 30-40 years and six females 30-40 years old. The results are presented in Table 5.12. The mean values for the Tri-index model indices in the subjects studied were found to be in the normal range. Again, hemoglobin concentration and hematocrit values are higher in males than females. Mean serum ferritin values, indicating iron stores, were also higher in the males than the females. In the females, three out of six had serum ferritin values below the cutoff point (<12 μg/l) and are considered as having depleted iron stores. Two of those three females had also abnormal values for STS (<16%). This group was presumably consuming different type of diets than the larger rural population studied. In the cities, people have more access to foods containing readily available dietary iron. Therefore, one would expect to see higher values for indicators of iron status (except for iron-binding capacity) in the small group from Rabat.

Table 5.12. Biochemical indices of iron status of adult men and women from Rabat.

	n	Hemoglobin (g/L)	Hematocrit (1)	MCHC (g/L)	Serum Fe (µmol/L)	TIBC (µmol/L)	STS (%)	Serum ferritin (µg/L)
Adult males	4	167 ± 30.0*	0.47 ± 0.07	380 ± 10.0	14 ± 5.0	58 ± 12	25 ± 8.0	57 ± 48 (14,126)**
Adult females	6	153 ± 17.0	0.42 ± 0.05	370 ± 10.0	15 ± 6.0	67 ± 9.0	23 ± 11	33 ± 54 (2,140)

* Mean ± SD

** The range is in parentheses

5.5 PREVALENCE OF IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA

The prevalence of iron deficiency in the population studied was to be determined using the Tri-index model and the mixed distribution analysis of hemoglobin. The Tri-index model combines MCHC, STS and SF. Iron deficient individuals have two or three abnormal values in this model. Iron deficiency has been defined by the existence of two or more abnormal indicators of iron status (Hercberg et al., 1986). The final stage of iron deficiency is associated with a significant decrease in circulating hemoglobin, and subjects are considered anemic only when the hemoglobin level (or hematocrit) has decreased below a cutoff level according to sex, age or other physiological conditions (Hercberg and Galan, 1985). The prevalence of anemia can also be defined in terms of the percentage of individuals with Hb values below a 95% reference range. Anemia can also be considered in terms of the depression of Hb concentration by the presence of common abnormalities such as iron deficiency or inflammatory disease even if that depression occurs within the "normal" reference range (Dallman et al., 1984).

5.5.1 Tri-Index Model

In the present study, the percentages of males and females classified as iron deficient, based on abnormal val-

ues for each of the biochemical indicators of iron status, individually and combined are shown in Table 5.13. The results show that when using hemoglobin concentration alone as an indicator of iron status, none of the males was classified as iron deficient. This is also true when using the MCHC. However, six females were anemic (Hb<120 g/L), and seven females had abnormal values for MCHC, an indicator of the final stage of iron deficiency. When using STS as an indicator of iron status, more males (10) and more females (34) were classified as iron deficient (second stage) than with the MCHC. The serum ferritin assay identified 2 males and 24 females with depleted iron stores (first stage of iron deficiency). These results confirm that iron deficiency cannot be correctly identified by using only one biochemical test such as hemoglobin. When identifying iron deficiency, a minimum of two independent biochemical measures should be used (Cook et al., 1976). Moreover, it is not easy to define normal values for hemoglobin concentration because of a marked overlap of hemoglobin values observed in normal and anemic subjects (Hercberg et al., 1987). The arbitrary definition of anemia based on hemoglobin concentration levels results in a large number of false positive and false negative findings (Cook et al., 1976). Therefore, more specific and more sensitive parameters than hemoglobin should be used to evaluate the iron status of populations. The MCHC is a red cell index that

Table 5.13. Percentages of males and females classified as iron deficient using different biochemical indicators of iron status individually and combined.

Groups	n	Biochemical indices				
		Hemoglobin (M<130, F<120g/l)	MCHC (<320g/l)	STS (<16%)	SF (<12 µg/l)	Tri-index*
Young males (17-30 yrs)	21	0(0)†	0(0)	4.8(1)	4.7(1)	0(0)
Older males (31-64 yrs)	26	0(0)	0(0)	34.6(9)	3.8(1)	0(0)
All males	47	0(0)	0(0)	21.3(10)	4.2(2)	0(0)
Young females (16-30 yrs)	30	13.3(4)	16.6(5)	60 (18)	53.3(16)	43.3(13)
Older females (31-55 yrs)	20	10 (2)	10 (2)	80 (16)	40 (8)	30 (6)
All females	50	12 (6)	14 (7)	68 (34)	48 (24)	38 (19)

† The number of subjects is in parentheses

* The Tri-index model combines MCHC, STS, and SF. Iron deficient individuals have two or three abnormal values in this model.

measures the concentration of hemoglobin in an average red corpuscle. This value is low in the final stage of iron deficiency; the normal values of MCHC are from 330-380 g/l (Simko and Cowell, 1984). Cook and coworkers (1976) reported that when the level of STS is below 15%, the synthesis of hemoglobin is impaired. They suggested that this measurement identifies approximately twice the number of iron deficient subjects as can be detected with the hemoglobin value. This latter index remains normal until there has been a total depletion of body iron stores. This observation is reflected by the data in the present study, and STS identified more than four times the number of iron

deficient subjects as detected by the hemoglobin concentration (Table 5.12).

The Tri-index model identified 19 (38%) females as iron deficient, and no males were classified as iron deficient. This observation is in agreement with other findings, indicating that iron deficiency is more prevalent among females than males (Cook et al., 1976; Cook et al., 1986; Bindra and Gibson, 1986). As indicated by the Tri-index model, 14 females had two abnormal values and 5 females had three abnormal values; thus, 19 subjects were classified as iron deficient.

Total iron binding capacity (TIBC) begins to increase as iron stores are depleted: TIBC > 370 μg/100 ml (66 μmol/l) and STS less than 15% were considered reflecting a latent hemoglobin iron deficit for adults (Baker and Demaeyer, 1979). In the presence of anemia, TIBC values > 400 μg/dl (72 μmol/l) are indicative only of iron deficiency anemia, not chronic disease (Bothwell et al., 1979). In the present study, 9 males and 27 females had TIBC values > 400 μg/dl (72 μmol/L), that is, about 20 and 54 percent of the males and females, respectively, were iron deficient. These percentages are similar to those found using the STS as an indicator of iron status.

In conclusion, 12% of the females were anemic and at least 38% were iron deficient and had depleted iron stores (first stage). In the males, about 20% have the second

stage of iron deficiency. The Tri-index model identified fewer individuals as iron deficient than did STS or SF. The least number of iron deficient subjects was identified by using hemoglobin concentration alone. This observation confirms that hemoglobin determination indicates the last stage of development of iron deficiency anemia.

Frequency distribution of hemoglobin, MCHC, STS and serum ferritin in males and females are presented in Figures 5.2 through 5.4. It is clear that there is no distinct separation between normal and abnormal values for any of these indicators of iron status, suggesting the need for using more than one iron index for assessing iron status in population studies.

5.5.2 Mixed Distribution Analysis of Hemoglobin

The relative prevalence of iron deficiency anemia is estimated by using mixed distribution analysis of hemoglobin (Hercberg et al., 1988), also termed hemoglobin percentile shift method (Dallman et al., 1984; Bindra and Gibson, 1986). Mixed distribution analysis of hemoglobin is based on the fact that the presence of iron deficiency anemia causes a lowering of the population's hemoglobin concentration. The relative prevalence of anemia is estimated as the Hb-percentile shift or change after excluding individuals with STS < 16% and SF < 12 μg/l from the entire population. In the present study, none of the males had both STS < 16% and SF < 12 μg/l, therefore the mixed

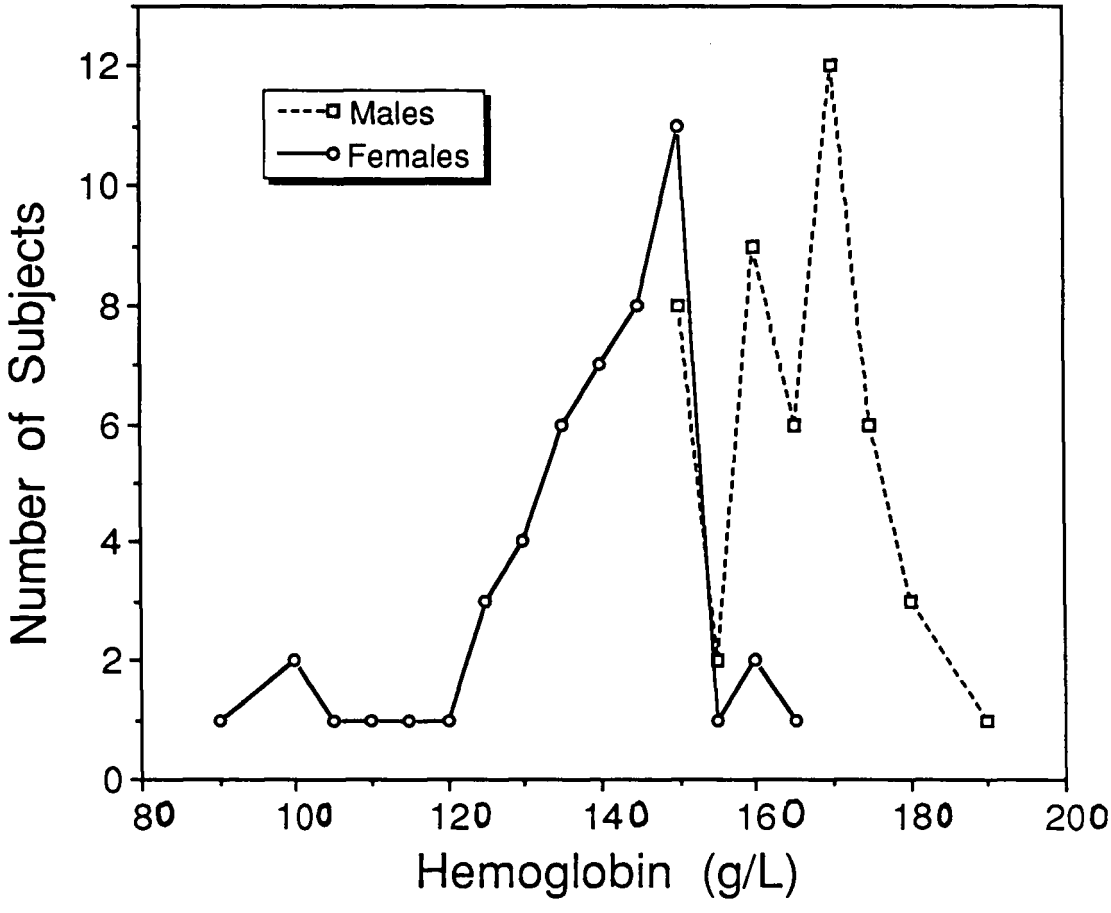

Figure 5.2. Frequency distribution of hemoglobin for males and females.

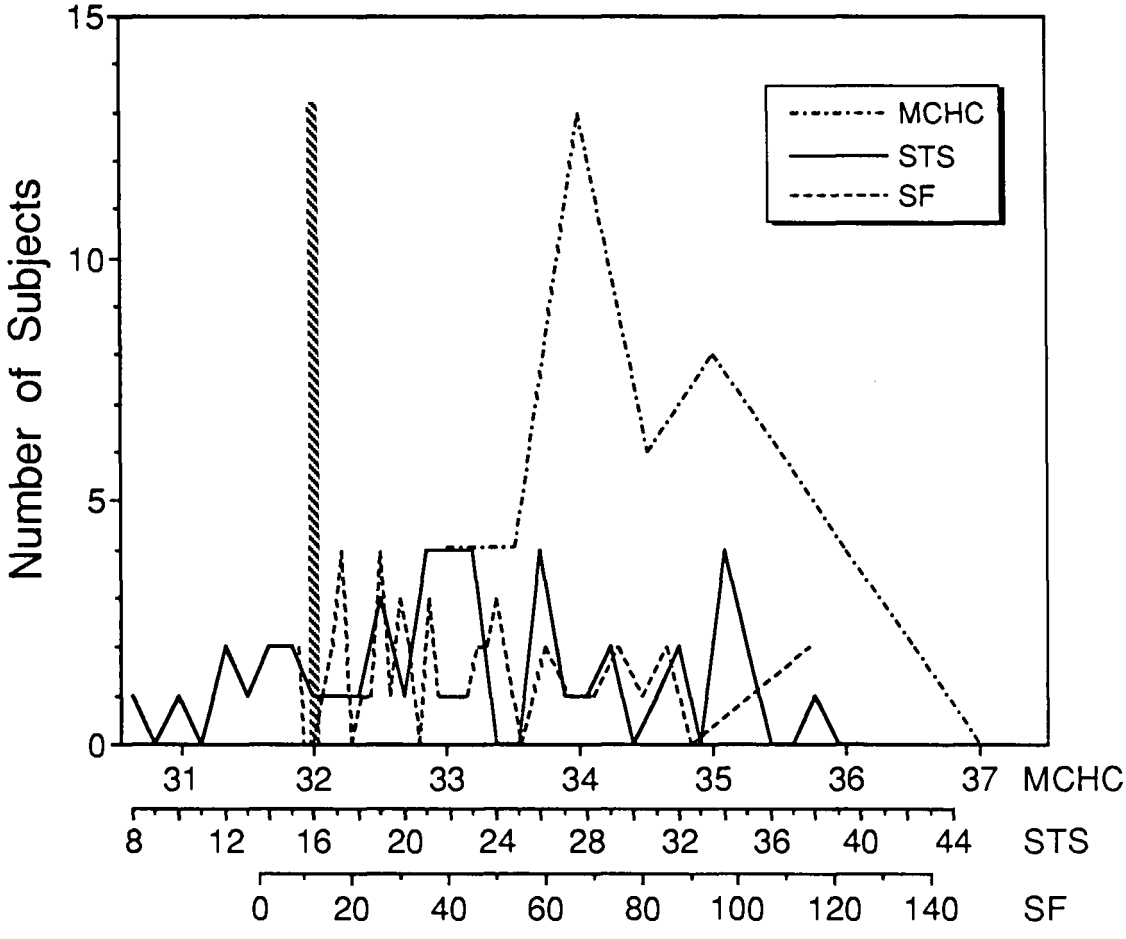

Figure 5.3. Frequency distribution of the Tri-index model indices for males.

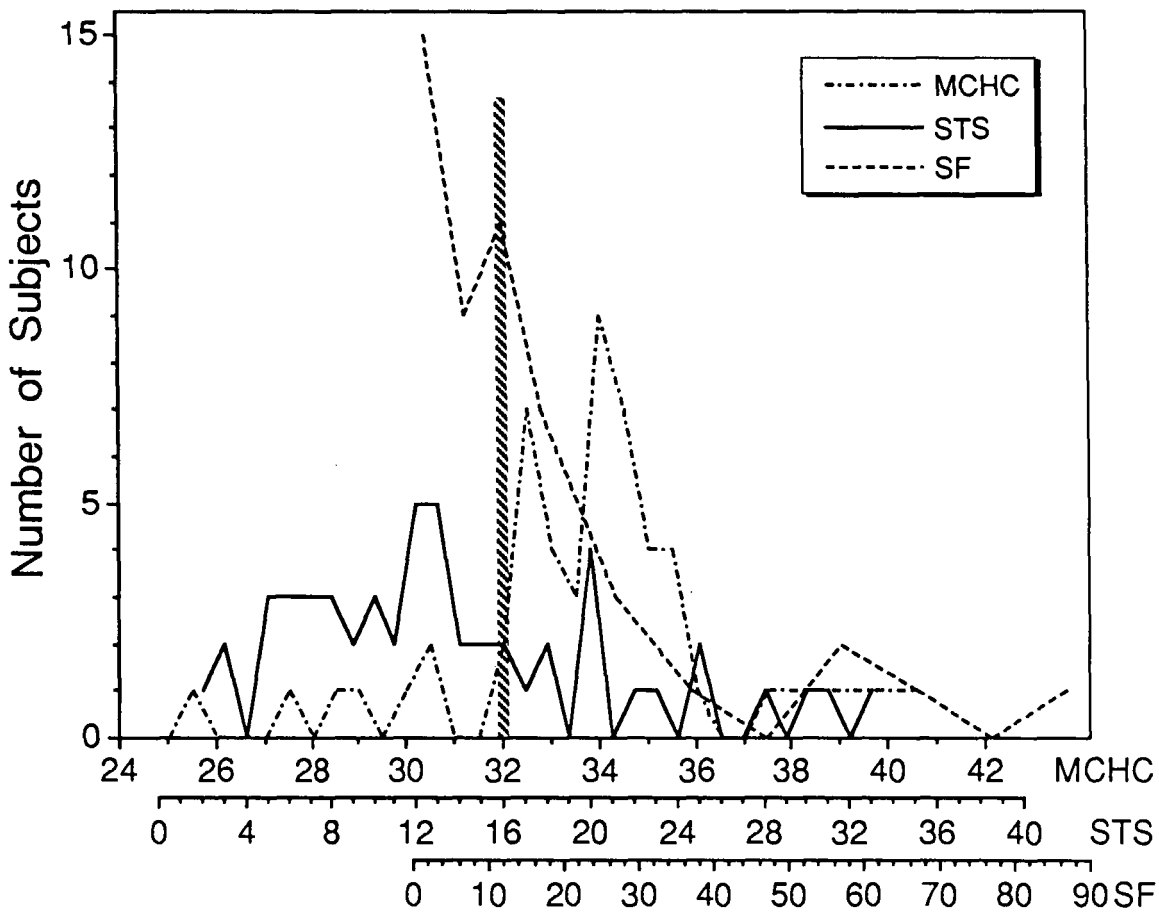

Figure 5.4. Frequency distribution of the Tri-index model indices for females.

distribution analysis will be applied only for the females. Table 5.14 shows the distribution of hemoglobin values for all the females (entire sample) and for those females with STS ≥ 16% and SF ≥ 12 μg/l (reference sample). The determination of the hemoglobin percentile (Hb -%) shift for females (Table 5.15) was based on the calculation of the median (50^{th} percentile) for hemoglobin in the entire sample and in the reference sample, which excludes the subjects with both STS < 16% and SF < 12 μg/l. Based on this, 33 females were considered to be "normal" and constitute the reference sample. The median (50^{th} percentile hemoglobin value) was 136 g/L for the entire sample and 140 g/L for the reference sample (Figure 5.5). This value of 140 g/l corresponds to the sixty-second percentile value for hemoglobin in the entire sample. The difference in percentiles is 12% and is considered the Hb percentile shift, and indicates the relative prevalence of anemia (Table 5.15). This figure of 12% is lower than that found with the Tri-index model (38%).

This difference between the two methods can be explained by the fact that the use of hemoglobin concentration identifies the late stage of iron deficiency anemia, while the indices of the Tri-index model identifies earlier stages. In the present study, a similar percentage (12%) (Table 5.13) for the prevalence of anemia in females was found by using the cutoff point for hemoglobin (<120

Table 5.14. Frequency distribution of Hb values for the entire sample and the reference sample of females.

Hemoglobin class-values (g/l)	Entire sample			"Reference" sample		
	Ni*	fi (%)**	Σfi(%)***	Ni	fi (%)**	Σ fi (%)
90-†	1	2	2			
95-	1	2	4			
100-	1	2	6			
105-	1	2	8	1	3.03	3.03
110-	2	4	12	0	0	3.03
115-	0	0	12	0	0	3.03
120-	1	2	14	1	3.03	6.06
125-	6	12	26	2	6.06	12.12
130-	1	2	28	0	0	12.12
135-	7	14	42	6	18.18	30.3
140-	8	16	58	5	15.15	45.45
145-	7	14	72	6	18.18	63.63
150-	10	20	92	10	30.3	93.93
155-	2	4	96	0	0	93.93
160-	1	2	98	1	3.03	96.96
165-	1	2	100	1	3.03	100
Total	50	100		33	100	

* Ni = number of subjects
** fi (%) = number of subjects as a percent of the total
*** Σ fi (%) = sum of the fi (cumulative frequency)
† The dash following each number indicates values from that number up to the next one.

Table 5.15. Hemoglobin percentile (Hb -%) shift for females.

Median (50^{th} percentile Hb) g/l		Hb -% of entire sample represented by reference sample's 50th percentile value	Relative prevalence of anemia*
Entire sample	Reference sample		
136	140	62nd percentile	12%

* Relative prevalence of anemia is estimated as the Hb-% shift after excluding individual females with STS < 16% and SF < 12 μg/l, from the entire sample.

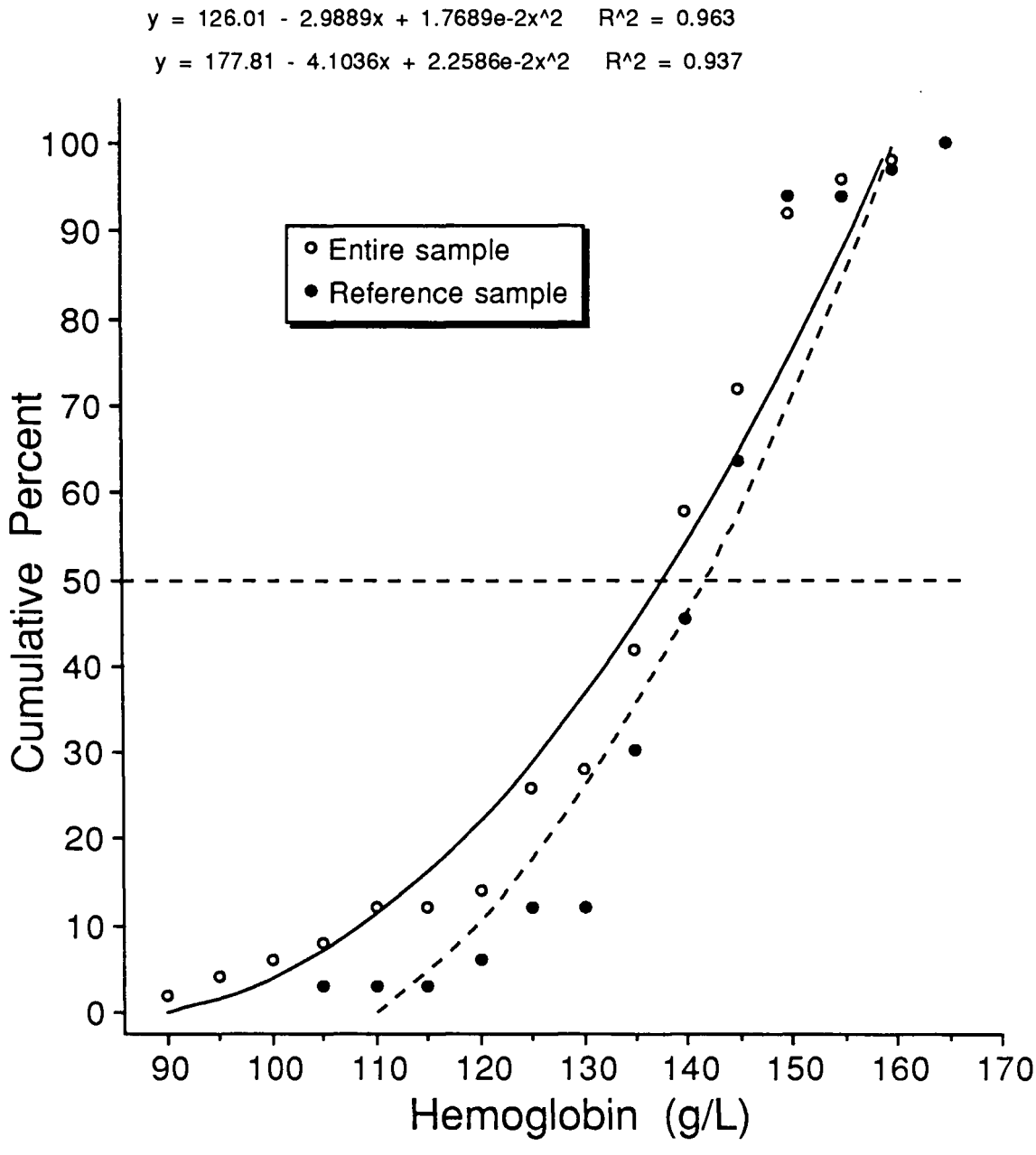

Figure 5.5. Cumulative frequency distribution of hemoglobin values for females.

g/l) determined by the INACG INACG, 1985) and by the WHO (WHO, 1968), and that found by using the mixed distribution analysis of hemoglobin. The rise in hemoglobin at the 50^{th} percentile (after exclusion of females with STS < 16% and SF < 12 μg/L) was 4.0 g/l (Table 5.15). There is a shift of the curve of hemoglobin towards higher values as illustrated in Figure 5.5.

We conclude that with both methods (Tri-index model, mixed distribution analysis of hemoglobin), the prevalence of iron deficiency is higher in females than males in the areas of Morocco sampled. The latter method identified the final stage of iron deficiency (anemia), while the former one identified the stages before the development of anemia (i.e., depleted iron stores and impaired iron transport).

5.6 **INFLUENCE OF DIETARY IRON ON IRON INDICES**

The relationship between dietary iron intakes (total iron intake, heme iron, non-heme iron, and available iron) and iron indices was studied in a subgroup of adult males (38) and adult females (49) from the population of adults previously described. Ten subjects were excluded because of lack of data on their food intake. Energy, protein and iron intakes (Table 5.16) were determined, for each subject, from the intakes of the subsequent family, by using the unit of consumption formula (UC) described earlier. This formula assumes that the food intake for adult females

Table 5.16. Mean daily intakes of energy, protein, and dietary iron in the males and females.

Groups	Young males	Older males	All males	Young females	older females	All females
n †	19	19	38	30	19	49
Caloric intake (Kcal/p)	2875 ± 424*	2798 ± 491	2837 ± 454	2258 ± 350	2309 ± 417	2278 ± 374
Protein intake (g/p)	98.4 ± 20.1	95.3 ± 21.4	96.8 ± 20.5	75.6 ± 14.5	81.3 ± 18.5	77.8 ± 16.2
Iron intake (mg/p)	22.3 ± 4.8	20.1 ± 5.0	21.2 ± 4.9	17.6 ± 3.2	17.8 ± 4.2	17.7 ± 3.6
Iron density (mg/1000Kcal)	7.75	7.10	7.40	7.30	7.70	7.70
Heme iron (mg/p)	0.58 ± 0.51	0.55 ± 0.50	0.56 ± 0.49	0.49 ± 0.24	0.52 ± 0.39	0.50 ± 0.30
Non-heme iron (mg/p)	21.7 ± 4.8	20.1 ± 4.8	20.9 ± 4.8	17.0 ± 3.3	17.3 ± 4.2	17.1 ± 3.7
Available iron (mg/p)	1.45 ± 0.35	1.30 ± 0.32	1.37 ± 0.34	1.10 ± 0.17	1.20 ± 0.25	1.10 ± 0.20

† Number of persons in sample providing intake data differs from samples providing biochemical data due to exclusion of case with missing value.

* Mean ± SD

(UC=0.8) is 20% less than that of adult males (UC=1.0) within a family. This is reflected in the results shown in Table 5.16.

The values found for caloric intake in males are similar to those reported to be available at the national level (2734 kcal/p/d). The protein intake in the present study in males was higher than the value reported at the national level (71 g/p/d). For iron, the values were similar to those found to be "available" at the national level (20 mg/p/d) (Strategie Alimentaire, 1989). The calculated iron density from the mean caloric and iron intake is from 7 to 7.75 (mg iron/1000 kcal). Slightly higher values (8 mg iron/1000 kcal) were reported in lacto-ovo-vegetarian East Indian immigrants to Canada (Bindra and Gibson 1986).

Correlation coefficients were determined between dietary iron intake and biochemical indices. In the males, a positive nonsignificant correlation was found between hemoglobin and heme iron (R^2= 0.001), and between hematocrit and heme iron (R^2= 0.024). In the females, the correlation coefficients were 0.01 between hemoglobin and heme iron; 0.001 between hematocrit and heme iron; and 0.005 between serum ferritin and heme iron. No significant correlations were found between dietary iron intake and biochemical indices. Similarly, others did not find any significant correlation between dietary iron and iron indices (Bindra and Gibson, 1986) or between serum ferritin concentration

and total iron intake (Dhur and Hercberg, 1989; Galan et al., 1985). Correlation coefficients of 0.02 and 0.13 between dietary iron and hemoglobin values have been reported in the Ten-State Nutrition Survey (Kerr et al., 1982).

Although there were no signficant correlations, in the present study and in other studies in the literature previously cited, food and nutrient intakes may influence biochemical indices of iron status. An elevated nutrient intake might not be expected to increase a biochemical value above an already adequate level, and an occasional nutrient intake below the reference standard might not be expected to reduce the biochemical value significantly. However, an inadequate intake should, over time, be reflected in lower biochemical indices of nutritional health (Kerr et al., 1982). In addition, the methods used to assess dietary intakes (including the 7-day weighed diet) are of a short term, while the biochemical indices are of longer term and might vary with non dietary factors. For instance, Kies and McEndree (1982) reported that vegetarians were found to better utilize iron from a vegetarian diet than were omnivores consuming the same vegetarian diet. They suggested that vegetarians may undergo physiological adaptation enabling them to somewhat better utilize iron than would be expected from iron bioavailability studies.

In the present study, the low availability of dietary iron (6%) (Table 5.7) may be partially responsible for the etiology of iron deficiency. This low availability of dietary iron is due to the typical diet, consumed mainly in rural areas, that is high in cereals and cereal products. These foods are high in phytates and fiber, components that inhibit iron absorption (Gilloly et al., 1984).

The higher prevalence of iron deficiency in females compared to males may be attributed to their lower iron intakes and higher iron losses. In addition, the low prevalence of iron deficiency in males can be explained by the fact that the men, especially in rural areas, get to eat more meat than women from outside the home. For instance, during celebrations (e.g., weddings or births.) the men are usually invited and then over time do eat more than the women; also on the days of the "souk", most men would have something to eat before they go home. The heme iron from meat, poultry and fish is better absorbed than the non-heme iron from grains. In view of these findings, it is not surprising that the prevalence of iron deficiency was found to be higher in females than males. This can be attributed to the differences in food intake and in physiological differences between males and females.

5.7 EFFECT OF TEA CONSUMPTION ON IRON INDICES

The relationships between tea consumption and iron indices (hemoglobin, hematocrit, MCHC, serum iron, TIBC, STS and serum ferritin) was studied. By using Pearson's correlation coefficients, a negative, but not significant, correlation was found between tea consumption and serum ferritin ($r = -0.19$, -0.09, -0.13 and -0.08, $p<0.05$ in the groups of young males, older males, young females and older females, respectively). Similarly, Galan and coworkers (1985) found that the dietary consumption of tea had a significant negative correlation ($r = -0.18$, $p < 0.05$) with serum ferritin in French females aged 17 to 42 years. This finding emphasizes the inhibitory effect of tea on iron absorption (Hallberg, 1981). In older females tea consumption had a significant negative correlation with hemoglobin ($r=-0.60$, $p<0.05$), hematocrit ($r= -0.80$, $p<0.01$), serum iron ($r= -0.53$, $p<0.05$), and serum transferrin saturation ($r= -0.57$, $p<0.05$). The effects of tea consumption on iron indices in the other groups were not significant. The effect of coffee consumption on iron indices was not significant and inconsistent.

5.8 EFFECT OF SMOKING ON IRON INDICES

The effect of smoking on iron indices was studied in 20 male smokers. Although not significant, a negative correlation was found between cigaret smoking and hemoglobin

(r= -0.24), hematocrit (= -0.18), total iron binding capacity (r= -0.30) and serum transferrin saturation (r= -0.31). The only significant correlation was between the number of cigarets smoked per day and serum iron (r= -0.48, $p<0.05$). No data were found in the literature regarding the effect of smoking on iron indices.

5.9 **GENERAL DISCUSSION**

The results found by using the Tri-index model (combining MCHC, serum transferrin saturation and serum ferritin) confirm that a minimum of two independent biochemical measures should be used to correctly identify iron deficiency (Cook et al., 1976; Bindra and Gibson, 1986).

The usual approach, of separating normal from iron deficient subjects on the basis of only one criterion, may involve errors in the diagnosis of both subjects (Hercberg et al., 1988). In the present study, the lowest percentage of iron deficient individuals was identified by using hemoglobin alone as an indicator of iron status. Similar values were found by using mixed distribution analysis of hemoglobin (i.e., 12% of the females were anemic). However, the Tri-index model identified zero percent of the males as iron deficient; few males (2) had lower serum ferritin values (<12 μg/L) indicating a depletion of their iron stores.

Higher prevalence of iron deficiency was observed among females, and may be partially attributed to inadequate intakes of readily available dietary iron. In the present study, available dietary iron represented only 6% of the total iron intake. This value is lower than that reported (10 to 15%) in industrialized countries (FAO, 1988). The lower availability of dietary iron was due to the nature of the diets consumed. For instance, cereals constitute the staple food of the population studied and represent the main source of dietary iron. The contribution of cereal to the total dietary iron supply was greater than 50%. Cereals contain substances (phytic acid, fiber, and polyphenols) that are known to depress iron absorption (Gillooly et al., 1984). In addition, the consumption of tea is high, and tea may be a potent inhibitor of iron absorption because of the presence of tannins in tea (Hallberg, 1981).

The higher prevalence of iron deficiency in females than males may be attributed to physiological factors (menstruation in females) and to the fact that in this society, the males, especially in rural areas, do consume more flesh foods outside the home than do the females. The latter consume only meals at different occasions such as celebrations (e.g., weddings, births) and at "souks".

These observations suggest that other studies should be conducted in other regions of Morocco for a better un-

derstanding of the prevalence and the cause(s) of iron deficiency, prior to conducting an intervention trial toward its eradication.

CHAPTER 6

SUMMARY AND CONCLUSIONS

The purpose of this study was to determine the iron status of a population of adults in a rural region of Morocco, and to determine the influence of the typical diet, consumed locally, on iron status.

The present study was conducted in a rural area at the Province of Chefchaouen, north of Morocco. As in most other rural areas, this region is characterized by the high consumption of foods produced locally. The diets are monotonous and cereal based. The iron supplied from foods is mostly non-heme iron. The consumption of flesh foods (meat, poultry and fish), providing readily available heme iron, is low.

Twenty-eight families were chosen in four villages. The food consumption of the families was determined for seven days. All foods and ingredients were weighed by trained personnel. Caloric, protein and iron intakes were determined by using food composition tables. From those families 47 adult males and 50 adult females participated in this study. The nutrient intake of each subject was determined from that of the subsequent family, based on the Unit of Consumption (UC) formula. This formula assumes

that the dietary intakes of males (UC=1.0) are 20% greater than those of the females (UC=0.8). Based on this assumption, the mean daily iron intake was 21.2 and 17.7 mg/person adult/day for males and females, respectively. When the younger members of the families were considered, this intake ranged from 15 to 17 mg/p/d. Cereal's contribution to the total dietary iron supply was from 52 to 56 percent in the four villages. The availability of dietary iron was estimated to be about 6%. This low availability of iron from foods was attributed to their contents of phytates, polyphenols and fiber.

The prevalence of iron deficiency in males and females was determined based on abnormal values of a Tri-index model (MCHC, STS, SF) and using mixed distribution analysis of hemoglobin. Using hemoglobin concentration alone, as an indicator of iron status, 6 (12%) females and no males were anemic (Hb < 120 g/l). This figure was the same as that found using mixed distribution analysis of hemoglobin. This identified the late stage of iron deficiency anemia. With STS as an indicator of iron status, 10 (21%) males and 34 (68%) females were classified as iron deficient (second stage). Only 2(≈4%) males and 24 (48%) females had depleted iron stores (SF < 12 μg/l) (first stage of iron deficiency). These results confirm that iron deficiency cannot be correctly identified using a single biochemical test such as hemoglobin, and that a minimum of two independent

biochemical measures should be used. With the Tri-index model (two or three abnormal values), 19 (38%) of the females were iron deficient. Based on abnormal values of TIBC (> 400 μg/dl, or 72 μmol/l) as an indication of iron deficiency anemia, 9 (19%) males and 27 (54%) females were iron deficient. In conclusion, 12% of the females were anemic and at least 38% were iron deficient and had depleted iron stores. In the males, 20% were at the second stage of iron deficiency. The least number of iron deficient subjects was identified using hemoglobin concentration alone. This observation provides further support that the use of hemoglobin alone underestimates the true prevalence of iron deficiency in population surveys and identifies only the late stage of deficiency. The high prevalence of iron deficiency noted particularly among females can be attributed to inadequate intakes of readily available dietary iron from meat, poultry and fish. In fact, in most rural areas, males consume more flesh foods outside homes than do females. Traditionally, in rural areas, the males do the shopping in the "souks" and consume different meals than are available at home. In addition, the males are usually invited in the villages for celebrations such as weddings and births, and consume more meat products than females. These differences between males and females are not reflected in the seven day-weighed intake method.

The lack of a strong correlation between dietary intake and biochemical indices of iron is not surprising. The biochemical indices may vary considerably with non-dietary factors. For instance, the limitations of the study include one failure to consider the possible presence of parasites, menstruation in females, and the consideration of other age groups. This study constitutes the first available information regarding iron status of an other wise healthy population of adults in Morocco. It is strongly recommended that other studies considering different age groups should be conducted in different regions of Morocco to better understand the etiology of iron deficiency anemia, prior to considering any fortification or supplementation trials towards the eradication of iron deficiency. It is also recommended that future studies apply a model that combines at least two independent biochemical measures of iron status, especially in groups of high risk such as pregnant and lactating women and growing children. An information campaign should proceed at the national level which encourages the consumption of foods (oranges, potatoes) containing enhancers of iron absorption at the same time as cereals are consumed.

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APPENDICES

Appendix 1

Questionnaire

Date:
Subject number:
Picture taken: Yes No
Investigator:

Background data

1. Village:
2. Name:
3. Sex: M F
4. Age (birth date):
5. Number of children: their ages:
6. How many times have you been pregnant (females):
7. How many live babies have you delivered:
8. Education:
9. Monthly income:
10. Occupation:
11. Do you smoke ? Yes No
    Number of cigarettes per day:
12. Does your family own their fields, what do you grow in the fields.
13. Destination of grown products
    - For family consumption
    - For sale
    - For exchange
    - Other

14. Does your family own any animals

    What kind of animals, how many of each

15. How long have you lived in this village:

16. How many people live in the household

17. Have you been sick or ill recently? Yes No

    If so - What was the illness?

    - For how long?

    - Did you receive any medication?

Anthropometric data

Weight (Kg)

Height (M)

Mid-arm circumference (cm)

Triceps skin fold

Teeth - Missing
- Caries

Blood pressure (mm Hg)
Systolic
Diastolic

Comments:

Blood drawing

1. Body temperature °C
2. Blood sample, volume ml

   Comments

3. Hematocrit %
4. Hemoglobin g/L
5. Comments about plasma or serum.

The household description

Names	Age	Sex	Weight	Height	Number of U.C*	Comments
Head						
Spouse						
Children						
-						
-						
-						
-						
Grand-parents						
-						
-						
-						
Relatives						
Others						

* U.C = Unit of consumption
  - for males ≥ 14 years, UC= 1.0
  - for females ≥ 10 years, UC= 0.8
  - for children UC= 0.3 + 0.05 × age (years).

Appendix 2

Approval Letter, Ministry of Health, Morocco

ROYAUME DU MAROC

MINISTERE DE LA SANTE PUBLIQUE

المملكة المغربية

وزارة الصحة العمومية

N° 229 DT/208

3 FEV 1989

LE MINISTRE DE LA SANTE PUBLIQUE

02 FEV 1989

Ministère de la Santé Publique
Bureau d'Ordre
Arrivée le 15 FEV 1989
Enregistré Sous n° 11443

04386

//-)

MONSIEUR LE DIRECTEUR DE L'INSTITUT AGRONOMIQUE ET VETERINAIRE HASSAN II

- Rabat -

+ Copie

OBJET : Demande d'autorisation pour mener une recherche scientifique.

REFER : Votre lettre n° 5388 du 7.12.88

Monsieur le Directeur,

Mes services ont étudié la demande d'autorisation de recherche que vous avez bien voulu m'adresser pour Mr. ALAOUI Larbi.

Le Ministère de la Santé Publique qui développe des programmes de lutte contre les maladies de carence ne peut qu'être intéressé par des recherches opérationnelles sur l'anémie ferriprives et peut apporter son aide sur le terrain pour la réalisation de cette enquête.

Cependant il est nécessaire que Mr. ALAOUI Larbi discute auparavant avec la Direction des Affaires Techniques la méthodologie, l'échantillon et les lieux d'enquête. Celle-ci avisera ensuite les responsables provinciaux et précisera leur participation.

Veuillez agréer, Monsieur le Directeur, l'assurance de mes sentiments distingués./.

Pour le Ministre
Le Secrétaire Général
du Ministère de la Santé

Appendix 3

Mean Daily Intakes of Foods of the Different Families (g/p/d)

Village	Family N°	Cereals	Dry legumes	vegetables	Fruits	Meat poultry fish and eggs	Milk and Milk products	Fat and oils	Sugar
Gharouzia	1	563.14	47.71	105.71	0.00	71.20	156.14	54.71	48.43
	2	380.86	37.14	141.86	0.00	116.00	103.43	56.00	30.43
	3	507.86	160.00	49.43	0.00	87.14	137.28	69.34	49.86
	4	358.16	72.45	88.16	0.00	21.63	73.37	35.61	33.16
	5	324.37	5.36	62.48	14.28	73.75	101.34	38.84	25.18
	6	478.57	64.28	222.71	114.28	90.00	93.14	37.71	41.43
	7	383.33	27.78	240.32	138.89	256.03	225.40	46.51	29.68
	8	457.14	101.02	182.55	20.41	88.16	68.37	67.35	32.14
	mean	431.7	64.5	136.7	36.0	100.5	119.8	50.8	36.3
	± SD	±82.7	± 48.4	±72.4	±56.9	±68.3	±52.0	±13.2	± 9.2
Dar Akouba	1	407.86	17.86	191.70	34.71	40.18	181.11	36.34	24.46
	2	340.36	83.30	177.34	10.71	85.55	199.48	49.84	26.69
	3	320.00	59.71	189.14	22.86	73.43	201.71	54.43	52.11
	4	280.95	114.28	131.43	0.00	52.62	80.00	39.05	40.48
	5	381.43	32.86	291.71	17.14	91.00	118.57	42.91	45.17
	6	312.32	53.03	204.82	35.71	28.03	102.86	35.89	41.23
	7	407.14	115.71	137.59	0.00	43.26	108.98	42.86	18.67
	8	362.50	62.50	128.66	21.43	60.53	0.00	39.03	48.12
	9	476.78	10.71	248.43	0.00	53.57	56.25	20.00	59.78
	mean	365.5	61.1	189.0	15.9	58.7	116.5	40.1	39.7
	± SD	±60.1	± 38.1	±54.8	±14.2	±21.1	±68.1	±9.7	±13.7
Lachaich	1	307.04	86.12	263.20	40.82	85.10	147.75	86.53	47.43
	2	490.31	40.41	244.18	0.00	84.69	178.98	83.98	42.33
	3	346.21	35.64	185.73	21.50	126.93	135.71	43.26	33.86
	4	487.44	45.29	124.73	0.00	136.74	84.37	66.39	37.35
	5	475.09	76.19	252.63	29.76	76.90	166.61	47.45	33.39
	mean	421.2	56.7	214.1	18.4	102.1	142.7	65.52	38.9
	± SD	±87.6	± 22.8	±58.3	±18.2	±27.6	±36.6	±20.0	± 6.0
Akarat	1	252.02	88.26	164.61	38.96	78.70	60.30	74.64	38.73
	2	366.42	29.76	178.31	35.71	19.58	102.36	56.70	44.88
	3	426.04	69.79	153.77	21.98	54.39	127.72	33.50	34.15
	4	714.28	212.93	169.14	90.48	69.88	96.78	89.81	70.83
	5	376.95	216.34	299.03	20.89	32.50	186.52	87.66	56.20
	6	425.90	122.07	119.49	0.00	34.81	56.42	50.08	30.64
	mean	427.0	123.2	180.7	34.67	48.3	105.0	67.0	45.90
	± SD	±154.5	±76.9	±61.4	±30.62	±23.2	±48.2	±22.0	±15.2

Appendix 4

Total Iron Intake, Heme Iron and Non-Heme Iron for the Families Studied

Village	Family N°	Fe intake (mg/p/d)	Heme Fe (mg/p/d)	Non-heme Fe (mg/p/d)
Gharouzim	1	19.19	0.32	18.87
n= 8	2	13.63	0.49	13.14
families	3	22.84	0.50	22.34
	4	13.86	0.104	13.756
	5	10.16	0.53	9.63
	6	19.04	0.80	18.24
	7	17.13	1.80	15.33
	8	17.3	0.47	16.66
	mean ± SD	16.6 ± 4.0	0.6 ± 0.5	16.0 ± 4.0
Dar Akouba	1	12.29	0.17	12.12
n= 9	2	16.50	0.17	15.77
	3	14.80	0.36	14.43
	4	19.39	0.48	18.91
	5	14.09	0.73	13.36
	6	12.27	0.16	12.11
	7	17.08	0.26	16.82
	8	16.97	0.52	16.45
	9	14.38	0.37	14.02
	mean ± SD	15.31 ± 2.4	0.4 ± 0.2	14.9 ± 2.3
Lachaich	1	19.03	0.81	18.22
n= 5	2	17.54	0.34	17.21
	3	14.30	0.68	13.62
	4	15.80	0.64	15.15
	5	18.17	0.48	17.69
	mean ± SD	17.0 ± 2.0	0.6 ± 0.2	16.4 ± 2.0
Akarat	1	9.01	0.31	8.70
n= 6	2	12.34	0.057	12.28
	3	12.78	0.24	12.54
	4	29.90	0.28	29.62
	5	25.02	0.19	24.83
	6	17.56	0.14	17.42
	mean ± SD	17.7 ± 8.1	0.2 ± 0.1	17.4 ± 8.1

Appendix 5

Dietary Iron Sources, Iron Supply and Percent of Total Supply

Village	Family N°	Total dietary Fe intake	Cereals Fe		Meat, Poultry and Fish Fe		Other sources of Fe	
			Q*	%	Q	%	Q	%
Gharouzim	1	19.19	12.07	62.89	0.79	4.12	6.33	33.00
	2	13.63	8.43	61.84	1.22	8.95	3.98	29.20
	3	22.84	11.17	48.90	1.25	5.47	10.42	45.62
	4	13.86	7.88	56.85	0.26	1.87	5.73	41.34
	5	10.16	7.74	76.18	1.33	13.09	1.09	10.72
	6	19.04	9.15	48.05	2.00	10.50	7.89	41.43
	7	17.13	8.36	48.80	4.50	26.26	4.27	24.92
	8	17.13	9.70	56.62	1.18	6.88	6.25	36.48
	mean	16.62	9.31	57.52	1.57	9.64	5.74	32.84
	± SD	± 3.96	±1.58	±9.54	±1.28	±7.61	±2.78	±11.26
Dar Akouba	1	12.29	8.97	72.98	0.43	3.49	2.89	23.51
	2	16.50	7.49	45.39	1.83	11.09	7.19	43.57
	3	14.80	7.04	47.56	0.91	6.14	6.85	46.28
	4	19.39	6.08	31.35	1.20	6.18	12.12	62.50
	5	14.09	8.39	59.54	1.82	12.91	3.86	27.39
	6	12.27	6.87	55.94	0.41	3.34	4.94	40.66
	7	17.08	8.95	52.40	0.65	3.80	7.47	43.73
	8	16.97	9.11	53.68	1.31	7.72	6.55	38.59
	9	14.38	10.49	72.94	0.92	6.39	2.55	19.82
	mean	15.31	8.15	54.65	1.05	6.78	6.08	38.45
	± SD	± 2.37	±1.35	±13.14	±0.53	±3.34	±2.20	±13.19
Lachaich	1	19.03	8.56	44.98	2.02	10.61	8.43	44.29
	2	17.54	10.78	61.45	0.86	4.88	5.91	33.69
	3	14.30	7.61	53.21	1.70	11.88	4.98	34.82
	4	15.80	10.06	63.67	1.61	10.18	4.12	26.07
	5	18.17	10.44	57.45	1.21	6.65	6.52	35.88
	mean	16.97	9.49	56.15	1.48	8.84	5.99	34.95
	± SD	± 1.90	±1.35	±7.41	±0.45	±2.94	±1.64	± 6.49
Akarat	1	9.01	5.01	55.60	0.77	8.54	3.22	35.73
	2	12.34	8.05	65.23	0.14	1.13	4.14	33.54
	3	12.78	9.36	73.23	0.59	4.61	2.82	22.06
	4	29.90	15.71	52.54	0.71	2.37	13.47	45.05
	5	25.02	8.31	33.21	0.48	1.91	16.23	64.86
	6	17.56	9.21	52.44	0.35	1.99	7.99	45.50
	mean	17.77	9.27	55.37	0.51	3.42	7.98	41.12
	± SD	± 8.13	±3.52	±13.59	±0.23	±2.77	±5.70	±14.48

Q*: Represents the quantity of iron from foods (mg/p/d).

Appendix 6

Mean Daily Intakes of Meat, Poultry and Fish (MPF), Ascorbic Acid, Iron, and Available Iron

Village	Family N°	MPF (g/p/d)	Ascorbic acid (mg/p/d)	Iron (mg/p/d)	Available Fe (mg/p/d)	Availability (%)
Gharouzim	1	51.71	24.50	19.19	1.12	5.83
	2	109.14	32.20	13.63	0.85	6.25
	3	67.71	16.67	22.84	1.32	5.78
	4	21.63	17.55	13.86	0.79	5.69
	5	70.89	18.12	10.16	0.73	7.18
	6	85.71	85.18	19.14	1.38	7.24
	7	246.82	86.57	17.13	1.45	8.46
	8	81.63	47.67	17.13	1.22	7.12
	mean	92.0	41.1	16.63	1.11	6.69
	± SD	±67.6	± 29.5	± 3.97	± 0.28	± 0.97
Dar Akouba	1	35.71	51.01	12.29	0.61	4.96
	2	77.49	40.82	16.50	2.42	4.66
	3	50.00	42.18	14.80	0.87	5.86
	4	52.38	35.21	19.39	0.87	4.48
	5	71.43	82.65	14.09	1.04	7.38
	6	17.85	64.04	12.27	0.76	6.19
	7	40.82	22.74	17.08	0.93	5.44
	8	50.71	31.06	16.97	1.025	6.04
	9	53.57	42.51	14.38	0.986	6.85
	mean	49.99	45.80	15.31	1.06	5.76
	± SD	±17.87	± 18.12	± 2.37	± 0.53	± 0.98
Lachaich	1	81.63	62.16	19.03	1.24	6.51
	2	71.44	42.87	17.54	1.22	6.95
	3	67.92	32.03	14.30	1.04	7.27
	4	121.52	18.96	15.80	1.04	6.58
	5	53.57	52.81	18.17	1.07	5.88
	mean	79.22	41.77	16.97	1.12	6.64
	± SD	±25.70	± 16.18	± 1.20	± 0.10	± 0.52
Akarat	1	77.40	48.05	9.01	0.67	7.43
	2	17.20	43.20	12.34	0.73	5.91
	3	49.45	46.47	12.78	0.81	6.33
	4	59.52	72.96	29.80	1.89	6.32
	5	30.79	70.62	25.02	1.41	5.63
	6	29.76	22.99	17.56	1.15	6.54
	mean	44.0	50.71	17.75	1.11	6.36
	± SD	± 22.3	± 18.66	± 8.10	± 0.47	± 0.62

Appendix 7

Correlation Coefficients Between Iron Indices in the Groups of Males and Females

Fe indices		Hct	MCHC	SFe	IBC	STS	SF
Hb	(1)*	0.8282	0.5039	NS	NS	NS	NS
	(2)	0.9423	0.4336	NS	NS	NS	NS
	(3)	0.8532	0.6496	NS	NS	NS	NS
	(4)	0.6488	0.8520	NS	NS	NS	0.5683
Hct			NS	NS	NS	NS	NS
			NS	NS	NS	NS	NS
			NS	NS	NS	NS	NS
			NS	NS	NS	NS	NS
MCHC				NS	NS	NS	NS
				NS	NS	NS	NS
				NS	NS	NS	NS
				NS	NS	NS	0.6395
SFe					NS	0.8866	NS
					NS	0.8933	NS
					NS	0.9633	NS
					NS	0.9375	NS
IBC						NS	NS
						NS	NS
						NS	NS
						-0.4626	-0.6188
STS							NS
							NS
							NS
							NS

* (1),(2),(3) and (4) represent, respectively, the groups of young males, older males, young females, and older females.

Hb: Hemoglobin

Hct: Hematocrit

MCHC: Mean corpuscular hemoglobin concentration

SFe: Serum iron

IBC: Iron binding capacity

STS: Serum transferrin saturation

SF: Serum ferritin

Appendix 8

Anthropometric and Biochemical Data for Individual Subjects in the Four Groups of Males and Females

Young males

A = Akarat; L = Lachaich; G = Gharouzim; D = Dar Akouba

Subject number	Subject code	Family and village	Sex	Age year	Weight (Kg)	Height (m)	W/Ht² Kg/m²	Blood Pressure D	Blood Pressure S	Hb (g/L)	Hct (%)	MCHC (%)	SFe (µg/dl)	TIBC (µg/dl)	STS (%)	SF (µg/L)
1	2	A5	M	22	68	1.74	22.46	60	120	160	44.5	35.8	111.0	383.0	29.0	19.6
2	6	A3	M	25	70	1.61	27.03	60	120	168	48.1	35.0	114.2	422.0	27.1	48.1
3	9	A1	M	22	55	1.69	19.257	60	120	174	47.8	36.5	105.4	397.4	26.5	36.2
4	13	A4	M	27	63	1.62	24.00	60	110	167	47.8	35.0	76.0	334.1	22.9	33.2
5	17	A2	M	22	67	1.79	20.91	60	120	173	49.0	35.3	106.0	336.6	31.5	50.1
6	20	A6	M	30	60	1.66	21.77	60	110	162	48.1	33.7	109.4	290.2	37.7	39.0
7	26	L2	M	29	63	1.755	20.58	80	120	149	42.8	34.8	52.6	395.1	13.3	18.1
8	27	L2	M	26	70	1.79	21.87	80	120	154	46.0	33.4	72.0	336.6	21.4	23.6
9	45	L5	M	22	68	1.74	22.46	60	110	182	50.5	36.0	110.2	325.0	33.9	51.2
10	48	D3	M	27	50	1.625	19.05	60	130	171	49.0	34.9	127.6	336.4	34.8	66.7
11	50	D3	M	30	65	1.64	24.16	60	120	172	50.5	34.1	86.5	387.7	22.3	29.8
12	53	D3	M	25	55	1.61	21.23	70	120	175	50.0	34.9	129.4	372.7	34.7	27.2
13	54	D4	M	25	48	1.60	18.75	70	120	174	50.8	34.1	172.2	412.4	41.8	9.7
14	56	D2	M	25	73	1.67	26.17	70	125	170	50.2	33.9	76.2	360.1	21.2	30.0
15	70	D2	M	21	69	1.75	22.53	50	110	161	48.5	33.0	110.7	325.5	34.0	70.5
16	72	G4	M	17	52	1.67	18.64	50	105	165	47.5	34.7	124.0	387.6	32.0	27.4
17	75	G2	M	25	64	1.64	23.80	60	110	177	49.0	36.1	107.4	475.6	22.6	26.2
18	81	G7	M	19	69	1.84	20.38	60	110	167	49.5	33.7	113.7	397.5	28.6	41.8
19	85	G8	M	17	69	1.74	22.79	60	110	158	47.0	33.6	75.8	351.2	21.6	127.0
20	90	-	M	29	65	1.69	22.75	70	130	159	47.8	33.3	102.4	465.9	22.0	18.8
21	91	-	M	20	64	1.67	22.948	50	110	160	45.2	35.4	112.1	348.7	32.1	33.5

Older males

A = Akarat; L = Lachaich; G = Gharouzim; D = Dar Akouba

Subject number	Subject code	Family and village	Sex	Age year	Weight (Kg)	Height (m)	W/Ht² Kg/m²	Blood Pressure D	Blood Pressure S	Hb (g/L)	Hct (%)	MCHC (%)	SFe (µg/dl)	TIBC (µg/dl)	STS (%)	SF (µg/L)
1	1	A1	M	49	60	1.63	22.58	70	110	152	42.0	36.2	88.5	336.6	26.3	18.2
2	5	A3	M	49	57	1.56	23.42	60	120	168	46.0	36.5	107.0	363.4	29.4	27.8
3	8	A1	M	43	52	1.71	17.78	70	110	172	49.0	35.1	57.1	392.7	14.5	45.5
4	12	A4	M	45	52	1.60	20.31	70	120	162	46.2	35.1	50.7	329.3	15.4	48.4
5	16	A2	M	40	60	1.71	20.52	70	110	170	49.8	34.0	70.0	334.1	20.9	63.1
6	19	A6	M	45	51	1.59	20.17	80	120	150	44.0	34.1	97.8	285.4	24.3	46.9
7	30	L3	M	60	55	1.78	17.36	60	110	157	45.5	34.5	54.2	340.2	15.9	24.6
8	37	L4	M	38	65	1.65	23.87	70	120	168	46.8	35.8	37.3	351.8	10.6	37.8
9	38	L4	M	34	54	1.655	19.36	80	120	152	44.8	34.0	23.2	293.8	7.9	75.7
10	42	L5	M	64	67	1.71	22.91	90	140	151	44.0	34.3	46.4	366.1	12.7	127.7
11	44	L5	M	33	63	1.69	22.06	70	120	163	47.8	34.1	80.4	421.8	19.0	9.5
12	59	D1	M	34	59	1.69	20.65	70	130	174	49.2	35.4	92.9	357.8	25.9	46.1
13	60	D1	M	40	50	1.58	20.03	60	120	172	48.0	35.8	92.1	415.0	22.2	80.9
14	62	D7	M	50	66	1.73	22.05	70	110	168	49.2	34.2	69.0	357.8	12.3	42.9
15	65	D8	M	50	67	1.72	22.64	70	120	153	45.0	34.0	66.9	283.3	23.6	52.5
16	68	D9	M	50	64	1.57	25.96	60	130	153	44.5	34.4	58.7	337.9	17.4	37.6
17	71	G4	M	42	69	1.69	24.16	60	120	192	56.5	33.9	80.2	310.5	25.8	92.5
18	77	G1	M	35	73	1.71	24.96	60	120	172	49.8	34.7	81.8	351.2	23.3	30.2
19	82	G7	M	45	68	1.70	23.53	80	150	175	51.2	34.2	81.6	397.5	20.5	87.7
20	86	-	M	54	69	1.565	28.35	70	130	180	49.8	36.1	132.3	412.2	32.1	60.2
21	87	-	M	45	74	1.75	24.16	50	110	178	50.0	35.6	70.2	385.4	18.2	86.2
22	89	-	M	38	60	1.69	21.00	60	120	163	49.2	33.1	55.6	390.2	14.2	15.9
23	92	-	M	37	57	1.60	22.26	50	120	166	48.2	34.4	80.6	424.4	19.0	17.3
24	93	-	M	36	54	1.64	20.07	60	120	176	50.2	35.0	78.2	336.6	23.2	78.3
25	94	-	M	38	63	1.575	25.56	60	120	167	48.8	34.2	62.9	424.4	14.8	17.8
26	95	-	M	41	54	1.69	18.90	50	100	155	46.8	33.1	60.5	317.1	19.1	31.5

Young females

A = Akarat; L = Lachaich; G = Gharouzim; D = Dar Akouba

Subject number	Subject code	Family and village	Sex	Age year	Weight (Kg)	Height (m)	W/Ht² Kg/m²	Blood Pressure D	Blood Pressure S	Hb (g/L)	Hct (%)	MCHC (%)	SFe (µg/dl)	TIBC (µg/dl)	STS (%)	SF (µg/L)
1	4	A5	F	20	55	1.60	21.48	50	110	150	42.2	35.5	53.9	431.7	12.5	12.6
2	7	A3	F	16	44	1.50	19.55	40	85	137	48.0	28.4	56.3	404.9	13.9	15.2
3	11	A1	F	20	52	1.625	19.81	60	110	115	34.5	33.2	29.8	456.1	6.5	8.6
4	15	A4	F	18	53	1.57	21.50	80	120	146	42.8	24.0	48.2	356.1	13.5	26.5
5	22	A6	F	25	46	1.54	19.39	80	130	144	35.2	40.8	76.8	343.9	22.3	20.9
6	24	L1	F	21	48	1.58	19.22	80	125	155	45.0	34.5	45.3	382.9	11.8	11.5
7	25	L1	F	20	58	1.59	22.94	80	125	166	46.8	35.4	79.2	465.9	17.0	13.5
8	29	L2	F	20	62	1.60	24.22	80	120	159	44.8	35.5	38.0	461.0	8.2	9.8
9	31	L2	F	18	53	1.61	20.44	80	130	144	42.2	34.1	25.9	485.6	5.3	5.6
10	34	L3	F	22	65	1.71	22.23	70	110	151	43.8	34.5	82.0	320.4	25.6	22.2
11	35	L3	F	20	52	1.65	19.10	80	120	145	43.0	33.7	33.1	342.4	9.7	22.7
12	36	L3	F	17	53	1.75	17.30	40	100	144	42.5	33.9	78.7	390.3	20.2	10.7
13	41	L4	F	30	45	1.55	18.73	60	120	121	35.5	24.2	31.5	354.4	8.9	14.8
14	46	L5	F	28	58	1.63	21.83	60	120	147	45.0	32.6	98.6	498.6	19.8	9.8
15	47	L5	F	29	56	1.54	23.61	50	100	147	44.0	33.3	47.2	474.7	9.9	5.4
16	49	D3	F	20	60	1.67	21.51	70	110	104	34.0	30.5	26.2	228.5	11.5	3.2
17	51	D4	F	22	55	1.57	22.31	60	120	91	32.8	27.7	37.3	434.8	8.6	2.8
18	52	D5	F	23	55	1.57	22.31	70	120	146	41.5	35.3	98.4	429.9	22.9	5.1
19	55	D3	F	29	58	1.67	20.79	60	110	129	40.2	32.0	46.0	437.2	10.5	2.9
20	57	D2	F	22	55	1.55	22.89	50	130	137	39.2	34.8	91.3	454.8	20.1	5.1
21	61	D6	F	24	55	1.58	22.03	70	140	144	41.8	34.3	56.4	434.9	12.9	4.0
22	64	D7	F	20	57	1.61	21.98	60	120	153	45.0	34.1	101.6	355.4	28.6	12.2
23	67	D8	F	24	60	1.52	25.96	60	105	150	44.8	33.5	123.1	407.6	30.2	14.0
24	69	D9	F	20	55	1.55	22.89	50	105	140	41.0	34.0	87.6	352.7	24.8	67.3
25	76	G2	F	26	49	1.57	19.88	60	140	160	46.8	34.6	123.9	370.7	33.4	25.1
26	78	G1	F	25	50	1.55	20.81	60	100	125	40.8	30.6	16.5	485.4	3.4	1.7
27	79	G1	F	20	60	1.67	21.51	70	110	132	40.5	32.6	44.6	409.7	10.9	11.7
28	80	D9	F	25	66	1.62	25.15	20	110	129	38.2	33.8	126.4	424.4	29.8	3.3
29	96	G6	F	23	58	1.65	21.30	60	110	106	35.2	30.1	8.8	509.2	1.7	66.0
30	97	G6	F	16	46	1.575	18.66	60	120	152	43.2	35.2	69.4	439.0	15.8	19.2

Older females

A = Akarat; L = Lachaich; G = Gharouzim; D = Dar Akouba

Subject number	Subject code	Family and village	Sex	Age year	Weight (Kg)	Height (m)	W/Ht² Kg/m²	Blood Pressure D	Blood Pressure S	Hb (g/L)	Hct (%)	MCHC (%)	SFe (µg/dl)	TIBC (µg/dl)	STS (%)	SF (µg/L)
1	3	A5	F	45	63	1.60	24.61	70	110	130	40.0	32.4	29.8	446.3	6.7	7.8
2	10	A1	F	40	59	1.65	21.67	60	100	138	41.8	33.0	64.3	439.0	14.7	15.8
3	14	A4	F	45	52	1.56	21.36	70	120	138	41.6	33.2	46.6	385.4	12.1	31.9
4	18	A2	F	39	59	1.64	21.94	60	100	152	43.8	34.7	46.1	361.0	12.8	22.9
5	21	A6	F	45	49	1.56	20.13	70	120	113	44.2	25.6	9.4	339.0	5.7	5.0
6	23	L1	F	49	71	1.67	25.45	70	110	151	42.8	35.2	46.1	370.7	12.4	67.0
7	28	L2	F	46	63	1.60	24.61	60	120	126	38.5	32.7	21.8	443.9	4.9	4.0
8	32	L3	F	50	50	1.58	20.03	70	130	151	40.2	37.6	31.5	265.9	11.9	87.6
9	33	L3	F	35	50	1.61	19.28	40	90	143	40.8	35.1	46.4	284.4	16.3	14.2
10	39	L4	F	34	55	1.56	22.60	50	100	150	43.8	34.3	34.8	426.2	8.2	16.0
11	40	L4	F	46	45	1.52	18.47	80	120	128	39.2	32.6	77.9	424.0	18.4	8.4
12	43	L5	F	55	55	1.55	22.89	70	120	153	43.0	35.7	50.5	388.0	13.0	34.4
13	58	D2	F	45	60	1.53	25.63	60	120	136	41.5	32.7	45.2	504.4	8.9	4.9
14	63	D7	F	42	46	1.55	19.14	60	140	142	43.8	32.3	80.9	342.5	20.6	10.6
15	66	D8	F	49	62	1.52	26.83	50	120	100	34.0	29.1	16.5	531.6	3.1	3.1
16	73	G4	F	38	50	1.59	19.77	50	100	150	41.8	35.9	50.4	341.5	14.7	46.6
17	74	G3	F	40	67	1.56	27.53	70	120	141	40.8	34.6	21.5	446.3	4.8	6.0
18	83	G7	F	40	60	1.59	23.73	60	100	148	44.2	33.5	68.5	373.7	18.3	16.1
19	84	G8	F	45	66	1.58	26.44	80	130	142	41.2	34.5	48.4	375.6	12.9	27.9
20	88	-	F	49	64	1.61	24.69	50	100	135	41.5	32.5	60.5	387.8	15.6	13.5

Appendix 9

Considerations When Conducting Nutrient Status Assessment in Rural Areas Based on Experience in the Present Study

The present study was conducted in a rural area located in the Occidental Rif in the northern part of Morocco. Necessary approvals were obtained from both the Ministry of Health and the Ministry of the Interior and Information. The objectives of the study were fully explained to local authorities, medical professionals and to the subjects of the investigation.

Dietary surveys were carried out for seven days by well-trained females. This was done in order to facilitate access to kitchens and to establish contact with local housewives. In some rural areas, dialects are spoken and the personnel who completed the survey should be familiar with the spoken dialects. All necessary equipment and supplies should be purchased ahead of time. Chemicals need to be stored properly. Tubes and vials, needed to manipulate blood samples, should be properly labeled. Extra clean tubes and vials should be available.

Fasting blood samples should be obtained using Vacutainer tubes instead of syringes. Sufficient amounts of blood sample should be obtained from each subject to assure sufficient serum to properly complete the nutrient status, depending upon the methods chosen. The selected methods need to be tested in the laboratory prior to the study.

Some subjects may agree to provide blood samples, but refuse at the time the blood is to be drawn. Therefore, it is recommended that a greater number of subjects be registered than are required to be able to replace those who withdraw at the last moment. In this case, the subjects, or families, were asked not change their dietary habits with respect to quantity or quality.

If blood samples are to be transported or stored for subsequent analysis, it is recommended that appropriate storage be used. For the present study, serum samples were transported in glass vials, put on ice for subsequent analysis of serum iron and iron-binding capacity, or plasma put in cryovials and placed over liquid nitrogen for storage subsequent to analysis of serum ferritin. All the samples should be properly labeled.

It is recommended that there be sufficient trained personnel to properly handle blood samples and to carry out

the analysis of the biochemical indices selected. The volumes of blood or sera should be accurately pipeted. All assays should be carried out in duplicate or in triplicate as cost and time allow. When the budget is limited, as in the case of the present study, and in most cases, it is necessary to assure that all equipment needed is available or can be used elsewhere. For example, in the present study, a liquid scintillation counter (Gamma counter) was required for the (RIA) determination of serum ferritin. This equipment was not available in our department, but was available in another department or in another close-by institution. Also, the liquid nitrogen was not available in our department. Thus, prior to blood drawing, the researcher was required to check that all the equipment and supplies needed were present on time.

In many developing countries, all of the chemicals may not be available in the country, but can be purchased in another country. It is recommended that the chemicals needed should be ordered well in advance of the study and that sufficient quantities are available. The chemicals should be stored properly.