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Iron status and anemia in a cohort of youths in Northern Norway: The Fit Futures Study

Master thesis (MED-3950)

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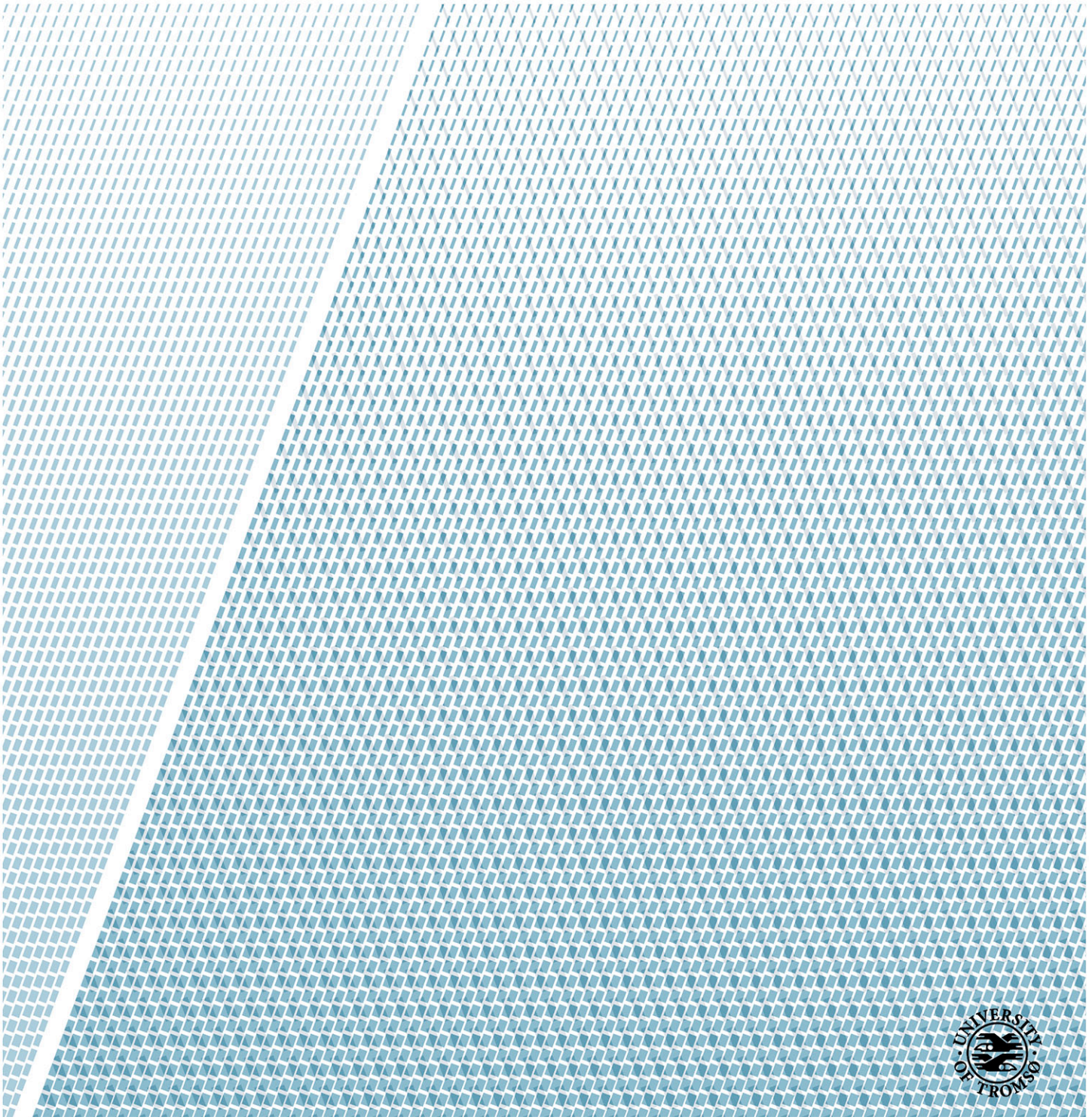

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1 ABBREVIATIONS

BMI	Body mass index
CDC	U.S. Centers for Disease Control and Prevention
CRP	C-reactive protein
DALY	Disability-adjusted life year
Fe	Iron
FF1	Fit Futures 1
FF2	Fit Futures 2
Hb	Hemoglobin
ID	Iron deficiency
IDA	Iron deficiency anemia
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
Mdn	Median
NAID	Non-anemia/non-anemic iron deficiency
NHANES	National Health And Nutrition Examination Survey
NIDA	Non-iron-deficiency anemia
RBC	Red blood cell
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TfR	Transferrin receptor
UNICEF	United Nations Children's Fund
UNN	University hospital of North Norway
UNU	United Nations University
WHO	World Health Organization

2 ABSTRACT

Background: Adolescents are at risk of developing iron deficiency (ID) and iron deficiency anemia (IDA) due to rapid growth and sexual maturation.

Objectives: Our objectives were to; (1) examine gender-specific distribution of hemoglobin and ferritin, and whether certain lifestyle factors influence on these; (2) describe the prevalence of iron deficiency, iron deficiency anemia and anemia, and; (3) investigate the applicability of the WHO-criteria for anemia in adolescents in our population.

Methods: Data is gathered from the Fit Futures survey. The survey was conducted in 2010-2011, inviting 1117 students in first year of upper secondary school, and repeated in 2012-2013 inviting all who participated in the former survey. Participants between ages 15-17 in Fit Futures 1 (FF1), with valid Hemoglobin (Hb) values, ferritin<500 and CRP<10 were included; 813 participants in FF1, 561 participants in Fit Futures 2 (FF2). 505 participants met inclusion criteria in both surveys. Participants were considered anemic if Hb<13 for males and Hb<12 for females (WHO guidelines), and iron deficient if ferritin<12. The prevalence of iron deficiency and anemia was compared across surveys.

Results: The prevalence of ID and IDA decreased from 9.5% and 4.9% in FF1 to 6.8% and 3.6% in FF2, respectively. Using the 2.5 percentile for Hb as cut-off, the prevalence of anemia was reduced by two thirds in females (from 20.3% to 6.2% in FF1 and 16.8% to 5.6% in FF2). The male prevalence was practically unchanged. Lifestyle factors (junk food, snacks, sweets, vitamin/mineral supplements, physical activity, menstrual cycle length and BMI-classification) have non-consistent effects on Hb and ferritin.

Conclusion: ID is common in female adolescents in Northern Norway. The 2.5 percentile cut-off for anemia corresponds well with the WHO-criteria in males, but not in females. Future research should apply the 2.5 percentile for Hb as cut-off value for anemia in adolescent females.

3 BACKGROUND

Iron deficiency is the most common nutritional disorder in the world [1], and the primary cause of anemia worldwide [2]. Just short of a third of the worlds population is suffering from anemia, approximately half of these as a result of iron deficiency [3]. Although iron deficiency can occur in every stage of human life, it is most prevalent among young children (i.e. toddlers) and women of childbearing age. Another group at risk of developing iron deficiency and iron deficiency anemia is adolescents. Due to the rapid growth spurt in the first half of the teenage years, adolescents undergo massive physiological changes. Expanding blood volume, rapid growth and sexual maturation all increase demands for dietary iron in both genders. In females, however, the elevated iron demand is superimposed with iron deficit due to menstrual blood loss. Furthermore there are various other physiological and pathological conditions that may contribute to the depletion of iron stores, such as malnutrition, vegan and vegetarian diets, chronic disease, overweight and strenuous physical activity.

Our aim in this master thesis is to examine gender-specific distribution of hemoglobin and ferritin, and the prevalence of iron deficiency, iron deficiency anemia and anemia in a population based study of adolescents from Northern Norway, and evaluate the applicability of the WHO criteria for anemia in this population. Our data are based on the *Fit Futures-study* from the municipalities of Tromsø and Balsfjord. Heiberg et Prytz [4] gave an account on the matter in their master thesis based on data from the *Fit Futures 1* study (2010-2011). In this paper we will examine data from Fit Futures 1 and the follow-up study *Fit Futures 2* (2012-2013) to monitor the development of iron deficiency and anemia in the population sample. We will also look into lifestyle factors, such as dietary habits and physical activity, and investigate whether or not they influence hemoglobin and ferritin levels in youths. There are to our knowledge very few, if any, population-based studies on iron deficiency in adolescent cohorts with more than one examination of each participant.

4 INTRODUCTION

4.1 The role of iron in the body

Iron is an essential micronutrient for humans, and is important in all the metabolic active cells in the body. It functions as a cofactor in many proteins, including enzymes, and is important for physiological functions [5]. To mention some, iron has a crucial role in binding and transport of oxygen, gene regulation, electron transfer reactions and regulation of cell growth and differentiation [6].

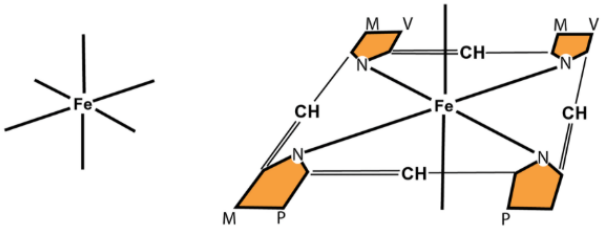
The total amount of iron in the human body depends on height, weight, sex, capacity for storing iron etc., and is approximately 3-4 g in adults. Seventy-five percent is bound in heme proteins such as myoglobin and hemoglobin. Hemoglobin is the oxygen carrying protein in the erythrocytes, and plays a crucial role in transport of oxygen from the lungs to the tissues [5, 7, 8]. The other main source of heme is in muscles, as a part of myoglobin, which stores oxygen in the tissues. Approximately twenty percent of body iron may be found as ferritin or hemosiderin, which constitute body depots in the liver and the reticuloendothelial system. The remaining iron is bound in enzyme systems. When transported in the blood, iron is bound to transferrin as ferric iron [9]. Less than one thousandth of the total body iron is found in plasma bound to transferrin [7].

In 2001, the World Health Organization (hereinafter referred to as WHO) published guidelines concerning iron deficiency anemia [10]. The publication aimed to emphasize the importance of sufficient dietary iron for humans, implying that the functional consequences of iron deficiency may impair cognitive performance and development, behavior, physical growth of children, immune system, physical activity and work performance, gastrointestinal functions, hormone production and metabolism.

4.2 Iron homeostasis

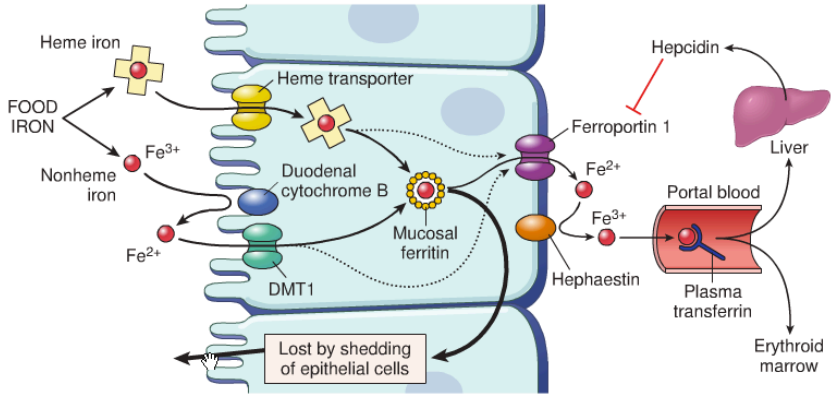
Iron balance is the difference between iron losses and iron absorption in the body. The amount of iron absorbed is the product of iron intake and bioavailability from the diet, supplements and contaminant iron [11].

Figure 1 Non-heme iron (left) and heme iron (right) (Source: Medscape [12])



Dietary iron exists as heme iron and non-heme iron (Figure 1). The absorption mechanism in the intestine, as well as bioavailability, differs between the two. This is demonstrated in Figure 2. Heme, which is a Fe^{2+}-ion encircled by a porphyrin ring, is derived mainly from hemoglobin and myoglobin, which is abundant in meat and fish [13]. About 15-35% of heme can be absorbed from the intestines. Non-heme iron is ingested as iron salts (for example $FeSO_4$) and is found in inorganic foods, vegetables and animal source foods. Only 2-10% non-heme is absorbed from the gastrointestinal tract. Various dietary factors affect absorption, and extensive research is done concerning this topic. To name a few, phytic acid, polyphenols found in coffee and tea [14], calcium and fiber impair absorption, whereas ascorbic acid (vitamin C) [15], meat and fish reinforce absorption [16, 17].

Figure 2 Duodenal epithelial cell uptake of heme and non-heme iron, and regulation of iron absorption (Source: Robbins Basic Pathology [18])



The main physiological loss of iron is due to shedding of intestinal epithelium and menstrual blood loss in females. In addition, a minor amount iron is lost daily through urine and sweat [19]. There is no known mechanisms for compensation of iron loss, other than increased

absorption from the intestine [7]. Obviously, this requires an adequate content of iron in the diet in otherwise healthy people with a well functioning iron metabolism system.

The peptide hepcidin, produced mainly by hepatocytes in the liver, is a ferroportin modulator and is considered to be the most important regulator of iron absorption with respect to total content of iron in the body. Ferroportin is important in transport of iron from the intestinal lumen to plasma. The production of hepcidin increases when iron requirements are met, thereby reducing intestinal absorption of iron, and vice versa decreases when the demands are not met [7].

Concerning adolescents, iron requirements increase in males and females as a result of growth. Namely because of expanding blood volume, increase in lean body mass (muscle and bone), sexual maturation and onset of menses in females [20].

4.3 Definition of anemia and iron deficiency

Iron deficiency is a state in which there is not enough iron in the body to maintain normal physiological functions. When iron balance is negative, the iron stores will slowly be reduced until they are depleted. At this point, if iron balance is not corrected, one will develop iron deficiency. When iron-deficient erythropoiesis occurs the hemoglobin can fall below threshold, causing iron deficiency anemia, and one will typically see smaller red blood cells (RBCs) with reduced hemoglobin concentration (hypochromic, microcytic anemia)[10].

The WHO defines anemia accordingly: *"anaemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status" [21].* The lab test performed to investigate and diagnose anemia is the measure of hemoglobin (Hb). Anemia is considered to be present when Hb concentration is under threshold of the values set by WHO, UNICEF[a] and UNU[b] [10]. Since the normal distribution of hemoglobin varies with age, gender, sex etc. there must be

[a] UNICEF – United Nations Children's Fund
[b] UNU – United Nations University

different cut-off levels for different populations. WHO defines anemia in non-pregnant women above 15 years as Hb <12,0 g/dl, and in men above 15 years as Hb <13,0 g/dl (Table 1).

WHO´s threshold values for anemia is based on the 2.5 percentile for Hb in healthy subjects, and was first presented in 1968. The cut-off values were originally based on four published references and a set of unpublished observations, and population groups such as young non-pregnant women were not represented in any of the populations. Later, the criteria have been validated by findings in the The Second National Health and Nutrition Examination Survey (NHANES II). The overall cut-off criteria for anemia have remained unchanged, except some minor changes regarding age group for children, cut-off value for children 5-11 years of age, persons living at altitudes higher than 1000 meter above sea level and smokers, based on data from the NHANES II and the US Centers for Disease Control and Prevention´s (CDC) Pediatric Nutrition Surveillance System in children living in mountainous states [22, 23].

Table 1 Hemoglobin cut-off values for anemia at sea level (g/dL) [23]

Population	Non-anemia	ANEMIA		
		Mild	Moderate	Severe
Children 6-59 months of age	≥ 11.0	10.0 – 10.9	7.0 – 9.9	< 7.0
Children 5-11 years of age	≥ 11.5	11.0 – 11.4	8.0 – 10.9	< 8.0
Children 12-14 years of age	≥ 12.0	11.0 – 11.9	8.0 – 10.9	< 8.0
Non-pregnant women (≥ 15 years of age)	≥ 12.0	11.0 – 11.9	8.0 – 10.9	< 8.0
Pregnant women	≥ 11.0	10.0 – 10.9	7.0 – 9.9	< 7.0
Men (≥ 15 years of age)	≥ 13.0	11.0 – 12.9	8.0 – 10.9	< 8.0

When assuming a normal distribution of Hb, the reference range is obtained by measuring Hb in a given reference group and placing cut-off values at two standard deviations (2 SD) to either side of the mean (also referred to as the 2.5 percentile). Validity of the reference range concerning Hb requires that people with nutritional deficiencies, chronic inflammatory disease, hemoglobinopathies and other common causes of anemia have been excluded from the reference population.

Several big population studies concerning normal distribution of Hb, such as the second and third National Health and Nutrition Examination Survey (NHANES II and III), have defined the reference population as participants with neither biochemical signs of inflammation nor iron deficiency, and anemia is then defined as present when values are below the 2.5 percentile of the population [24].

In 2005, Skjelbakken et al. studied the gender-specific distribution of hemoglobin in an adult population of Northern Norway (ages 24-104), comparing the WHO´s criteria for anemia with the 2.5 percentile for hemoglobin in their reference population. The study showed that the WHO criteria for anemia gave a higher prevalence of anemia in women, than the 2.5 percentile for hemoglobin. In men there was little difference [24].

4.4 Diagnostics and clinical features of iron deficiency

The diagnosis of iron deficiency is primarily based on laboratory measurements of biochemical iron indicators [25]. Unfortunately, to diagnose non-anemic iron deficiency there is no consensus on a single standard test. As the different biochemical iron indices show different aspects of iron metabolism, viewing several parameters together can be helpful in diagnosing iron deficiency.

Table 2 Iron stores on the basis of serum ferritin concentration (Source: WHO [10])

Status of iron stores	Serum ferritin (µg/L)			
	< 5 years of age		< 5 years of age	
	Male	Female	Male	Female
Depleted iron stores	< 12	< 12	< 15	< 15
Depleted iron stores (in the presence of infection)	< 30	< 30	-	-

Ferritin molecules are the primary intracellular stores of iron in the body. However, they are also acute phase proteins released by hepatocytes in the event of an inflammation. Ferritin correlates with the relative total body iron stores, presuming there is not an ongoing infection

or inflammation. A low value reflects depleted stores and is a precondition before iron deficiency. In a Danish study, ferritin appeared to be the strongest predictor of Hb in blood donors [26]. Different cut-off levels for depleted iron stores are used, and WHO suggests serum ferritin <15 µg/l as standard cut-off level for men and women above 5 years of age [10], presented in Table 2. In the NHANES studies, Looker et al. used serum ferritin <12 µg/l as cut-off level. In a cross-sectional study on the prevalence of iron deficiency in Swedish youths [27], Hallberg et al. suggested using serum ferritin <16 µg/l as cut-off value for iron deficiency.

As ferritin is an acute phase protein, it is important to identify any possible infection by measuring inflammation parameters, for example C-reactive protein (CRP). There is also great interindividual variations in ferritin, which complicates interpretation further [7].

Iron deficiency also results in a decrease in serum iron, elevation in transferrin level, and a reduction in transferrin saturation. There is an overlap in all these parameters between iron-deficient people and non-deficient ones, as well as circadian variability. This lessens the usefulness of these parameters. Other parameters used are serum transferrin receptor and erythrocyte protoporphyrin [10]. Serum transferrin receptor (TfR) will increase in the case of iron deficiency, and will not be affected by inflammation. However, serum TfR is not a part of the blood panel for diagnosing iron deficiency in Norway yet [7]. Erythrocyte protoporphyrin is not measured in this study, and will not be further discussed.

Figure 3 Normal peripheral blood smear with normocytic, normochromic red blood cells (Source: American Association of Hematology [28])

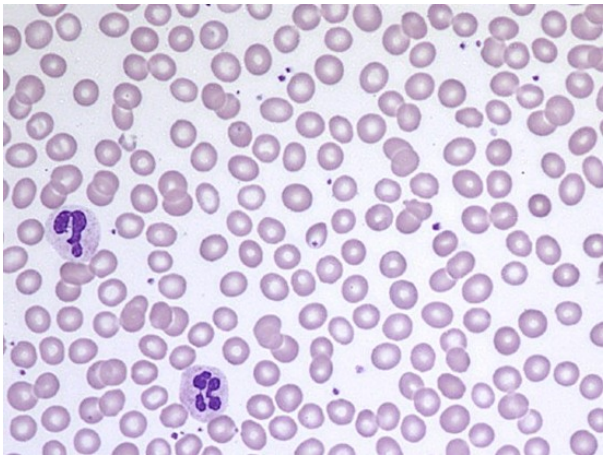
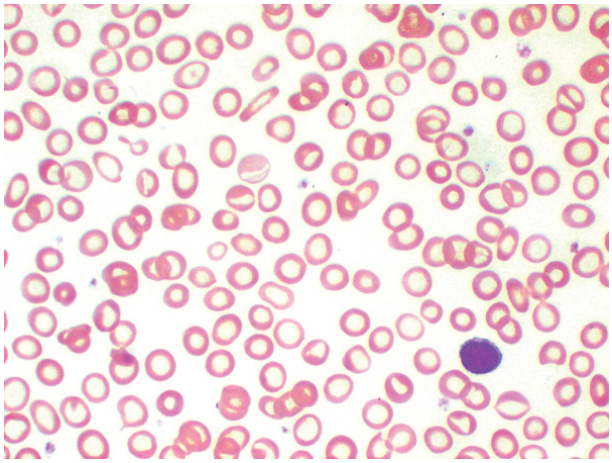


Figure 4 Peripheral blood smear with microcytic, hypochromic red blood cells (Source: Medical Laboratories Portal [29])



Red blood cell indices are measures of volume, mean corpuscular volume (MCV), and content of hemoglobin, mean corpuscular hemoglobin concentration (MCHC). MCV and MCHC are the most sensitive red blood cell indices for diagnosing iron deficiency [10]. Red blood cell indices are used in morphological classification of anemia. Iron deficiency anemia typically causes a decrease of both measures, hence classified as microcytic, hypochromic anemia [7]. Microscopy of a peripheral blood smear will show small and pale erythrocytes, as in Figure 4. In comparison, Figure 3 shows a normal peripheral blood smear with normocytic, normochromic erythrocytes. Mean red cell indices for female and male adolescents are presented in Table 3.

Table 3 Mean red cell indices for youths according to the WHO (cut-off value at -2 standard deviations) [10]

RBC indices	Female			Male		
	12 - 14,9 years	15 - 17,9 years	> 18 years	12 - 14,9 years	15 - 17,9 years	> 18 years
RBC count (-2 SD)	4.47 (3.9)	4.48 (3.9)	4.42 (3.8)	4.71 (4.1)	4.92 (4.2)	4.99 (4.3)
MCV (fl) (-2 SD)	86 (77)	88 (78)	90 (81)	85 (77)	87 (79)	89 (80)
MCH (pg) (-2 SD)	29.4 (26)	30.0 (26)	30.6 (26)	29.1 (26)	29.9 (27)	30.5 (27)
MCHC (g/l) (-2 SD)	34.1 (32)	33.9 (32)	33.9 (32)	34.4 (32)	34.4 (32)	34.5 (32)

According to the WHO, the single best indicator for detecting iron deficiency anemia is serum ferritin, as long as there is no infection present [10]. However, there is no international consensus of use of biochemical parameters to diagnose iron deficiency, as each indicator has its own limitation because of poor sensitivity or specificity, or because it is modified by other conditions than iron deficiency [20], and big studies concerning iron deficiency have used different approaches. Hallberg et al. defined iron deficiency as low serum ferritin. Looker et al. defined iron deficiency as two of three abnormal values of the following laboratory tests: transferrin saturation, serum ferritin and erythrocyte protoporphyrin. In *"Assessing the iron status of populations"* (2004), the WHO suggested an approach based on measurements of serum ferritin and sTfR in combination with CRP. This approach was adopted by Zimmermann

[30], and in the HELENA study, Ferrari et al. considered it the most appropriate method in diagnosing iron deficiency [25].

Clinical symptoms of iron deficiency and anemia depend on timeframe and severity, and symptoms that can be identified in clinical examination are often scarce. Symptoms of iron deficiency are generally only seen in severe, long-lasting cases. Examples of symptoms are brittle nails and hair, spoon shaped nails, atrophy of the tongue, angular stomatitis, dysphagia and glossittis.

When Hb-levels are slowly decreasing over time, hemodynamic compensation and enhancement of the oxygen carrying capacity of the blood can occur, and anemia may not give any symptoms. With a more rapid decrease in Hb, more severe symptoms will occur. Common non-specific symptoms of anemia are fatigue, headaches, faintness, breathlessness, palpitations, and, in the elderly, cardiorespiratory problems may be precipitated. Clinical signs of anemia are pallor, tachycardia, systolic flow murmur and in extreme cases cardiac failure [19].

4.5 Etiology and epidemiology of iron deficiency anemia

Anemia is caused by a variety of conditions (such as parasitic infections, hemoglobinopathies, nutritional deficiencies, cancers, acute and chronic infections) and it affects approximately a quarter of the world's population [31]. Although helminth infections, malaria, tuberculosis and HIV/AIDS are great contributors to the prevalence of anemia in undeveloped countries, iron deficiency is by far considered the leading cause, contributing to 50% of anemia cases worldwide [3]. Iron deficiency is, as described earlier, a result of negative iron balance (i.e. when the amount of ingested and absorbed iron does not meet the physiological requirements of the individual). This typically occurs in phases of rapid growth. In infancy, when the abundant supplies of transplacentally transmitted iron to the fetus are depleted at around four to six months after birth, the risk of iron deficiency increases. Breast milk only supplies about 30% of the daily requirements of infants and it is generally recommended to introduce solid, iron-fortified foods by the age of six months to avoid development of iron deficiency [20, 32].

Toddlers are vulnerable up to about 18-24 months of age when the pace of growth decreases and diet is richer. Pregnant women are also at risk of developing iron deficiency, due to the high iron demands of the fetus. Other vulnerable groups are adolescents, due to rapid growth, and women of childbearing age due to the excessive iron loss with menses. The main cause of iron deficiency, which is not due to nutritional inadequacy or menstrual blood loss, is loss of hemoglobin because of bleeding, usually occult through gastrointestinal ulcers, malignancies or parasitic infections [33, 34]. H. pylori infection has also been associated with iron deficiency [35, 36]. Other causes include hemoglobin loss through urine (hemoglobinuria, for example after intravascular hemolytic anemia), hemosiderinuria and pulmonary hemosiderosis, as well as malabsorption (for example due to poorly treated celiac disease or surgical removal of parts of the gastrointestinal tract).

According to the Third National Health and Nutrition Examination Survey (NHANES III), 9% of toddlers, 9% of adolescent girls and 11% of women of childbearing age were iron deficient, among whom 3%, 2% and 5% had iron deficiency anemia, respectively [37]. Hallberg et al. [27] studied a population of Swedish adolescents (15-16-year-olds, n = 427) and found the prevalence of iron deficiency to be 15% in boys and 40% in girls, whereas Eskeland and Hunskaar [38] in a pilot study on Norwegian adolescents (14-15-year-olds, n = 176) found the prevalence to be 30% and 25% (among whom 8% and 4% had iron deficiency anemia), respectively. These studies support the understanding that iron deficiency is widely prevalent in certain population groups, even in developed countries.

4.6 Iron deficiency and anemia in a global perspective

Iron deficiency is the most common and widespread nutritional disorder in the world [1] and has substantial effect on public health. In fact, iron deficiency was rated number 9 of 26 risk factors in terms of global disease burden [39]. 841 thousand deaths per year and approximately 35 million disability-adjusted life years (DALYs) are attributable to iron deficiency anemia [3], the majority of which are suffered in South-East Asia and Africa where dietary iron bioavailability in many places is low due to monotonous plant-based diets [40]. Pregnancy

anemia is overall the greatest contributor to the disease burden by increasing the risk of maternal mortality and perinatal mortality. WHO-guidelines currently recommend daily iron and folic acid supplementation to all pregnant women throughout pregnancy [41], and intermittent iron supplementation of children (pre-school age and school age) in areas where anemia is highly prevalent [42]. Studies have shown that interventions can have great impact; by increasing population mean hemoglobin by 1 g/dL the risk of maternal mortality is reduced by 25%, and perinatal mortality and overall risk of child mortality is reduced by 28% and 24%, respectively [43, 44]. However, pregnancy related complications of anemia are far greater threats to health in undeveloped countries contra developed countries where the greater disease burden lies in direct sequelae of iron deficiency (i.e. reduced cognitive and physical development in children, and physical endurance and work productivity in adults) [3].

5 MATERIAL AND METHODS

Study population

Data material was gathered in the *Fit Futures* youth survey. The survey is a supplement to the *Tromsø Study* that has been conducted in the municipality of Tromsø, Northern Norway, a total of six times since 1974, the seventh study being conducted in 2015. The Tromsø Study, a population-based prospective study including only adults age 20 and above, has been widely successful. Yet the youth population of Tromsø was not included until the Fit Futures Study was conducted for the first time in 2010-2011 (*Fit Futures 1*). 1117 students in the first year of upper secondary school in the neighboring municipalities Tromsø and Balsfjord were invited, and a total of 1038 youths (508 girls, 530 boys) participated in the study (92.9 % of invitees). The study was repeated in 2012-2013 (*Fit Futures 2*), inviting all attendants from the former study. Now in third year of upper secondary school, 694 of the students that attended the Fit Futures 1 survey participated in Fit Futures 2 (378 girls and 216 boys), giving an attendance rate of 66,9 % of the invited. The Fit Futures study is the most comprehensive study on somatic health in a cohort of youths in Northern Norway ever conducted.

Examinations and questionnaires were completed during school hours in a research lab at the University Hospital of North Norway (UNN). Pupils were transported by bus from their school to the research center. Other means of transportation were arranged for participants that had either dropped out of school or attended workplace apprenticeships. Transport expenses were covered in whole by the Fit Futures survey. The Regional Committee For Medical and Health Research Ethics, the Norwegian Data Protection Authority and the Norwegian Directorate of Health have all approved the study. All participants gave written informed consent. In Fit Futures 1, participants below age 16 acquired written consent from a parent or legal guardian. No participants were below age 16 in Fit Futures 2.

In this paper we aim to evaluate hemoglobin values and iron status in youths in Fit Futures 1 contra Fit Futures 2 (hereinafter referred to as *FF1* and *FF2*, respectively). We therefore constructed the following inclusion criteria in our research. Any participant outside the age range 15-17 years in FF1 (and hence age range 17-19 years in FF2) was excluded from our

study. Subjects with invalid or missing entries for hemoglobin, ferritin or CRP in FF1 or FF2 were also excluded. To prevent spurious ferritin values to impact on our research due to ongoing infection or inflammation in any of the participants, we excluded subjects who had a CRP value of 10 or above in either of the two surveys. In FF2 we also excluded one female participant with a clearly pathological ferritin value above 700 μg/L. Ferritin values under 500 μg/L for either gender were accepted, although threshold values suggested for iron overload have varied widely [45]. We were left with a sample of 813 participants (390 females and 423 males) with complete data and CRP < 10 in FF1, 561 participants (303 females and 258 males) in FF2 and 505 participants (275 females and 230 males) with complete data in both surveys.

Measurements

Past medical history was investigated in a clinical interview with a nurse. Height (meters) and weight (kilograms) were measured, and BMI (kg/m^2) was categorized in accordance with WHO growth reference data for BMI in adolescents [46]. As we only knew the participants' age in whole years and not months, we used BMI-categories for 15 years + 0 months, 16 years + 0 months and so on up to 19 years + 0 months. The following non-fasting blood samples were collected: high-sensitivity C-reactive protein (CRP), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), serum iron (Fe), serum ferritin and serum transferrin. Based on Hb and ferritin levels, we grouped participants according to their iron status; participants with low Hb (female Hb < 12 g/dL, male Hb < 13 g/dL) and low ferritin (< 12 μg/L) as iron deficiency anemia (IDA), participants with low ferritin and normal Hb (female Hb ≥ 12 g/dL, male Hb ≥ 13 g/dL) as iron deficient (without anemia), participants with low Hb and normal ferritin (≥ 12 μg/L) as anemia (non-iron deficient), and lastly participants with normal Hb and ferritin as normal iron status. In some analyses we dichotomized iron status groups as iron deficiency (iron deficiency anemia and non-anemic iron deficiency as one group) or iron replete (normal iron status and non-iron deficient anemia as one group). Participants with low Hb (males < 13, females < 11.5) were given notice and recommended further evaluation either at their general practitioner or at the pediatric outpatient clinic at UNN, depending on severity.

Information on participants' lifestyle was collected in self-administered electronic questionnaires, covering physical activity, food habits, ethnicity and self-rated health. Participants were asked how often they eat junk food, chocolate/sweets and snacks, which were grouped accordingly: "rarely/never", "1-3 times per month", "1-3 times per week", "4-6 times per week" and "every day". Questions on physical activity habits included frequency per week ("never", "less than once a week", "1 day a week", "2-3 days a week", "4-6 days a week", "almost every day"), hours per week ("none", "0.5 hour", "1-1.5 hours", "2-3 hours", "4-6 hours", "7 hours or more") and the intensity of the physical activity ("not hard at all", "a bit hard", "quite hard", "very hard", "extremely hard"). Participants were asked to rate their own health ("very bad", "bad", "neither good nor bad", "good" or "excellent") and say whether or not they use vitamin or mineral supplements ("no", "sometimes" or "yes, daily"). The FF1 survey included questions for females on menstrual cycle length (in days) and menstrual cycle regularity ("always regular", "usually regular" and "irregular"), but these data were not yet available in the FF2 dataset at the time of writing this thesis.

Statistical analyses

All statistical analyses are sex stratified, unless otherwise specified. Mean and standard deviations are calculated for continuous variables, while categorical variables are presented in numbers and percentages. Paired-samples t-tests were conducted to examine change in mean hemoglobin levels from FF1 to FF2. For ferritin, the Wilcoxon signed rank test was used for analyzing difference in median values between FF1 and FF2. To analyze differences in distribution of blood indices between genders, the independent samples t-test and Mann-Whitney U test were performed for hemoglobin and ferritin, respectively. McNemar's Chi^2-test was used to examine differences in iron status classes in Fit Futures 1 versus Fit Futures 2. To investigate changes in Hb and ferritin between FF1 and FF2 based on iron status groups from FF1 we used the Kruskal-Wallis H test. We used Kruskal-Wallis H tests or regular ANOVAs to explore effects of lifestyle factors on ferritin and Hb. Histograms, Q-Q plots and Shapiro-Wilks test were all used to examine normality of distributions. All statistical analyses were performed

using the Statistical Package of Social Sciences software (SPSS version 22). Significance levels were set at $p < .05$.

The work process

Authors prepared separate project descriptions during winter 2014. The original plan was to do two separate master theses, but as we saw it superfluous to write two separate papers on the same subject and data material, we paired up to write one larger thesis instead. A renewed project description was worked up and handed in to the faculty in April 2015.

Professor Flægstad, our supervisor, provided the SPSS software and an incomplete dataset from FF1 that we could use for practice in the summer of 2014. Through the fall and winter, while in clinical practice, we practiced using data material in SPSS, creating graphs and figures and conducting statistical analyses. We also looked up relevant literature through searches in PubMed and other databases.

In March 2015, after clinical practice was over, we started working together, intensifying our literature search and writing up a draft on the introduction to our thesis. Originally we were supposed to receive the complete dataset for FF1 and FF2 in August/September 2014, but this was delayed due to various reasons until early April 2015. The data material used in this thesis was collected and processed by the Fit Futures survey group, whose leader is Associate Professor Anne-Sofie Furberg at the Department of Community Medicine, UiT. Furberg was available to answer any questions we had concerning the Fit Futures survey. We were provided a raw data file for SPSS upon request. Some minor changes and additions to the dataset were necessary, for example creating grouping variables for continuous variables and the like.

Throughout April and May we worked with the dataset in SPSS, producing and interpreting results. A results draft was worked up, followed by a draft on material and methods, then discussion and conclusion. The authors have contributed equally to this paper. We had counseling appointments with Prof. Flægstad on a regular basis through this period. A final draft was produced the second last weekend of May, and last counseling from our supervisor was held the following week. Our master thesis was completed May 29th, 2015.

6 RESULTS

We have organized our results in three main sections. The first section presents analyses of data material from FF1, the second section presents analyses of the data material from FF2, and the last section presents analytical comparisons of the two surveys.

6.1 Fit Futures 1

A sample of 813 adolescents (390 females and 423 males), that were between ages 15 and 17 had valid data for both hemoglobin and ferritin and a CRP value less than 10, were included in our analyses from FF1. Values are mean (±SD) unless otherwise specified. 46 participants were 15 years old, 653 were 16 years old, and 114 were 17 years old. Mean body mass index was 22.26 (± 3.97) for females and 22.18 (± 3.86) for males.

Distribution of Hb and Ferritin in FF1

The mean Hb for females was 12.63 g/dL (± 1.02), and for males 14.57 g/dL (± .88), giving a mean difference between genders of 1.94 g/dL (95% CI 1.81 – 2.07, p < .001). The median value of ferritin was 48 µg/L (observed range: 5 - 230) for men, and 24 µg/L (observed range: 2 - 144) for females, giving a median difference of 24 µg/L between genders (p < .001). Data of mean blood values for both genders can be found in Table 4 and Table 5.

In evaluation of whether Hb and ferritin was normally distributed in our population, we used visual methods given the large sample size. By inspection of histograms (Figure 5 and Figure 6) and Q-Q Plots, the distribution of Hb was approximately normally distributed for both genders, while it was clearly not for ferritin for neither females nor males.

Figure 5 Normal distribution of Hb in females

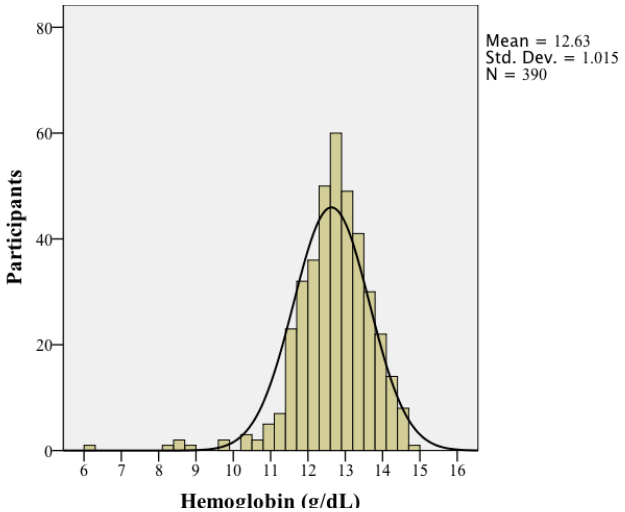


Figure 6 Normal distribution of Hb in males

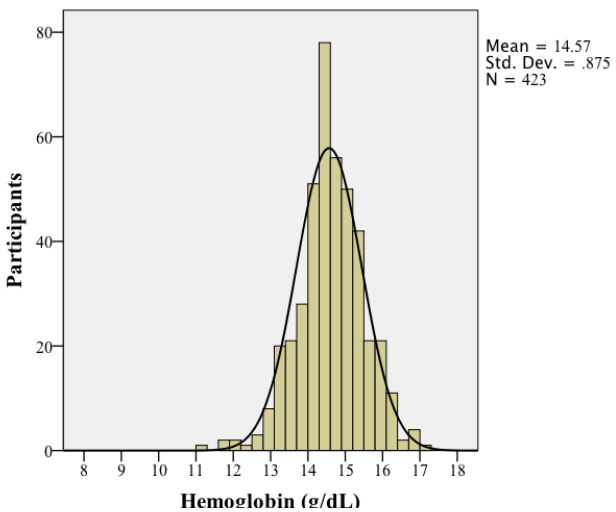


Table 4 Distribution of blood indices in females in FF1

Females	N	Mean	SD	Range	Minimum	Maximum
Hemoglobin (g/dL)	390	**12.63**	**1.02**	8.70	6.00	14.70
MCHC (g/dL)	385	**33.38**	**.74**	7	29	36
MCV (fl)	387	**86.30**	**5.25**	45	53	98
Ferritin (ug/L)	390	**29.87**	**21.68**	142	2	144
Transferrin (g/L)	390	**3.02**	**.48**	3.00	1.90	4.90
Fe (µmol/L)	390	**15.30**	**7.40**	59	0	59

Table 5 Distribution of blood indices in males in FF1

Males	N	Mean	SD	Range	Minimum	Maximum
Hemoglobin (g/dL)	423	**14.57**	**.88**	6.00	11.00	17.00
MCHC (g/dL)	418	**33.67**	**.56**	4	31	35
MCV (fl)	423	**87.05**	**3.41**	29	68	97
Ferritin (ug/L)	423	**54.24**	**32.44**	225	5	230
Transferrin (g/L)	423	**2.85**	**.37**	2.40	1.90	4.30
Fe (µmol/L)	423	**18.72**	**7.00**	54	3	57

Prevalence of anemia, iron deficiency and iron deficiency anemia

The total prevalence of iron deficiency for males and females was 1.7% and 17.9%, respectively. 0.7% of males and 9.5% of females had iron deficiency anemia. The overall prevalence of anemia was, among females, 20.3%, and in males 3.1%. In Table 6, iron status for participants is presented. 8.4% of females and 0.9% of males were iron deficient with Hb-value within normal range for gender.

Table 6 Prevalence of iron deficiency, iron deficiency anemia and anemia in males and females in FF1

Iron status	Females		Males		Total	
	n=390	Prevalence	n=423	Prevalence	n=813	Prevalence
ID (non-anemia)	33	8.4%	4	0.9%	37	4.6%
IDA	37	9.5%	3	0.7%	40	4.9%
Anemia (non-ID)	42	10.7%	10	2.4%	52	6.4%
ID (total)*	70	17.9%	7	1.7%	77	9.5%
Anemia (total)**	79	20.3%	13	3.1%	92	11.3%

** ID and IDA combined. **IDA and non-ID anemia combined.*

2.5 percentile as cut-off value for anemia

We wanted to compare the prevalence of anemia according to the WHO criteria to the -2 SD cut-off value (-2 standard deviations, also referred to as the 2.5 percentile) for Hb in our population. We chose to use adolescents without iron deficiency or inflammation as our

reference population, and excluded 77 subjects with ferritin <12 μg/L. In females, the -2 SD cut-off was Hb <11.4 g/dL and in males Hb <12.9 g/dL. According to the 2.5 percentile, the prevalence of anemia was 6.2% in females and 2.4% in males. The WHO cut-off value gave a three times higher prevalence of anemia compared to the 2.5 percentile of Hb in women, whereas for males the difference in prevalence was only 0.7% (Table 7).

As the WHO´s cut-off value for anemia gave a higher prevalence of anemia than the -2 SD for Hb in our population, we wanted to test the properties of the WHO´s cut-off value. We chose to use the -2 SD cut-off for Hb in our population as "gold standard", and calculate the sensitivity, specificity and positive predictive value (PPV) of the WHO criteria (Table 8 and Table 9). WHO´s cut-off value gave a sensitivity of 100%, a specificity of 85.0% and a PPV of 30.4% in females. In males the WHO criteria gave a sensitivity of 100%, a specificity of 99.3% and a PPV of 76.9%.

Table 7 Prevalence of iron deficiency, iron deficiency anemia and anemia in males and females in FF1 when using 2.5 percentile as cut-off value

Iron status	Females		Males		Total	
	n=390	Prevalence	n=423	Prevalence	n=813	Prevalence
ID (non-anemia)	53	13.6%	4	0.9%	57	7.0%
IDA	17	4.4%	3	0.7%	20	2.5%
Anemia (non-ID)	7	1.8%	7	1.7%	14	1.7%
ID (total)*	70	17.9%	7	1.7%	77	9.5%
Anemia (total)**	24	6.2%	10	2.4%	34	4.2%

** ID and IDA combined. **IDA and non-ID anemia combined.*

Table 8 Diagnostic test properties of the WHO criteria in females in FF1

Females		-2 SD cut-off		Total
		Anemia	Non-anemic	
WHO cut-off	Anemia	24	55	79
	Non-anemic	0	311	311
Total		24	366	390

Table 9 Diagnostic test properties of the WHO criteria in males in FF1

Males		-2 SD cut-off		Total
		Anemia	Non-anemic	
WHO cut-off	Anemia	10	3	13
	Non-anemic	0	410	410
Total		10	413	423

Lifestyle factors

A sex stratified one-way ANOVA was conducted to examine the differences in mean Hb levels between BMI-groups in FF1. Participants were grouped according to age-adjusted gender-specific BMI-categories: underweight (females n = 0, males n = 5), normal weight (females n = 301, males n = 304), overweight (females n = 62, males n = 78), obese (females n = 20, males n = 31) and severely obese (females n = 6, males n = 5). There was missing data on height and weight for one female, which brought the total number of participants for this analysis to 812. Data was assumed normally distributed for all groups with n > 60. For groups with n ≤ 60 a Shapiro-Wilk test was run, which proved all groups normally distributed (p > .05). Hb levels were not significantly different between BMI-categories, neither for females (F(3, 385) = 2.197, p = .088) nor for males (F(4, 418) = 2.225, p = .066), data is shown in Table 10.

Table 10 Mean hemoglobin levels in FF1 according to BMI-classification for each gender. Standard deviations (SD) and 95% confidence intervals are displayed

Gender	BMI-class in FF1	n	Mean	SD	95% CI
Females	Normal weight	301	12.57	1.03	12.45 – 12.69
	Overweight	62	12.77	1.00	12.52 – 13.02
	Obese	20	12.83	.72	12.49 – 13.16
	Severely obese	6	13.42	.73	12.65 – 14.18
	Total	389*	12.63	1.02	12.53 – 12.73
Males	Underweight	5	14.52	.76	13.58 – 15.46
	Normal weight	304	14.49	.85	14.40 – 14.59
	Overweight	78	14.79	.85	14.59 – 14.98
	Obese	31	14.73	1.00	14.36 – 15.09
	Severely obese	5	14.86	1.28	13.27 – 16.45
	Total	423	14.57	.88	14.48 – 14.65

**one female had missing data for height and weight.*

The median ferritin values tended to increase with groups with a higher BMI-classification. Data is shown in Table 11. A Kruskal-Wallis H test was conducted to examine this trend. Medians varied significantly between BMI groups for females (p = .012) but not for males (p = .765). A post hoc pairwise comparison between groups was run for the female sample, but there were no statistically significant differences.

Table 11 Median ferritin values in FF1 according to BMI classifications

Gender	BMI Classification	n	Median
Female	Normal weight	301	23.0
	Overweight	62	27.0
	Obese	20	37.5
	Severely obese	6	38.5
	Total	389*	24.0
Male	Underweight	5	37.0
	Normal weight	304	47.5
	Overweight	78	49.0
	Obese	31	46.0
	Severely obese	5	76.0
	Total	423	48.0

*One female had missing data for weight and height in FF1.

Mean hemoglobin and median ferritin levels for both genders were tested either with an ANOVA or a Kruskal-Wallis H test, depending on the assumption of normally distributed data being in each variable group, in regard to lifestyle factors submitted by each participant in the questionnaire. There were no statistically significant variations in Hb means between groups according to junk food, chocolate/sweets, snacks, hours of physical activity per week or intensity of physical activity. In females there was no significant variation in Hb according to menstrual cycle length, or menses regularity. There was however a statistically significant variation between groups of physical activity frequency per week among males ($p = .013$) but not females ($p = .243$). Only one male participant was in the “never” physically active group, and was excluded for this analysis. A post hoc analysis (Bonferroni corrected for multiple comparisons) showed that there was a higher Hb in males who were physically active 2-3 days per week (mean Hb = 14.68) compared to males who were physically active 4-6 days per week (mean Hb = 14.19)(mean difference = .49, 95% CI .085 - .897, $p = .007$), but not between any other physical activity frequency groups. There was also statistically significant variance between groups that took vitamins or not. In females there was statistically significant variance between groups ($p = .034$). Participants who sometimes took supplements had slightly lower Hb than the two other groups, but there was no significant difference when groups were compared pairwise. In males there was statistically significant variance in means in those who use vitamin

supplements daily (mean Hb = 14.38) and those who don't or only sometimes take supplements (mean Hb = 14.61), p = .047.

Variance in median ferritin values between the same groups of lifestyle factors was assessed using a Kruskal Wallis H test. There were statistically significant differences in distributions between groups according to frequency of eating chocolates and sweets in males (p = .029) but not in females (p = .985). A pairwise comparison revealed significant differences between the "rarely/never" group (mean rank = 131.64) and the "1-3 times per week" group (mean rank = 213.72, p = .011), as well as the "1-3 time per month" group (mean rank = 208.99, p = .036). There were also differences in distributions between males grouped according to frequency of eating snacks (p = .039). Ferritin values tended to increase with more frequent intake of snacks, but pairwise comparisons were not statistically significant. Neither were there any differences in ferritin between female snacking groups. However, females that were frequently physically active tended to have higher ferritin values (p = .019), this was not the case for males (p = .483). The pairwise comparisons revealed a statistically significant difference in distributions between groups of females physically active "1 day a week" (mean rank = 104.31) and "almost every day" (mean rank = 171.92, p = .024). There were no statistically significant differences in ferritin medians between groups of males nor females according to lifestyle factors junk food, hours of physical activity, intensity of physical activity, menstrual cycle length, menses regularity, vitamin/mineral supplements or self-rated health.

6.2 Fit Futures 2

In FF2, 561 participants out of 694 met our inclusion criteria, with valid measures for Hb and ferritin, and CRP < 10. The sample consisted of 303 females and 258 males. Because we wanted to use the same participants as in FF1, and not exclude additional adolescents because of age-criteria, the 15-17 years-old participants in FF1 were included for our analysis of FF2 data. We assumed all participants were exactly two years older in FF2 than the age reported in FF1. Age was distributed accordingly; 23 participants were 17 years old, 459 participants were 18 years old and 79 participants were 19 years old. There was no statistically significant difference in

mean age between FF1 and FF2 samples (p = .258). The mean BMI was 22.89 (± 3.84) for females, and 23.49 (± 4.16) for males in FF2 (n=561).

Distribution of Hb and Ferritin in FF2

In males, the mean Hb level was 14.83 g/dL (± .85), and in females 12.68 g/dL(± .88), giving a mean difference of 2.15 g/dL (95% CI 2.01 – 2.30, p < .001). The median values of ferritin were higher significantly higher in males (79.5 μg/L) than in females (33 μg/L, p<0.001). Data of mean blood values for both genders can be found in Table 12 and Table 13.

The distribution of Hb is approximately normal as assessed by inspection of histograms (presented in Figure 7 and Figure 8) and Q-Q Plots for females and males. By inspection histogram and Q-Q plots of ferritin, there was not normal distribution for either gender.

Figure 7 Normal distribution of Hb in females

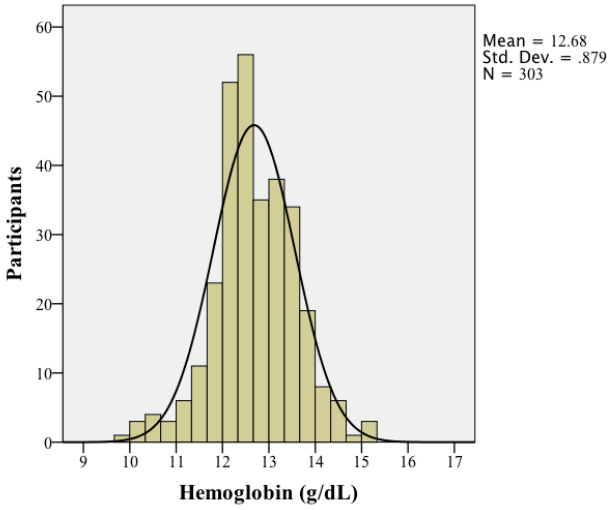


Figure 8 Normal distribution of Hb in males

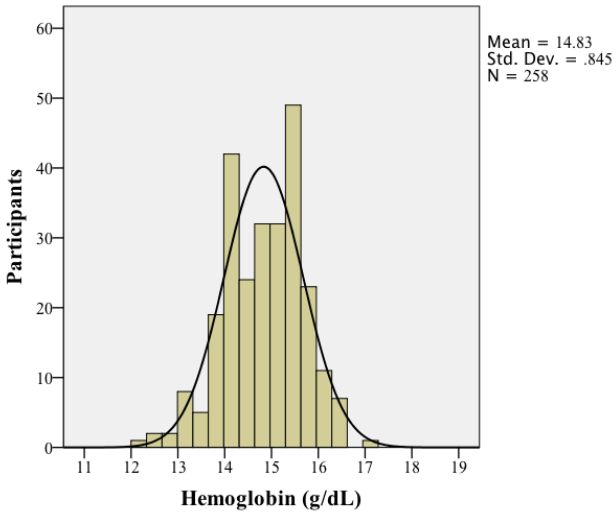


Table 12 Distribution of blood indices in males in FF2

Males	N	Mean	SD	Range	Minimum	Maximum
Hb (g/dL)	258	**14.83**	**.85**	4.80	12.30	17.10
MCHC (g/dL)	258	**33.56**	**.95**	5.00	31.00	36.00
MCV (fl)	258	**88.54**	**3.32**	22.00	78.00	100.00
Ferritin (ug/L)	258	**92.15**	**55.33**	439	8	447
Transferrin (g/L)	258	**2.62**	**.29**	1.80	1.80	3.60
Fe (μmol/L)	258	**19.64**	**7.22**	35.00	5.00	40.00

Table 13 Distribution of blood indices in females in FF2

Females	N	Mean	SD	Range	Minimum	Maximum
Hb (g/dL)	303	**12.68**	**.88**	5.40	9.70	15.10
MCHC (g/dL)	303	**32.84**	**1.12**	7.00	29.00	36.00
MCV (fl)	303	**89.17**	**4.34**	32.00	70.00	102.00
Ferritin (ug/L)	303	**39.43**	**28.51**	186	3	189
Transferrin (g/L)	303	**2.94**	**.48**	3.20	1.90	5.10
Fe (µmol/L)	303	**17.24**	**7.47**	40.00	3.00	43.00

Prevalence of anemia, iron deficiency and iron deficiency anemia

The total prevalence of iron deficiency for males and females was 0.8% and 11.9%, respectively. 0.4% of males and 6.3% of females had iron deficiency anemia. The overall prevalence of anemia was 16.8% among females, and in males 1.9%. 5.6% of females and 0.4% of males were iron deficient with Hb-value within normal range. In Table 14, iron status for participants is presented.

Table 14 Prevalence of iron deficiency, iron deficiency anemia and anemia in males and females in FF2

Iron status	Females		Males		Total	
	n=303	Prevalence	n=258	Prevalence	n=561	Prevalence
ID (non-anemia)	17	5.6%	1	0.4%	18	3.2%
IDA	19	6.3%	1	0.4%	20	3.6%
Anemia (non-ID)	32	10.6%	4	1.6%	36	6.4%
ID (total) *	36	11.9%	2	0.8%	38	6.8%
Anemia (total)**	51	16.8%	5	1.9%	56	10.0%

**ID and IDA combined. **IDA and non-ID anemia combined.*

2.5 percentile for Hb as cut-off value for anemia

We wanted to compare the WHO criteria for anemia with the 2.5 percentile for Hb in our population. The reference population is based on subjects in FF2 without iron deficiency or inflammation (n=523). The -2 SD cut-off for Hb is in females Hb <11.4 g/dl, and in males Hb <13.1 g/dL. By use of the 2.5 percentile as cut-off value, 5.6% females and 1.9% males have anemia (Table 15). In other words, the prevalence in females was reduced by two thirds compared to use of the WHO criteria. In males the prevalence of anemia was the same with either criteria.

We wanted to test the properties of WHO´s cut-off value, as the prevalence of anemia differed for females by use of the 2.5 percentile, as presented in Table 16. The 2.5 percentile was used as a golden standard, and the sensitivity, specificity and PPV of the WHO´s reference range were calculated. WHO´s cut-off value gave a sensitivity of 100%, a specificity of 88.11% and a PPV of 33.33% for females. Regarding males, the 2.5 percentile differ from the WHO´s cut-off value only by 0.1 g/dL, and in our data material that results in the exact same prevalence (1.9%) and perfect test properties.

Table 15 Prevalence of iron deficiency, iron deficiency anemia and anemia in males and females in FF2 when using 2.5 percentile as cut-off value

Iron status	Females		Males		Total	
	n=303	Prevalence	n=258	Prevalence	n=561	Prevalence
ID (non-anemia)	25	8.3%	1	0.4%	26	4.6%
IDA	11	3.6%	1	0.4%	12	2.1%
Anemia (non-ID)	6	2.0%	4	1.6%	10	1.8%
ID (total) *	36	11.9%	2	0.8%	38	6.8%
Anemia (total)**	17	5.6%	5	1.9%	22	3.9%

**ID and IDA combined. **IDA and non-ID anemia combined.*

Table 16 Diagnostic test properties of the WHO criteria in females in FF2

Females		-2 SD cut-off		Total
		Anemia	Non-anemic	
WHO cut-off	Anemia	17	34	51
	Non-anemic	0	252	252
Total		17	286	303

Liftestyle factors

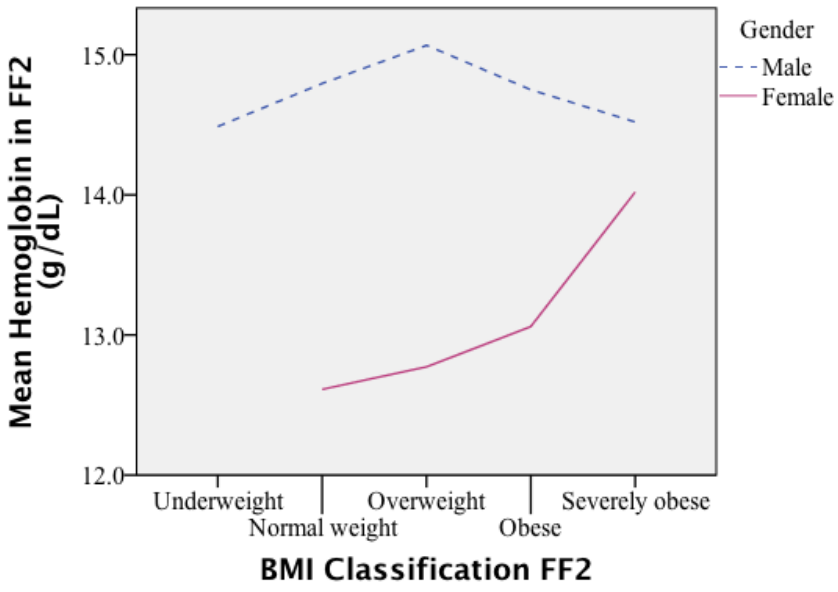
Hb-values in FF2 were compared between BMI groups using a one-way ANOVA. The BMI groups were: underweight (nil females and eight males), normal weight (240 females and 177 males), overweight (43 females and 52 males), obese (15 females and 16 males) and severely obese (five females and five males). Data was assumed normally distributed for groups with more than 60 subjects, while smaller groups ($n \leq 60$) were proved normally distributed with a Shapiro-Wilk test ($p > .05$). Means, standard deviations and 95% confidence intervals are shown in Table 17. Hb levels were significantly different between BMI-categories in females ($p = .001$), but not in males ($p = .162$). The post hoc analysis revealed a statistically significant difference in

Hb means between the female groups “normal weight” and “severely obese” (p = .002), and “overweight” and “severely obese” (p = .014). The hemoglobin variation between BMI groups in both genders is demonstrated in Figure 9.

Table 17 Mean hemoglobin levels in FF2 according to BMI-classification for each gender. Standard deviations (SD) and 95% confidence intervals are displayed

Gender	BMI-class	n	Mean	SD	95% CI
Females	Normal weight	240	12.61	.89	12.50 – 12.72
	Overweight	43	12.77	.75	12.54 – 13.00
	Obese	15	13.06	.69	12.68 – 13.44
	Severely obese	5	14.02	.89	12.91 – 15.13
	Total	303	12.68	.88	12.58 – 12.78
Males	Underweight	8	14.49	1.10	13.57 – 15.41
	Normal weight	177	14.79	.81	14.67 – 14.92
	Overweight	52	15.07	.84	14.83 – 15.30
	Obese	16	14.75	.87	14.28 – 15.22
	Severely obese	5	14.52	1.39	12.80 – 16.24
	Total	258	14.83	.85	14.73 – 14.94

Figure 9 Means plot demonstrating the variation in hemoglobin means for each gender according to BMI classifications



Conducting a Kruskal-Wallis H test, we assessed variation of median ferritin values according to BMI groups in FF2 (Table 18). As in FF1, the ferritin medians tended to increase with increasing BMI classifications in both genders. In females there was statistically significant variation in

ferritin medians (p = .004) while there was not for males (p = .761). However, the post hoc analysis in the female groups for pairwise comparisons showed no statistically significant variations between groups.

Table 18 Median ferritin values in FF2 according to BMI classifications

Gender	BMI Classification	n	Median
Female	Normal weight	240	30.0
	Overweight	43	44.0
	Obese	15	45.0
	Severely obese	5	55.0
	Total	303	33.0
Male	Underweight	8	81.5
	Normal weight	177	82.0
	Overweight	52	77.5
	Obese	16	92.0
	Severely obese	5	118.6
	Total	258	79.5

As for the FF1 data, mean hemoglobin and median ferritin levels for both genders were compared group wise according to lifestyle factors submitted by the participants in the questionnaire. ANOVAs or Kruskal Wallis H tests were conducted, depending on the assumption of normally distributed data in groups being violated or not. In FF2 there was no statistically significant variance in Hb means in any of the following lifestyle factors: junk food, chocolates/sweets, snacks, physical activity (neither in frequency, weekly hours nor intensity), vitamin/mineral supplements and self-rated health. Data on menstrual cycle were not available in the FF2 survey.

Kruskal Wallis H tests were used when examining variations in ferritin medians according to lifestyle factors. There were statistically significant variations in distributions of ferritin values in different junk food frequency groups in males (p = .047), not in females (p = .581). A post hoc analysis was conducted, but the pairwise comparison revealed no significant differences between groups. The trend, however, was higher median values in male groups that eat junk food more frequently, except for the “every day” group, which consisted of only one male participant. Another analysis was conducted, excluding this one participant, but rendered not

statistically significant for the male groups (p = .057). Statistically significant differences were found in median values of male groups (p = .019) according to whether or not they use vitamin or mineral supplements, but not in female groups (p = .707). A pairwise comparison revealed broader distribution of ferritin values in the group that did not use vitamins (mean rank = 137.59) versus the group that used vitamins daily (mean rank = 106.20, p = .038). The group that used vitamins "sometimes" showed no significant variation compared to any of the two other groups. For males there was also a statistically significant difference in variation of ferritin values according to how the participants rated their own health (p = .020), but not for females (p = .189). One male case was excluded for this analysis, due to him being the only participant rating his health "very bad". A post hoc analysis was conducted for the male groups and found statistically significant differences in ferritin medians between the "excellent" health group (*Mdn* = 65.5, mean rank = 98.31) and the "good" health group (*Mdn* = 85.0, mean rank = 129.98, p = .037) as well as the "neither good nor bad" health group (*Mdn* = 86.0, mean rank = 136.66, p = .035), but not between any other group. There was no statistically significant variance in ferritin medians in groups according to the following lifestyle factors in FF2: chocolates/sweets, snacks and physical activity (neither in frequency, weekly hours nor intensity).

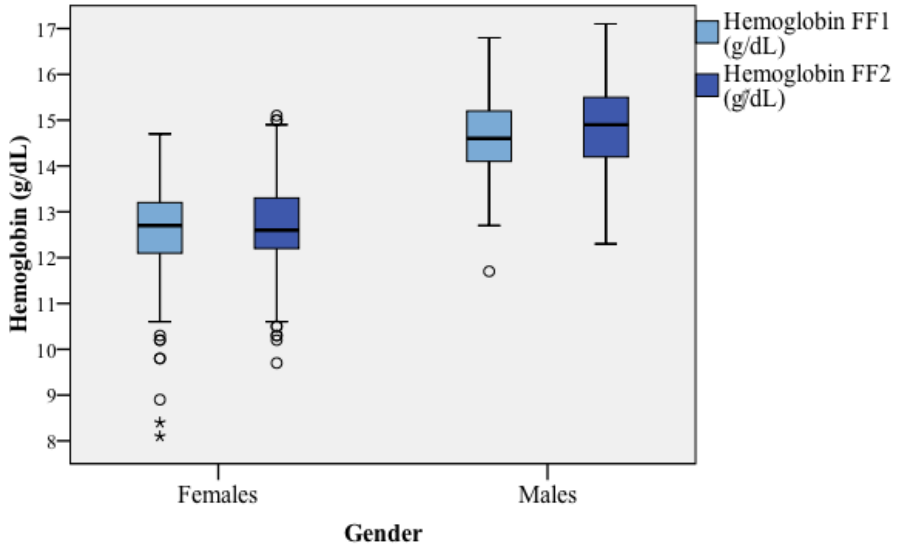
6.3 Development in hemoglobin, ferritin and iron status between FF1 and FF2

To examine the development of Hb and ferritin values from Fit Futures 1 to Fit Futures 2 we included only those participants with a complete dataset for ferritin and Hb in both surveys. A sample of 505 participants (275 females and 230 males) met our inclusion criteria for the following analyses. We wanted to ensure that this smaller sample was representable statistically for the two larger samples in FF1 (n = 813) and FF2 (n = 561). We ran sex stratified one-sample T-tests on mean Hb levels in the small sample versus the mean Hb levels in the larger samples for FF1 and FF2. There were no discrepancies, with p > .05 for all tests. For BMI we also conducted a similar test of the total sample means in FF1 and FF2 against the means in the smaller sample. There was no statistically significant difference between the small and large sample for BMI in FF1 (p = .278), nor in FF2 (p = .481). For ferritin we tested the total sample

medians in the larger samples against the sample medians from FF1 and FF2 in the small sample with a Wilcoxon signed-rank test, which was not statistically significant for neither FF1 (p = .236) nor FF2 (p = .170).

Figure 10 presents the distributions of hemoglobin values in FF1 and FF2, for females and males, respectively. Mean Hb values were lower in FF1 (females 12.61 ± .99, males 14.60 ± .81) compared to FF2 (females 12.68 ± .89, males 14.81 ± .86). The hemoglobin change for each gender was assessed using paired-samples T-test. The participants' hemoglobin values increased over the two-year period by .068 for females (CI -.028 to .165) and .213 for males (CI .122 to .303). The increase for males was statistically significant (p < .001) while it was not for females (p = .164).

Figure 10 Distributions of hemoglobin (g/dL) in females (n = 275) and males (n = 230) in Fit Futures 1 and Fit Futures 2 compared



As the data for ferritin were not normally distributed for either gender in FF1 or FF2, a related-samples Wilcoxon signed rank test was conducted. Ferritin values increased for both genders over the two-year period (Figure 11). Of the 505 participants, 206 males and 193 females had increased ferritin values in FF2 compared to FF1, whereas 22 males and 76 females had reduced ferritin values and no change was seen in two males and six females. The median increase in ferritin was statistically significant for both males and females, at 32.00 µg/L (p < .001) and 6.00 µg/L (p < .001) respectively.

Figure 11 Median serum ferritin values in females and males in Fit Futures 1 and Fit Futures 2 compared. 95% confidence intervals displayed

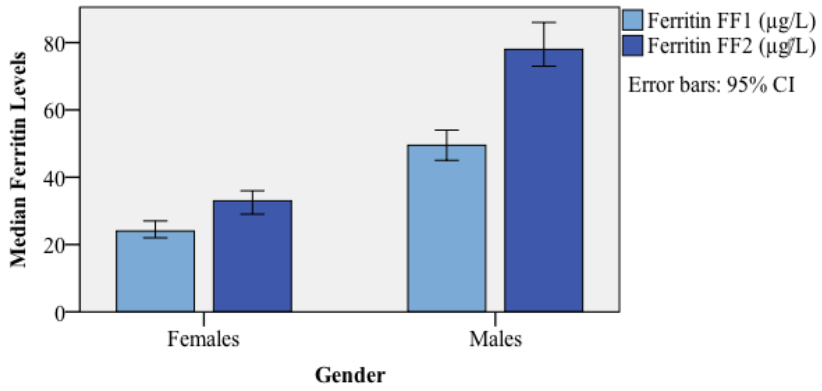


Table 19 presents the number of iron deficient (ferritin < 12 µg/L) and iron replete (ferritin ≥ 12 µg/L) participants in our sample (n = 505) in FF1 and FF2. The prevalence of ID in this sample was 10.7% (1.7% of males and 17.5% of females) in FF1 and 7.3% (0.9% of males and 12.7% of females) in FF2. Over the two-year period between the surveys, 21 participants (one male and 20 females) had remained iron deficient, while 31 iron deficient participants (three males and 28 females) had become iron replete and 16 iron replete participants (one male and 15 females) had become iron deficient. McNemar's test was used to examine the difference in proportions between the two surveys. The decrease in iron deficiency for both genders combined was statistically significant (p = .040). However, a sex stratified analysis showed no statistical significance for either males (p = .625) or females (p = .066).

Table 19 Cross table presenting prevalence of iron deficiency in Fit Futures 1 and Fit Futures 2 compared. Numbers for both genders combined (% of total)

		Iron status in Fit Futures 2		Total
		Iron deficient	Iron replete	
Iron status in Fit Futures 1	Iron deficient	21	31	52 (10.7%)
	Iron replete	16	437	453 (89.3%)
Total		37 (7.3%)	468 (92.7%)	505 (100%)

Table 20 Cross table presenting prevalence of iron deficiency, iron deficiency anemia and non-iron deficiency anemia in females in FF1 and FF2 compared (% of total)

Females		Blood status in Fit Futures 2				Total
		Normal	Non-anemia ID (NAID)	Non-ID Anemia (NIDA)	Iron deficiency anemia (IDA)	
Blood status in Fit Futures 1	Normal	170	5	15	3	193 (70.2%)
	Non-Anemia Iron Deficiency (NAID)	19	5	1	0	25 (9.1%)
	Non-ID Anemia (NIDA)	14	1	13	6	34 (12.4%)
	Iron deficiency anemia (IDA)	7	6	1	9	23 (8.4%)
Total		210 (76.4%)	17 (6.2%)	30 (10.9%)	18 (6.5%)	275 (100%)

We further examined the change in prevalence of non-anemic iron deficiency (NAID), iron deficiency anemia (IDA) and non-iron deficiency anemia (NIDA) between the two surveys. In lack of a better term we will call these "blood status groups". For males (n = 230), the prevalence of NAID, IDA and NIDA in FF1 was 0.9% (n=2) for each and in FF2 0.4% (n=1), 1.7% (n=4) and 0.4% (n=1), respectively. The proportions in males were considered too small to analyze statistically. In females, however, the prevalence of NAID, IDA and NIDA in FF1 was 9.1%, 8.4% and 12.4%, and in FF2 6.2%, 6.5% and 10.9%, respectively. Data is presented in Table 20. The prevalence of NAID, IDA and NIDA in females had all decreased over the two-year period between surveys. A McNemar-Bowkers test was run to examine the prevalence changes in females, which was statistically significant, p = .004.

To identify exactly where these changes had occurred in the females, we compared the blood status groups in FF1 in terms of hemoglobin change ($Hb_{FF2} – Hb_{FF1}$) and ferritin change ($ferritin_{FF2} – ferritin_{FF1}$). As the values of these differences were not normally distributed within each blood status group, a Kruskal-Wallis H test was conducted. Distributions of the Hb- and ferritin differences were not similarly shaped among the blood status groups, as assessed by visual inspection of boxplots (Figure 12 and Figure 13), therefore we could not infer on

differences in medians between groups, only on the distributions as a whole. Distributions of Hb and ferritin-changes were statistically significantly different between blood status groups for hemoglobin ($H(3) = 59.098$, $p < .001$) and ferritin ($H(3) = 9.684$, $p = .021$). Subsequently, pairwise comparisons were performed with a correction for multiple comparisons. This post hoc analysis revealed significant differences in distributions of Hb-change between the IDA group (mean rank = 239.04) and normal group (mean rank = 116.88)($p < .001$), the IDA group and ID group (mean rank = 167.12)($p = .010$), the IDA group and anemia group (mean rank = 168.13)($p = .006$), the normal group and ID group ($p = .018$) as well as the normal group and anemia group ($p = .003$), but not between the ID group and anemia group ($p = 1.00$). The difference in distributions of ferritin-change was statistically significant between the anemia group (mean rank = 110.34) and ID group (mean rank = 173.74)($p = .015$), but not between any other blood status groups (IDA group mean rank = 126.98, normal group mean rank = 139.56).

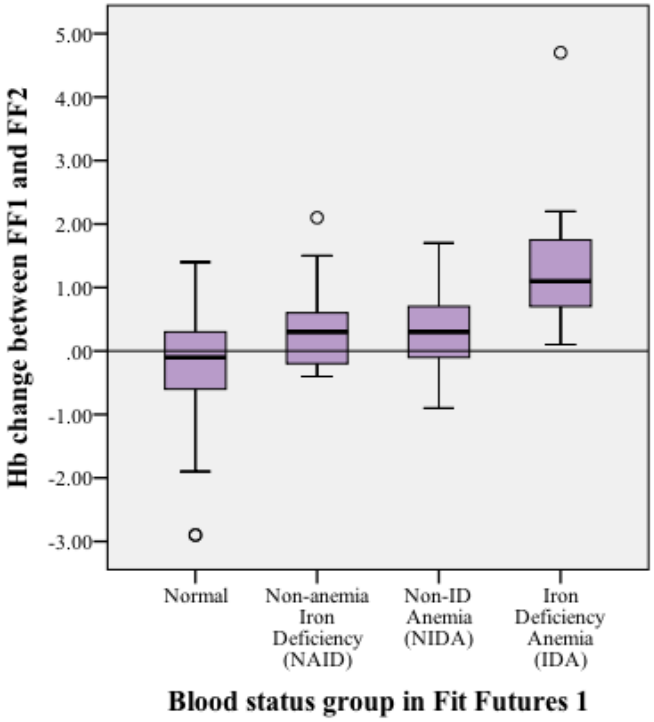


Figure 12 Hb-change between FF1 and FF2 according to blood status groups in FF1; normal (n = 193), NAID (n = 25), NIDA (n = 34) and IDA (n = 23)

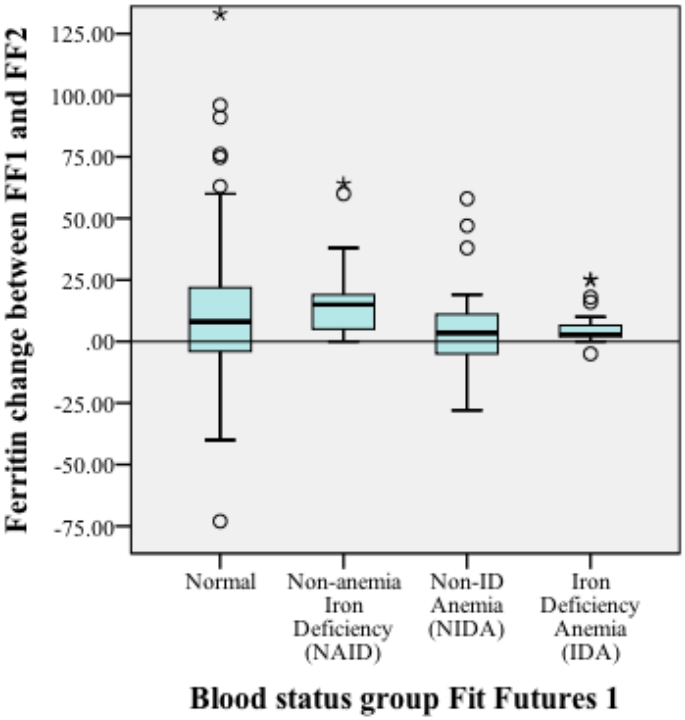


Figure 13 Ferritin-change between FF1 and FF2 according to blood status groups in FF1; normal (n = 193), NAID (n = 25), NIDA (n = 34) and IDA (n = 23)

7 DISCUSSION

To our knowledge there are few, if any, population-based studies on iron deficiency and anemia in adolescent cohorts with repeated measurements. In FF1 we had a high participation rate (92.9%), and out of the attending participants in FF1, 66.9% participated in FF2. The high response rate reinforces our results for FF1. The lower rate in FF2 somewhat increases the likeliness of non-response bias. In this master thesis, we wanted to examine gender-specific distribution of hemoglobin and ferritin, and the prevalence of iron deficiency, iron deficiency anemia and anemia in a population of adolescents in Northern Norway, and evaluate the applicability of the WHO criteria for anemia in this population. We also wanted to investigate whether or not certain lifestyle factors influence Hb and ferritin levels in our sample population.

In this cohort of adolescents from Northern Norway the prevalence of iron deficiency in FF1 was 17.9% in females and 1.7% in males. In FF2, the prevalence was 11.9% in females and 0.8% in males. These findings are consistent with that of some studies considering this age group [37, 47, 48], while considerably lower than others [27, 38]. Yet it is clear that iron deficiency is a common condition among female adolescents in this population. A decrease in prevalence of ID and IDA between FF1 and FF2 was expected, as the pubertal growth spurt is more likely to have passed in most participants in the second survey, two years after the first one [20]. In females, the prevalence remained relatively high in FF2, which is likely to be a result of higher iron demands due to menstrual blood loss. The prevalence of non-ID anemia, however, did not differ much between the two surveys. For both genders combined it remained stable at 6.4%.

For the sample that met inclusion criteria in both surveys (n = 505), there was significant reduction in the number of iron deficient participants between FF1 and FF2. However, sex stratified analyses showed no significant difference in males while in females the difference trended towards a significant p-value (p = .066). It is plausible that this analysis would be statistically significant for females, given a larger sample size. When stratified further into "blood-status" groups, there was statistically significant reduction in the number of ID, IDA and non-ID anemic females. Coherently, ferritin values increased significantly in both genders between FF1 and FF2, but to a greater extent in males than females. A similar trend was

described by Milman et al. in a Danish study [49]. Despite the increased iron stores, mean Hb values did not increase significantly for females, only in males. It is clear that the risk for developing IDA steadily decreases in male adolescents as they near adulthood, whereas the risk in females is sustained.

Of the 505 participants that met inclusion criteria in both studies, we found that 20 out of 48 females and one out of four males that were iron deficient in FF1 remained so two years later, in FF2. More specifically, nine of the 23 females that had IDA in FF1 remained so in FF2, while six of the 23 became iron deficient. Among the 25 females who had ID without anemia in FF1, only five had ID in FF2. This raises questions about possible detrimental consequences of long-term ID and IDA in youths. The relationship between IDA and developmental delay in infants has been widely explored [50-55], and studies on adolescents have reported adverse effects on cognitive functions in ID subjects that are reversible when subjects are treated with iron-supplements [56, 57]. Lower math scores in iron deficient adolescents has been noted [58, 59]. One study from Taiwan reported increased risk of a variety of psychiatric disorders [60]. Other non-hematological consequences are reduced physical endurance and work capacity [61-63], alteration in immune activity [64, 65], and increased risk of developing cancers [66]. Increased risk of pregnancy complications in women with IDA have been described, including prematurity, low birth weight, perinatal and maternal mortality, but evidence of the effect of routine prenatal iron supplementation in pregnant women with IDA is inconclusive [67].

Among the blood status groups, the participants who had IDA in FF1 had the greatest increase in Hb levels two years later in FF2, followed by the anemic (non-ID) and iron deficient (non-anemic) group, while the group of participants that had neither anemia or ID had the lowest change in Hb. This corresponds well with our observations that 13 of 23 participants with IDA in FF1 did not have anemia in FF2. One could hypothesize that those 13 participants in the interim may have been treated with iron supplements. However, the IDA group did not have the greatest increase in ferritin, which would be expected had a majority of them been treated with iron supplements. The participants with ID without anemia in FF1 had the highest mean rank score for ferritin change, but it was only significantly higher than the rank score of the non-ID anemia group, not the two other groups. Participants that had low Hb values in FF1 (males <

13.0, females <11.5) were recommended to seek medical attention for further evaluation, but whether or not any of the participants with ID or IDA in FF1 were given iron supplements during the interval before FF2 is unknown.

Surprisingly, in both FF1 and FF2, the majority of anemic subjects did not have iron deficiency. The etiology of anemia in these cases is unknown. However, when using the 2.5 percentile for Hb as cut-off value for anemia, instead of the WHO cut-off value, the prevalence was altered, as presented in Table 7 and Table 15. This puts in question whether or not the WHO criteria for anemia is applicable to this population.

In differentiation between healthy and diseased in a population, reference intervals are important decision-making tools for the clinician, and the reference intervals should reflect normal physiological development for age and gender [68]. The WHO criteria for anemia are well established [69, 70], and frequently referred to in the literature. Despite the WHO criteria being nearly universally accepted, the generalizability of these cut-off values has been opposed in several publications emphasizing the need for population specific hematological criteria [71-74].

By using the WHO criteria, 20.3% and 16.8% females in FF1 and FF2 have anemia, and hence appears to be widely prevalent. Using the 2.5 percentile of Hb as cut-off for anemia, only 6.2% of females had anemia in FF1, and 5.6% of females had anemia in FF2. The difference in prevalence of anemia between the WHO criteria and the 2.5 percentile in males was small, and essentially not important. Our findings are consistent with the discrepancies between the WHO criteria and the 2.5 percentile in females reported by Skjelbakken et al., who found the 2.5 percentile for females above 24 years of age to be 11.4 g/dL in the population of Northern Norway (n = 13689) [24]. A Swedish study (2013) that investigated population-based pediatric reference intervals found the 2.5 percentile of Hb in 12-18 year olds to be Hb < 11.2 g/dL in females (n = 136), and Hb <12.6 g/dL in males (n = 117) [68]. This study and the study by Skjelbakken et al. agree with our findings that the WHO threshold value for anemia in adolescent females is too high in our population, and that the 2.5 percentile renders a more correct prevalence of anemia.

In evaluation of diagnostic test properties of the WHO criteria, the sensitivity was 100% in both surveys, and the specificity relatively high, 84.97% and 88.11%. The PPV on the other hand was low, 30.38% and 33.33%, namely because the WHO criteria in both surveys gave a three times higher prevalence of anemia in females compared to the 2.5 percentile. Our participants were not examined nor questioned about symptoms that can exist with anemia, but they were asked, ”How do you in general consider your own health to be?” There was no significant difference in Hb between groups of self-rated health for either gender. However, anemia presents with rather unspecific or no symptoms at all [19], and we cannot relate this finding to evaluate the accuracy of the WHO criteria for anemia.

As iron deficiency is the most common cause of anemia worldwide, and adolescence is a high-risk period of life for developing iron deficiency [2, 75], the majority of subjects with anemia in our population were expected to suffer from ID. However, using WHO´s cut-off value for anemia, the majority of anemic subjects did not have iron deficiency (only 43% of the anemic participants in FF1 and 36% in FF2 had IDA) (Table 6 and Table 14). When using the 2.5 percentile as cut-off value for anemia 59% of the anemic participants had IDA in FF1 and 54% in FF2. The surprisingly low proportion of anemic cases being ID in both surveys further questions the applicability of the WHO criteria, and supports use of the 2.5 percentile as cut-off value for anemia in the adolescent population in Northern Norway.

Several studies have identified overweight and obese individuals – children, adolescents and adults alike – as at risk for developing iron deficiency [76-79]. On the contrary, our results show significantly increasing median ferritin values with higher weight groups in females in both FF1 ($p = .012$) and FF2 ($p = .004$). However, a widely accepted hypothesis on the etiology of increased risk of ID in obesity is low-grade inflammation due to the excess adipose tissue [80-82]. The mediator of this effect is believed to be the peptide hepcidin, responsible for regulating expression of the iron transporter ferroportin-1, which is increased during inflammation [83]. As ferritin is an acute-phase protein, it may be elevated in the overweight and obese, therefore it is not considered an ideal marker for iron deficiency in overweight and obese persons. Hence, it is difficult to determine whether the increased ferritin values in

heavier-weight participants in our study are indeed a result of greater iron stores, or elevated because of inflammation.

In the present study we observed trends that Hb values increased in heavier-weight individuals of both genders in FF1 and in females in FF2. This trend was statistically significant in females in FF2 ($p = .001$). The main differences were between the severely obese group ($14.02 \pm .89$) and the overweight ($12.77 \pm .75$, $p = .014$) and the normal weight group ($12.61 \pm .89$, $p = .002$). These results are concordant with existing literature [24, 84, 85]. It is likely that the trends observed in both genders in FF1 would be significant as well, had the sample size been greater.

We investigated whether or not a variety of lifestyle factors had any influence on Hb and ferritin levels in our sample population. In the FF1 data, we found that males who were physically active 4-6 days a week had lower mean hemoglobin than those who were active 2-3 days per week (.49, 95% CI .09 - .90, $p = .007$). As this finding was not consistent through both surveys, we question the reliability of our results. However, lower Hb values and increasing prevalence of anemia among strenuously trained male adolescents has been described in one study [86]. Vitamin and mineral supplements also seemed to have an effect on Hb values in FF1, but not in FF2. In females, there was no significant variance in Hb when groups were compared pairwise. In males, however, we found that those who did not or only sometimes took vitamin/mineral supplements had higher mean hemoglobin values than those who did take vitamin/mineral supplements. Intake of supplementary vitamin C would be expected to increase iron stores, but as we do not have any information on the contents of the supplements the participants have been taking, any attempt at explaining the observed effect would be mere speculation. None of the other lifestyle factors were found to influence Hb levels.

Ferritin values were, in FF1, found to increase with frequency of snacking and sweets consumption in males and with exercise frequency in females, and in FF2 increase with junk food consumption and decrease with vitamin/mineral supplement use in males. Males who rated their own health better were also found to have lower ferritin levels in FF2. Overall, none of the lifestyle factors had consistent influence on Hb or ferritin values in both FF1 and FF2, therefore we consider the reliability of these results questionable. Further research is

warranted to conclude on whether or not the abovementioned lifestyle factors have any influence on Hb or ferritin values in adolescents. Research from the NHANES study on iron deficiency in obese, female adolescents did not find dietary iron intake, age of menarche, poverty status or physical activity to be independent predictors of iron deficiency [82].

In FF1, Hb and ferritin values did not differ significantly between females according to their menstrual cycle length or regularity of menses. Similar data was not yet available in the FF2 survey. However, other menstrual factors, such as heaviness of flow, may contribute to depletion of iron stores, but data was not available to assess this question.

There are several limitations to this study. Firstly, the sample population decreased from 1038 to 694 participants from FF1 to FF2. Due to restrictive inclusion criteria the sample available for statistical analyses further shrunk to 813 and 561 participants, FF1 and FF2 respectively, and the number of participants that met our inclusion criteria for both samples was 505. However, we believe our sample sizes are sufficient to produce reliable research, albeit suffering somewhat decreased statistical power. Second, our only criteria for diagnosing iron deficiency was serum ferritin < 12 μg/L. Other studies have utilized higher cut-off values (ferritin < 13, 15 or 16 μg/L), or a ferritin cut-off value in combination with different blood tests (for example serum protoporphyrin, transferrin saturation and serum iron) [27, 37, 48, 87]. However, using 12 μg/L as cut-off value is conservative and we are confident that we do not over-report numbers of iron deficiency in this population. Serum ferritin is known to accurately reflect body iron stores when there is no concurrent inflammation [88]. In inflammatory states, such as obesity and chronic disease, however, ferritin may be elevated and therefore less useful to assess iron status. In the overweight and obese that tend to have a higher mean ferritin value it may give a falsely low prevalence of ID. Ferritin also rises in the event of an infection, hence we excluded participants with a CRP of 10 or more. However, it has been noted that ferritin parallels CRP concentrations in acute and chronic disease, but with recovery there is a sharp drop in CRP levels while ferritin recedes more slowly [89, 90]. This could result in under-reporting of occurrence of iron deficiency. Thirdly, the use of iron supplements was not assessed. We do not know whether a larger proportion of those who had ID in FF1 used iron supplements before FF2, compared to those who were not iron deficient. As the greatest

increase in Hb levels between surveys was seen in participants who had IDA in FF1, this is a plausible explanation. And finally, smoking status was not assessed in our research. As smokers are known to have higher Hb-levels than non-smokers, this could lead to increased mean Hb levels.

8 CONCLUSIONS

Iron deficiency is prevalent in approximately one of six female adolescents in our population according to WHO criteria, and seems to prevail through a considerable part of adolescence in some youths. Due to the potentially harmful effects of iron deficiency anemia, this population group should be given special attention to ensure iron requirements are met. Further research is warranted on the effect of lifestyle factors in this population group. In the female adolescent population of Northern Norway, the WHO criteria for anemia do not correspond with the 2.5 percentile for hemoglobin, whereas it does in males. Use of the WHO criteria in clinical practice may lead to medicalization of adolescents with physiologically low Hb levels. Future research on anemia in this population should apply the 2.5 percentile as cut-off value for anemia in female adolescents.

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