Faculty of Health Sciences

The relation between iron deficiency and anaemia and HbA1c levels in Norwegian adolescents. The Tromsø Study: *Fit Futures*

Lise Dyhrberg Pettersen

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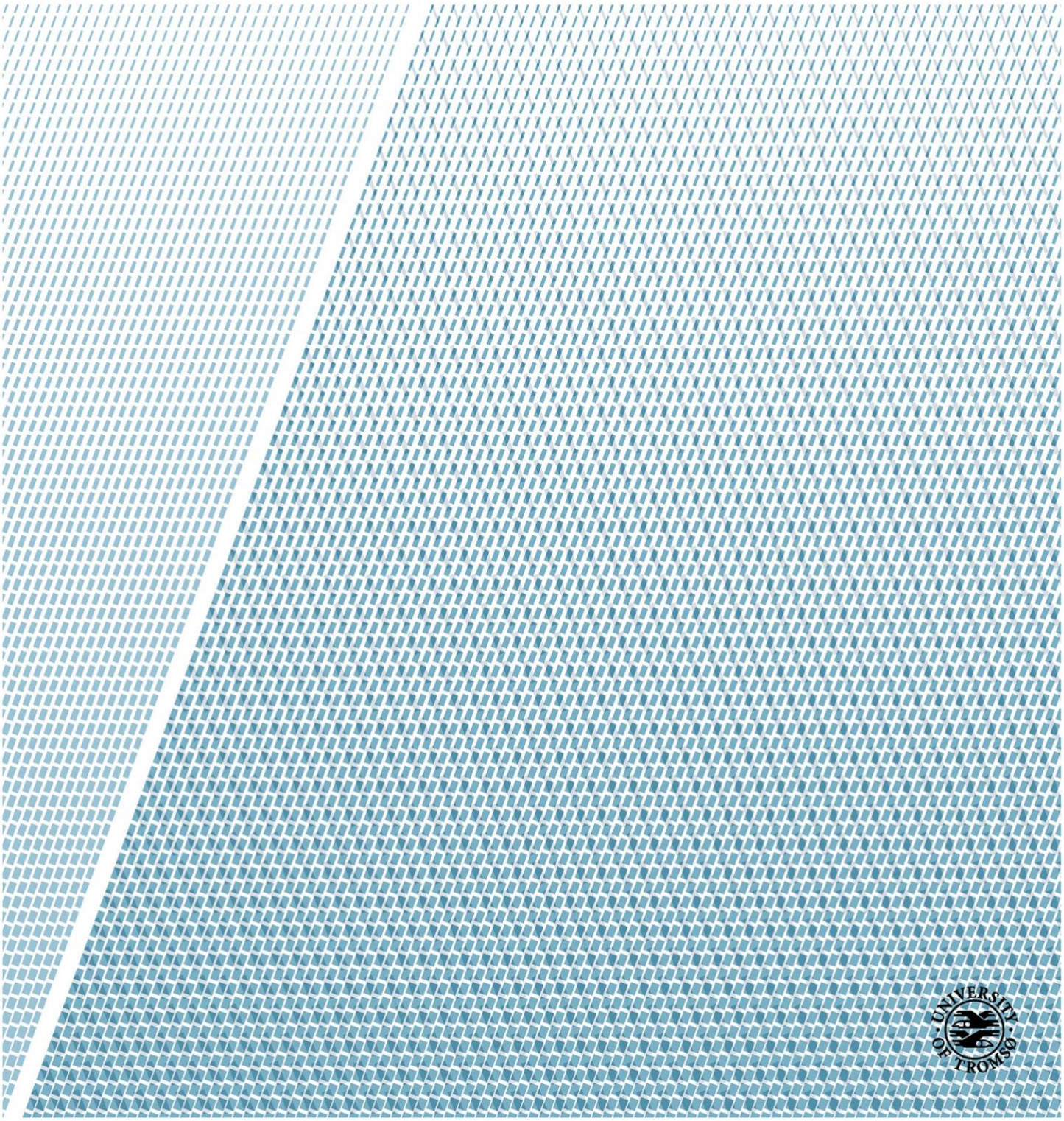

Preface

Purpose of the thesis

The purpose of the thesis is to study the relation between iron deficiency, iron deficiency anaemia and non-iron-deficiency anaemia and HbA1c levels, in Norwegian adolescents without diabetes. Can abnormalities in iron- and/or erythrocyte indices, which are frequent during adolescence, cause spuriously abnormal values of HbA1c in adolescents? This study will hopefully result in contributing information that may expand awareness concerning the use of HbA1c assays in adolescents.

The prehistory of the project

The medical fifth year thesis by Christine Bjørsvik from 2015 (1) assessed the normal distribution and determinants of HbA1c in non-diabetic Norwegian adolescents, by performing an analysis of data from the Tromsø Study: *Fit Futures 1* (TFF1). As will be discussed later, it was the finding of higher HbA1c in anaemic adolescents vs. non-anaemic adolescents, that gave the idea of exploring this issue further.

Resources

No extra resources demanding further applications to the administration at Faculty of Health Sciences was needed.

Project contributors

The supervisor of this master thesis is associate professor Guri Grimnes (MD., Endocrinologist, UNN Tromsø). A second collaborator is professor Trond Flægstad (MD., Paediatrician, UNN Tromsø). The supervisor's scientific experience and medical knowledge has been valuable for the project. She has contributed with the project idea, applications for making data disposable from the TFF1, guidance in the use of statistical analyses, and helped along the way with the project. The second collaborator has received drafts of the master thesis and contributed with a paediatric point of view. A great thank you to Guri Grimnes and Trond Flægstad for their essential contribution to this project.

The working progress

The work with the master thesis started in September 2016. When project contributors and subject for the master thesis was decided, the project protocol could be written and finalized. The project protocol and applications to REK Nord (2016/2072) and the Tromsø Study (the Data and Publication Committee) was submitted by the end of October 2016.

In January-May the following year, alongside the studies and exams, the work with the master thesis consisted of getting updated on relevant literature. In June-August 2017 I worked with revising my knowledge in statistics and SPSS, and initiated the performance of statistical analyses. In September 2017-June 2018 the work consisted of performing statistical analyses, further writing and finalizing the thesis for submission.

Lise Dyhrberg Pettersen

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Summary

Introduction

Diabetes represents a large global health challenge, with increasing prevalence – also in adolescents. HbA1c is used as a diagnostic test for diabetes. Iron deficiency and anaemia are frequent during adolescence, and it is of clinical value to be aware of possible coherence between these and HbA1c in adolescents. To shed light on this issue, the aim was to study the relation between iron deficiency, iron deficiency anaemia and non-iron-deficiency anaemia and HbA1c levels, in Norwegian adolescents without diabetes.

Methods

Data from The Tromsø Study: *Fit Futures 1*, where approximately 1000 adolescents from first year of high school attended throughout the schoolyear of 2010/2011. There are data on iron status, blood count and HbA1c, as well as questionnaire information on anthropometric measures, diabetes, use of antibiotics, etc. In a cross section design, the relation between iron deficiency and anaemia and HbA1c was described. IBM SPSS Statistics version 24 was used for statistical analyses in this study. Descriptive statistics was used, and glycaemic measures between groups were compared by parametric comparison (independent samples t-test) and non-parametric comparison (independent-Samples Mann-Whitney U test) as adequate.

Important results

This study found significant higher HbA1c levels in adolescents with anaemia and iron deficiency, compared to adolescents without these conditions, respectively. Some interesting gender differences in the subgroups of anaemia and ID were also found. It was not found significant higher fasting glucose levels and HOMA-IR in iron deficient- or anaemic adolescents.

Conclusion and consequences

The study shows that HbA1c must be assessed with caution in adolescents, when anaemia or iron deficiency are present. This is of clinical importance because of the relatively high occurrence of these conditions, especially in girls at this age.

Introduction

Adolescents

Adolescents constitute an age group with an estimated global prevalence of approximately 1.2 billion worldwide, and even though they make up a large share of the world population, the research on adolescent health has shown a tendency to lay behind research on both children and research on adults (2). WHO defines adolescents as individuals in the period of time between childhood and adulthood, more specifically between the age of 10-19 years (3).

Well known, this transitional period is a vulnerable time in life, where these young individuals go through biological and social changes. They are vulnerable to lifestyle and environmental effects, potentially giving permanent changes on their life course and risk profiles. Hence, adolescents constitute dynamic individuals undergoing transition, and further research on this patient group is of interest.

Diabetes

Diabetes mellitus (DM) is a heterogenic disorder characterized by hyperglycaemia caused by deficient insulin secretion, insulin action, or a combination. Prolonged hyperglycaemia is associated with long term complications involving several organ systems. The majority of DM cases can be subdivided into type 1 and type 2, the first one characterized by absolute lack of insulin secretion and the second with insulin resistance accompanied by progressive deficiency of insulin secretion (4, 5). DM may be diagnosed by either HbA1c $\geq$ 6.5 %, fasting plasma glucose level $\geq$ 7.0 mmol/l, or a plasma glucose level $\geq$ 11.1 mmol/l – either random or after oral glucose tolerance test (2-hour plasma glucose level after a 75g oral glucose load). It must be noted that, if absence of hyperglycaemic symptoms, a second test is required to confirm the diagnosis (5).

Studies from 2016 show that the estimated number of adults living with DM worldwide has nearly quadrupled from 1980 to 2014, with a rise from 108 million to 422 million in this period (6). The diabetic burden has increased faster in low- and middle-income countries compared to high-income countries (6). An increase in DM type 2 related to obesity has been found in adolescents (7).

Prediabetes is a term for the presence of higher-than-normal blood glucose levels, but not high enough to meet the diagnostic criteria for DM. It is interpreted as a state of high risk of developing DM and associated complications (8). Despite the lack of general consensus regarding prediabetes, however, with the presence of either HbA1c 5.7-6.4 %, fasting plasma glucose level 5.6-6.9 mmol/l or after oral glucose tolerance results (with 2-hour plasma glucose level after a 75g oral glucose load) of 7.8-11.0 mmol/l, prediabetes may be considered (5). The prevalence of prediabetes among adults in the UK rose from 11.6 % to 35.3 % from 2003-2011 (8). Population based studies on adolescents aged 12-19 in the US show an increase in prediabetes from 7 % in 1999-2000 to 13.1 % in 2005-2006 (9, 10).

With increasing prevalence of DM, one would assume a coherent increase in the use of its diagnostic tests, such as glycated haemoglobin (HbA1c).

HbA1c

HbA1c became in 2011 recommended as a diagnostic tool for DM by the World Health Organization (WHO), with a cut point of 6.5 % (≥ 6.5 %) for diagnosis, but with a value beneath this cut point unable to exclude DM when other glucose tests are used (11).

The HbA1c concentration emerge from a non-enzymatic process in which glucose attaches to haemoglobin (Hb). Nathan et al found, in a group of both diabetic- and non-diabetic individuals, a strong correlation between the HbA1c results at weeks 8 and 12 with the continuous glucose monitoring results for the past 8 and 12 weeks, a time period which corresponds to the approximate life span of an erythrocyte (12). In addition, a strong correlation between HbA1c and development of DM-related complications has been found (13). A decrease in HbA1c is likely to diminish the risk of DM-related complications in type 2 diabetic patients (14).

In addition to possessing diagnostic value, HbA1c is also used for assessment of glycaemic control and used in diabetic therapy together with other glycaemic control measurements (15).

The reliability on HbA1c has been questioned, not only regarding the use in children and adolescents (16, 17), but also regarding the influencing factors capable of giving increased or decreased HbA1c results, some of them adapted by the WHO (11) from Gallagher et al (18) including erythropoiesis, altered Hb, glycation, erythrocyte destruction and assays.

In the Standards of Medical Care in Diabetes from 2014 the American Diabetes Association (ADA) acknowledges the questions on the use of HbA1c in diagnosing children and adolescents, but concludes that they continue recommending HbA1c for diagnosis in these age groups (16, 17).

Iron deficiency, iron deficiency anaemia and non-iron-deficiency anaemia

Iron deficiency (ID) is prevalent in a significantly large share of the population in almost every country in the world, with every age group being vulnerable, thus, ID and anaemia have been increasingly emphasized as a global health problem (19).

ID may be explained as a condition with an absence of mobilizable iron stores and signs of reduced iron supply to the body tissues, including the erythrocytes and their precursors (19). The best considered test for detection of ID is serum ferritin levels, with a widely accepted cut point of <15 μg/l, when measured in the absence of infection (19). It must always be considered that ferritin levels can be increased by any infectious or inflammatory process, and can only be interpreted when these conditions are absent (19).

In developed countries, the cause of ID is usually chronic blood loss, especially uterine and gastrointestinal losses, and dietary deficiency rarely stands as the only cause (20). During adolescence, especially females after menarche often do not consume enough iron to fulfil the needs caused by physiological losses (i.e. uterine loss), often leading to an ID peak among these young females (19).

Increased demands of iron in particular clinical groups, such as adolescents and in menstruating women, account for a high risk of iron deficiency anaemia (IDA) (20). IDA represents the most severe end of the iron depletion scale. According to WHO's criteria for anaemia, it is considered to be present when Hb is less than 120 g/l in women (non-pregnant, above 15 years) and less than 130 g/l in men (above 15 years) (19).

Anaemia, as a general term for reduction in Hb concentration of the blood below normal for age and sex, also include conditions with normal iron levels; non-iron deficiency anaemia (non-ID anaemia). The microcytic, hypochromic anaemias include IDA, thalassaemia, anaemia of chronic disease (some cases), lead poisoning, and sideroblastic anaemia (some cases). The normocytic, normochromic anaemias include many haemolytic anaemias, anaemia of chronic disease (some cases), a consequence of acute blood loss, renal disease,

mixed deficiencies, and bone marrow failure (e.g. as consequence of chemotherapy or infiltration by carcinoma, etc.). The macrocytic anaemias include megaloblastic (vitamin B12 or folate deficiency) and non-megaloblastic (alcohol, liver disease, myelodysplasia, aplastic anaemia, etc.) anaemias (20).

Known association between abnormal iron-/erythrocyte indices and HbA1c

The International Expert Committee reported in 2009 that conditions capable of affecting erythrocyte turnover, may also affect HbA1c concentrations, and notifies clinicians to be aware of this fact (21, 22).

The WHO consultation states that iron is one of the factors capable of influencing HbA1c measurement, in a way that ID may lead to an increase in HbA1c, whereas iron administration may lead to a decrease in HbA1c (11).

English et al made a systematic review in 2015 (23) on the effect of anaemia and abnormalities in erythrocyte indices on HbA1c levels in adults without DM. This review found that HbA1c is likely to be affected by ID and IDA with a spurious increase in HbA1c values. Kim et al found significant higher HbA1c levels in iron deficient women vs. non-iron deficient women (n=10 535), independent of fasting glucose levels, even though no association was found at HbA1c levels higher than 6.0 % (this possibly due to smaller groups) (24). Ford et al (n=8296) found that individuals with normal Hb and low iron indices had borderline higher HbA1c levels than individuals with normal Hb and normal iron indices, though the study was limited by few cases of ID and/or anaemia (25). Koga et al (n=104) found that both ID and IDA were associated with upward shifts in HbA1c levels, and it was seen a significant negative association between iron metabolism indices and HbA1c (26).

In addition, more recent studies have shown results worth mentioning. A case control study from 2016 (n=122) concluded that the effect of IDA on HbA1c levels is dependent on the degree of anaemia. HbA1c levels were significant higher in moderate and severe anaemia, but mild anaemia did not show significant effects on HbA1c (27).

A cross-sectional study from 2017 (n=150) found statistically significant higher HbA1c in the IDA-group compared to the non-anaemic group of individuals, and that the HbA1c level increased when the severity of anaemia worsened (28).

However, another review and meta-analysis from 2015 by Cavagnolli et al (n=10773) showed no significant differences in HbA1c in the presence of ID or IDA (29). Though, they found an inter-study heterogeneity among the four studies included. Considering only the studies of Kim et al (24) and Son et al (30), the difference in HbA1c was about zero but not significant, but considering only the studies of Coban et al (31) and Christy et al (32), it was observed a significant positive difference. Since the available studies showed opposite results, the analysis remained inconclusive for the effect of ID and/or IDA on HbA1c in non-diabetic individuals.

A recent article by Renz et al (n=231) from 2018 concluded that iron supplementation during pregnancy did not affect HbA1c levels and had no clinical impact on the interpretation of results in the absence of anaemia or the presence of mild anaemia, even though interpretation of HbA1c results in pregnant women still requires caution during iron therapy with moderate and severe anaemia (33). Thus, the findings so far are inconclusive.

Regarding non-ID anaemia and relation to HbA1c, English et al also found that non-ID anaemia may lead to a decreased HbA1c value (23). Ford et al (n=8296) found downward shifts in HbA1c when low Hb and normal iron indices were present, compared to normal individuals (with normal Hb and normal iron status) (25).

To my knowledge, only a minority of studies on the relation between HbA1c and abnormal iron-/erythrocyte indices are based on young patients.

A recent two-center prospective observational study from 2018 (n=227) studied iron status and its association with HbA1c levels in Dutch children with DM type 1. They found that HbA1c levels were not associated with ID, which may be explained by the relatively mild deprived iron status in the patients (34).

Other studies on young patients include one study by Tarim et al (35) based on paediatric patients (n=68), another study by Aslan et al (36) based on patient groups with mean ages 10±12 years, 17±15 years and 30±13 years (n=98), a study by Hardikar et al (37) based on young adults with mean age 21.6 years (n=116) and finally, one study by El-Agouza et al (38) based on university students (n=730). None of these studies were based exclusively on adolescents. Thus, to my knowledge, most research on the relation between iron-/erythrocyte indices and HbA1c is based on adults, and it is therefore difficult to interpret these results as applicable on other age groups, such as adolescents.

Interestingly, with little research on this issue in adolescents, in the medical fifth year thesis by Christine Bjørsvik (1) based on data from the Tromsø Study: *Fit Futures 1*, it was found higher HbA1c concentrations in anaemic adolescents (aged 15-17 years) vs. non-anaemic adolescents, where anaemia was defined by <12 g/dl in girls and <13 g/dl in boys (data not published).

Presentation of issue

The finding of higher HbA1c level in anaemic vs. non-anaemic adolescents (1) created the idea to further study the relation between iron- and erythrocyte indices and HbA1c levels in adolescents. An association has been indicated, but the studies regarding this issue are conflicting and restricted to small patient groups, and further studies are needed to contribute to clarity on this issue (23). It was therefore of interest to investigate this issue further, especially in adolescents.

The issue was refined to study the relation between ID, IDA and non-ID anaemia and HbA1c levels in Norwegian adolescents. Only non-diabetic individuals were included, to avoid abnormal HbA1c- and glucose measurements. To study if a difference between HbA1c levels, depending on anaemia or iron status, could represent differences in glucose handling rather than erythrocyte characteristics, the fasting glucose and insulin resistance between the groups were also compared.

Materials and method

Nomenclature

TFF1: The Tromsø Study: *Fit Futures 1*

Hb: Haemoglobin

HbA1c: Glycated haemoglobin

ID: Iron deficiency

IDA: Iron deficiency anaemia

Non-ID anaemia: Non-iron-deficiency anaemia

n: Number of individuals

IQR: Interquartile range

SD: Standard deviation

Study population and data material

The Tromsø Study: *Fit Futures 1* (TFF1) is a cross-sectional population based study of adolescents, with focus on lifestyle and health. TFF1 is a collaborative effort between the University Hospital of Northern Norway (UNN, Tromsø), UiT The Arctic University of Norway (UiT, Tromsø) and the Norwegian Institute of Public Health (FHI, Oslo). TFF1 was performed in first year upper secondary school students from the two neighbour municipalities Tromsø and Balsfjord in Northern Norway in 2010/2011. The school nurse/contact person gave oral and written information about the study to potential participants. Information about the study was also available for the students and parents online. All students interested in participating in the study signed up online with a personal code. From the invited cohort, 1038 adolescents attended (508 girls, 530 boys), which gave a participation rate of 92,9 %. The examinations took place in the Clinical Research Unit at UNN Tromsø, performed by trained nurses. More details on the study population of TFF1 is found in (39).

Some exclusions were made from the data material, before the analyses were initiated. Diabetic individuals were excluded by questionnaire. Individuals who had answered "Yes"

(=1) on the question “Do you have diabetes?” were excluded. “Sysmis” on the variable “Do you have diabetes?” were interpreted as not having DM (=0), to include possible participants who had not answered. The WHO definition of adolescents 10-19 years was used (2). Individuals without HbA1c, Hb and ferritin measurements were excluded.

Methods and examinations

The methods of the present study consist of statistical analyses of data material from TFF1, a cross-sectional population based study of adolescents. The examinations in TFF1 were performed by trained nurses, and existed of physical examinations (e.g. height, weight), blood samples (e.g. Hb, ferritin, HbA1c) and questionnaire (self-reported e.g. diabetes, infection, intake of antibiotics).

During the medical examination, the adolescents were weighed and measured wearing light clothes and no shoes. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm by using a Jenix DS 102 stadiometer (Dong Sahn Jenix, Seoul, Korea. Current diabetes status of the participants was obtained by questionnaire asking, “Do you have diabetes?”. Current infection status was obtained by questionnaire asking, “Do you have any kind of infection (e.g. respiratory, urinary tract, skin)?” and “Have you taken any antibiotics (tablets or oral suspensions, nasal ointments, eye drops or eye ointment applicated in the nose/eye) the last 24 hours?”. HbA1c EDTA whole blood was determined by high performance liquid chromatography using an automated analyser (Variant II®, Bio-Rad Laboratories Inc., Hercules, CA, USA). Haemoglobin was determined by photometric method using an automated hematology system (Sysmex XE2100 (Sysmex, Kobe, Japan) and Coulther LH 750 (Beckman Coulter, Brea, California, USA)). Ferritin was measured by Modular E170 from Roche Diagnostic. CRP was measured by Modular P from Roche Diagnostic.

The participants in the highest 10-percentile of HbA1c in TFF1 and an equal-sized randomly selected group from the remaining participants were invited to a follow-up survey where an oral glucose tolerance test (OGTT) was performed and a total of 200 attended. The fasting glucose and fasting insulin from this group were used for analyses in this present study. Serum glucose measurements were performed using the hexokinase assay (Modular P, Roche Diagnostics, Mannheim, Germany). Serum insulin were measured using an electrochemiluminescence sandwich immunoassay (ECLIA, Modular E, Roche Diagnostics, Mannheim, Germany).

Homeostasis model assessment insulin resistance (HOMA-IR) was used to find the insulin resistance. The following equation was used: HOMA-IR = (I_0 x G_0) / 22.5, where I_0 is fasting serum insulin (mIU/l) and G_0 is fasting serum glucose (mmol/l) (40, 41). The fasting serum insulin had to be converted from pmol/l to mIU/l (42) in a new equation: HOMA-IR = ((I_1/6,945) x G_0) / 22.5, where I_1 is fasting serum insulin (pmol/l) and G_0 is fasting serum glucose (mmol/l).

Statistical methods

IBM SPSS Statistics version 24 was used for statistical analyses in this study. Descriptive statistics was used, and glycaemic measures between groups were compared by parametric comparison (independent samples t-test) and non-parametric comparison (independent-Samples Mann-Whitney U test) as adequate. Normal distribution was checked for by visual inspection of histograms. The normally distributed independent variables are presented as mean (+/- SD) and the not normally distributed independent variables are presented as median and interquartile range (IQR). Gender-specific analyses were used, because of the known difference in occurrence of anaemia and ID. P-value <0,05 was used as significance level.

Variables and variable definitions

We used the following variables in the analyses: sex, age (years), body height (cm), body weight (kg), haemoglobin (g/dl), ferritin (µg/L), HbA1c (%), fasting glucose (fasting serum, mmol/l), fasting insulin (fasting serum, pmol/l), high-sensitive CRP (serum), known DM (yes or no), infection (yes or no), intake of antibiotics during the last 24 hours (yes or no).

Table 1 presents the variables of haematological conditions made in SPSS. Based on ferritin and Hb, the participants were categorized into the following haematological conditions: anaemia (with/without ID), ID (with/without anaemia), IDA, non-ID anaemia and ID (without anaemia). Hence, the participants' HbA1c levels could be compared between e.g. anaemic and non-anaemic adolescents.

Ferritin is also known as an acute phase protein. To avoid confounding by infectious state, we also performed sensitivity analyses after exclusion of infection factors. Therefore, there were made additionally two groups regarding ID: ID; infection excluded and ID; infection and anaemia excluded.

Table 1: Variable definitions in girls and boys. Hb=haemoglobin. ID=iron deficiency. IDA=iron deficiency anaemia.

	Girls	Boys
Anaemia (with/without ID)	Hb <12 g/dl	Hb <13 g/dl
IDA	Ferritin <15 µg/L and Hb <12 g/dl	Ferritin <15 µg/L and Hb <13 g/dl
Non-ID anaemia	Ferritin ≥15 µg/L and Hb <12 g/dl	Ferritin ≥15 µg/L and Hb <13 g/dl
ID (with/without anaemia)	Ferritin <15 µg/L	Ferritin <15 µg/L
ID (without anaemia)	Ferritin <15 µg/L and Hb ≥12 g/dl	Ferritin <15 µg/L and Hb ≥13 g/dl
ID; infection excluded	Ferritin <15 µg/L and infection today=no, antibiotics=no and high sensitive CRP <5.	Ferritin <15 µg/L and infection today=no, antibiotics=no and high sensitive CRP <5.
ID; infection and anaemia excluded	Ferritin <15 µg/L, Hb ≥12 g/dl, infection today=no, antibiotics=no and high sensitive CRP <5.	Ferritin <15 µg/L, Hb ≥13 g/dl, infection today=no, antibiotics=no and high sensitive CRP <5.

Ethical conflicts

The participants have signed an informed consent prior to the examinations, and if the participants were below the age of 16 years at the time of examination, the parents also had to sign. The participants could withdraw from the study at any time. The data collection and storage from *Fit Futures 1* has been approved by REK and by the Data Inspectorate. A separate approval for this study by REK was obtained (2016/2072).

Implementation of the thesis

The first task was to find and study relevant literature, to find out what todays knowledge showed on the issue. Finding relevant literature was made by searching for key words such as “hba1c anaemia” and “hba1c iron deficiency” in PubMed. The project protocol was written and submitted after approvals from REK. Following this, the performance of statistical analyses was initiated. The results were brought into text, and the thesis was finalized.

Results

Basic characteristics of the study population

Prior to the exclusions, the dataset consisted of 1038 participants. After the exclusion of participants with diabetes (n=4), participants >19 years (n=36) and those with missing blood samples on HbA1c (n=171), ferritin (n=1) and Hb (n=4), the final dataset included 824 participants (377 girls and 447 boys). Two participants had two of the exclusion criteria.

The basic characteristics of the study population (n=824) are presented in table 2. The decimals were rounded up/down to the nearest tenth/hundredth. None of the participants had HbA1c ≥6.5 %.

Table 2: Basic characteristics of the study population. HbA1c=glycated haemoglobin. SD=standard deviation. IQR=interquartile range. Min=minimum. Max=maximum.

	Girls	**Boys**
Age (years)	Median: 16,0 (IQR 0) Min: 15,0 Max: 19,0	Median: 16,0 (IQR 0) Min: 15,0 Max: 19,0
Height (cm)	Mean: 164,7 (SD 6,6)	Mean: 177,1 (SD 6,7)
Weight (kg)	Median: 58,4 (IQR 11,35)	Median: 68,1 (IQR 16,40)
Haemoglobin level (g/dL)	Mean: 12,7 (SD 1,0) Min: 6,0 Max: 14,7	Mean: 14,6 (SD 0,9) Min: 11,0 Max: 17,4
Ferritin level (ug/L)	Median: 25 (IQR 26) Min: 2 Max: 160	Median: 49 (IQR 42) Min: 5 Max: 231

HbA1c level (%)	Mean: 5,28 (SD 0,30) Range: 2,40 Min: 3,90 Max: 6,30	Mean: 5,29 (SD 0,26) Range: 1,80 Min: 4,10 Max: 5,90

Prevalence of ID, IDA and non-ID anaemia

The prevalence of the different haematological conditions in the study population is presented in table 3. A noticeable feature of the study population is that the prevalence of the haematological conditions is clearly higher in girls compared to boys.

Table 3: Prevalence (%) of ID, IDA and non-ID anaemia in girls, boys and the total study population. The percent is rounded up to the nearest number with one decimal. ID=iron deficiency. IDA=iron deficiency anaemia.

	Prevalence in girls	Prevalence in boys	Total prevalence study population
Anaemia (with/without ID)	19,9 % (75/377)	2,9 % (13/447)	10,7 % (88/824)
IDA	10,3 % (39/377)	1,1 % (5/447)	5,3 % (44/824)
Non-ID anaemia	9,5 % (36/377)	1,8 % (8/447)	5,3 % (44/824)
ID (with/without anaemia)	23,6 % (89/377)	3,8 % (17/447)	12,9 % (106/824)
ID (without anaemia)	13,3 % (50/377)	2,7 % (12/447)	7,5 % (62/824)
ID; infection excluded	25,3 % (77/304)	4,6 % (16/351)	14,2 % (93/655)

ID; infection and anaemia excluded	17,4 % (42/242)	3,2 % (11/339)	9,1 % (53/581)
Healthy (no ID, no anaemia)	66,8 % (252/377)	94,4 % (422/447)	81,8 % (674/824)

HbA1c in relation to ID, IDA and non-ID anaemia

The HbA1c variable was assessed as normally distributed, and therefore parametric comparison was used. The HbA1c level in adolescents with anaemia was compared to the HbA1c level in adolescents without anaemia, and so on. The results are presented in table 4.

Significant higher HbA1c levels was found in both genders in several groups; anaemia (with/without ID), ID (with/without anaemia) and ID; infection excluded.

In the anaemia (with/without ID) group, the mean HbA1c was 5,44 % in girls vs. 5,24 % in girls without this condition (p-value 0,000). For boys in the same group, the mean HbA1c was 5,45 % vs. 5,28 % in boys without this condition (p-value 0,026). In the ID (with/without anaemia) group, the mean HbA1c was 5,34 % in girls vs. 5,26 % in girls without this condition (p-value 0,020). For boys in the same group, the mean HbA1c was 5,49 % vs. 5,28 % in boys without this condition (p-value 0,001). In the ID; infection excluded group, the mean HbA1c was 5,34 % in girls vs. 5,24 % in girls without this condition (p-value 0,010). For boys in the same group, the mean HbA1c was 5,48 % vs. 5,28 % in boys without this condition (p-value 0,003).

In girls, it was found significant higher HbA1c levels in non-ID anaemia and IDA, compared to girls without these conditions, respectively. The mean HbA1c was 5,38 % in girls with non-ID anaemia vs. 5,24 % in girls without this condition (p-value 0,006). The mean HbA1c was 5,49 % in girls with IDA vs. 5,24 % in girls without this condition (p-value 0,000). The mean HbA1c was higher in boys in these groups as well, but not statistical significant.

In boys, statistical significant higher HbA1c levels was found in ID (without anaemia) and ID; infection and anaemia excluded, compared to boys without these conditions, respectively. The mean HbA1c was 5,49 % in boys with ID (without anaemia) vs. 5,27 % in boys without this condition (p-value 0,005). The mean HbA1c was 5,47 % in boys with ID; infection and

anaemia excluded vs. 5,27 % in boys without this condition (p-value 0,013). The mean HbA1c was slightly lower for girls in the ID (without anaemia) group and slightly higher for the ID; infection and anaemia excluded group, compared to girls without these conditions, respectively, but none of them were statistical significant.

The exclusion of infection did not affect the significance of the results. The groups ID (with/without anaemia) and ID; infection excluded, both showed significant difference in HbA1c for both girls and boys. The groups ID (without anaemia) and ID; infection and anaemia excluded, both showed significant difference in HbA1c in boys, but not in girls.

Table 4: HbA1c levels compared among the haematological groups in girls and boys. Parametric analysis; Independent samples t-test. SD=standard deviation. HbA1c=glycated haemoglobin (%). ID=iron deficiency. IDA=iron deficiency anaemia.

		Girls		**Boys**	
		Mean HbA1c (SD)	P-value	Mean HbA1c (SD)	P-value
Anaemia (with/without ID)	Yes	5,44 (0,29)	0,000	5,45 (0,21)	0,026
	No	5,24 (0,29)		5,28 (0,27)	
IDA	Yes	5,49 (0,26)	0,000	5,48 (0,26)	0,083
	No	5,24 (0,29)		5,27 (0,26)	
Non-ID anaemia	Yes	5,38 (0,30)	0,006	5,43 (0,20)	0,108
	No	5,24 (0,29)		5,27 (0,26)	
ID (with/without anaemia)	Yes	5,34 (0,31)	0,020	5,49 (0,26)	0,001
	No	5,26 (0,29)		5,28 (0,26)	
	Yes	5,23 (0,31)	0,837	5,49 (0,27)	0,005

ID (without anaemia)	No	5,24 (0,29)		5,27 (0,26)	
ID; infection excluded	Yes	5,34 (0,31)	0,010	5,48 (0,26)	0,003
	No	5,24 (0,29)		5,28 (0,26)	
ID; infection and anaemia excluded	Yes	5,23 (0,31)	0,913	5,47 (0,27)	0,013
	No	5,23 (0,29)		5,27 (0,26)	

Fasting glucose in relation to ID, IDA and non-ID anaemia

The variable "fasting glucose" was assessed as not normally distributed. Therefore, non-parametric comparison was used for this variable. Fasting glucose was compared among the haematological groups, in girls and boys. It was compared between anaemic adolescents vs. non-anaemic adolescents, and so on. The null hypothesis was retained in all the analyses, hence, no significant difference in fasting glucose between the groups was found.

HOMA-IR in relation to ID, IDA and non-ID anaemia

The variable "HOMA-IR" was assessed as not normally distributed. Therefore, non-parametric comparison was used for this variable. HOMA-IR was compared among the haematological groups, in girls and boys. It was compared between anaemic adolescents vs. non-anaemic adolescents, and so on. The null hypothesis was retained in all the analyses, hence, no significant difference in HOMA-IR was found between the groups.

Discussion

Discussion of important findings

The aim was to study whether there was any association between HbA1c and markers of anaemia and ID in adolescents. The findings are discussed in the following.

For both genders, statistically significant higher HbA1c levels was found in anaemia (with/without ID), ID (with/without anaemia) and ID; infection excluded, compared to girls and boys without these conditions, respectively. The difference in mean HbA1c (%) was about 0,1-0,2 % between adolescents in these groups and adolescents without these conditions, respectively. The differences in HbA1c are not conspicuously large, and one would assume that such differences would probably not be of great meaning in a regular, diagnostic situation. Still, there is a difference. Theoretically, it could potentially be of clinical value with the presence of borderline HbA1c values in a diagnostic situation. Thus, one might think that it could be favourable to exclude the presence of anaemia or ID, in a situation of borderline HbA1c values.

In girls, we found statistical significant higher HbA1c levels in non-ID anaemia and IDA, compared to girls without these conditions, respectively. The mean HbA1c was higher in boys as well in these groups, but not statistically significant. There were only a few boys with non-ID anaemia and IDA, and perhaps is this the cause of the not significant results in boys.

In boys, statistical significant higher HbA1c levels was found in ID (without anaemia) and ID; infection and anaemia excluded, compared to those without these conditions, respectively. The mean HbA1c was higher for girls with ID; infection and anaemia excluded as well, but not statistical significant, even though the prevalence was clearly higher in girls vs. boys.

The significant higher HbA1c levels in the groups of non-ID anaemia, IDA, ID (without anaemia) and ID; infection and anaemia excluded, also showed a difference in mean HbA1c (%) of about 0,1-0,2 %.

Regarding fasting glucose levels, no significant difference was found between the groups of haematological conditions and those without these conditions, respectively. Regarding HOMA-IR, no significant difference was found between the groups either. If the higher HbA1c levels in the groups with ID and anaemia are caused by a real glucose dysregulation,

one might wonder why a similar association between fasting glucose/HOMA-IR and the groups were not found. Finding that only HbA1c was significant associated with anaemia and ID, as opposed to other determinants of glucose regulation, supports that it is some other cause in the erythrocyte, rather than a real dysregulation of glucose, that owes the association.

As discussed, earlier studies have found significant association between HbA1c and iron- and erythrocyte indices. To my knowledge, the major part is based on adults, as opposed to the present study which includes adolescents exclusively. In the present study it was found significant higher HbA1c levels in anaemic adolescents, as it was found in the medical fifth-year thesis of Bjørsvik (1), also based on data material from TFF1. The present study went further on this finding, by also assessing ID and different types of anaemia and their relation to HbA1c levels.

Even though most earlier studies on this subject seemingly are based on adults, it is of interest to discuss their findings despite of different age groups. Are there similarities in the findings between studies on adults and the present study's findings? The finding of significant higher HbA1c levels in anaemic girls and boys in this study, share similarities to a case-control study where it was found that HbA1c levels were significant higher in moderate and severe anaemia, but that mild anaemia did not show significant effects on HbA1c (27). Though, in the present study, the degree of anaemia was not assessed. In the present study, the finding of significant higher HbA1c levels in iron deficient girls and boys, without significant higher fasting glucose levels, are similar of the findings in the review of English et al, where it was found significant higher HbA1c levels in iron deficient women vs. non-iron deficient women independent of fasting glucose levels (23, 24). Hence, the present study clearly share similarities with earlier studies, in the manner of higher HbA1c levels in anaemic and iron deficient individuals.

On the other hand, earlier studies have also found findings dissimilar of the present study. In 2015, a review and meta-analysis on the effect of ID and/or IDA on HbA1c in non-diabetic individuals by Cavagnolli et al, showed no significant differences in HbA1c in the presence of IDA or ID (29). Hence, this review and meta-analysis shows opposite shifts in HbA1c in the presence of IDA and ID compared to the present study, even though regarding IDA we only found significant higher HbA1c in girls.

Interestingly, the present study found statistical significant higher HbA1c levels in girls with non-ID anaemia, compared to girls without this condition. This is a contrast to previous studies, such as the studies by Ford et al (n=8296). They found that HbA1c in non-ID anaemia showed opposite results compared to ID and IDA, namely downward shifts in HbA1c when low Hb and normal iron indices are present, compared to normal individuals (with normal Hb and normal iron status) (25). With contrary findings and limited research, the relation between non-ID anaemia and HbA1c levels remains inconclusive.

Gender differences

The prevalence of the haematological conditions, both anaemia and ID, is solely higher in girls compared to boys in the study population (table 3). This is most probably explained by greater physiological losses of iron in girls (due to uterine loss), combined with inadequate iron supplements through diet.

Interestingly, in boys, statistical significant higher HbA1c levels was found in ID (without anaemia), compared to boys without this condition. On the contrary, for girls, the mean HbA1c was lower for ID (without anaemia) compared to girls without this condition, but not statistically significant. Even though the prevalence of ID (without anaemia) was smaller in boys than in girls, the difference in HbA1c was only statistical significant in boys.

However, in the other haematological groups, girls and boys showed shifts in HbA1c in the same direction. But, for some results – whether the results were significant or not – varied between the genders.

What these differences between girls and boys are caused by, cannot be answered by this study. Some theories could be the difference in prevalence of the haematological conditions, difference in degree of ID/anaemia (Hb and ferritin levels), differences in HbA1c levels or generally physiological differences between the genders. Regarding the first theory, table 3 shows noticeable differences in prevalence between girls and boys. Regarding the second and third theory, table 2 shows noticeable differences in ferritin- and Hb level between the genders, but only a slightly difference in HbA1c level between the genders.

The importance of infection

Since ferritin is also an acute phase protein, we made separate analyses in subgroups of ID after exclusion of known infection, antibiotic use and elevated CRP level. I wanted to see if these factors affected the results in any way. However, for those with ID (with/without anaemia), there was similar results in the subgroups without infection. Thus, HbA1c was higher in both girls and boys independent of the presence of infection. In the groups with ID (without anaemia), the results were also similar after exclusion of those with infection. Thus, HbA1c was higher in boys with ID (without anaemia) – independent of infection, whereas no significant difference was seen in girls.

If we look at the mean HbA1c, it did not differ noticeably after exclusion of infection factors (table 4). The size of the ID groups before and after exclusion of infection factors, were diminished by only a few participants. No more than twelve iron deficient participants were excluded from each of the groups (table 3). Hence, the results were not seemingly affected by exclusion of infection factors on the ID variable.

Other factors that may possibly affect HbA1c

One study found that high HbA1c levels in non-diabetic individuals may be associated with chronic kidney disease (CKD) (43). A study regarding iron supplementation, found that iron supplementation during pregnancy did not affect HbA1c levels and had no clinical impact on the interpretation of results in the absence of anaemia or the presence of mild anaemia, even though interpretation of HbA1c results in pregnant women still requires caution during iron therapy with moderate and severe anaemia (33). However, WHO have stated that iron administration may lead to a decrease in HbA1c levels (11).

Even though an association has been indicated, iron supplementation and CKD were not accounted for in this present study. Given the age and inclusion by a healthy population based study, we can probably count on a low prevalence of CKD in the study population of the present study.

The results in relation to the purpose of the thesis

The findings of significant higher HbA1c levels among anaemic and iron deficient adolescents without DM were highly interesting, as the aim of the study was to further study

the relation between iron- and erythrocyte indices and HbA1c levels, in Norwegian adolescents without DM. With several indications of a relation between these factors, it was of interest to look at this even further, to see if this study would strengthen these indications or if the study would show something completely different.

The results in relation to methods

Participants with DM were excluded by a self-report in a questionnaire. When number of participants of the study are of this size (n=824), one might wonder if there still were unintentionally included some individuals with diabetes who were not aware of their diabetes (i.e. not yet discovered). With that said, HbA1c showed no value ≥6.5 %, which, to some extent, minimizes the risk of including participants with diabetes.

Strengths of the study

The number of participants included in this study after exclusions were 824, out of 377 was girls and 447 was boys. This is an appreciable number of participants, compared to many other studies.

The present study also had a lot of information, both from blood samples and questionnaire, e.g. which made it possible to look at the importance of infection regarding ferritin and ID.

Limitations of the study

The present study is a cross sectional study, which automatically gives the study some limitations. A cross sectional study is a snapshot of the reality, and do not follow participants over time. That means that the results can only present association, but not causality.

Unfortunately, the analyses were limited to the 824 with available blood samples. We have no reason to believe that those without blood samples had a higher prevalence of anaemia or ID, or different HbA1c-levels than those included. We therefore consider this a type of non-differential bias.

In addition, we have performed multiple statistical comparisons, which may increase the risk of type 1 errors.

Possible implications of the results

The interesting findings of this study will hopefully contribute to shed light and expand the knowledge regarding the use of HbA1c as a diagnostic tool, especially in adolescents. The higher HbA1c levels found in the anaemic and iron deficient groups constitute a relatively small difference, but still, it could theoretically be of clinical value in a diagnostic situation with the presence of borderline HbA1c values. This study will hopefully make more clinicians aware of HbA1c, including the factors that possibly may affect its results. Perhaps could this lead to fewer mistaken interpretations of HbA1c in patients.

Conclusion

The aim was to study the relation between ID, IDA and non-ID anaemia and HbA1c levels in adolescents. I wanted to see if abnormalities in iron- and/or erythrocyte indices, which are frequent during adolescence, could cause spuriously abnormal values of HbA1c in adolescents. We found significant higher HbA1c levels in adolescents with anaemia and ID, compared to adolescents without these conditions, respectively. Some interesting gender differences in the subgroups of anaemia and ID were also found. It was not found significant higher fasting glucose levels or HOMA-IR in adolescents with anaemia or ID.

The study shows that HbA1c in adolescents, with anaemia or ID present, must be assessed with caution. This is of clinical importance because of the relatively high occurrence of anaemia and ID, especially in girls at this age. Further studies on this subject are needed to contribute to knowledge and increased awareness, also regarding degrees of anaemia, and in diabetic patients – in which HbA1c may be used as an assessment of glycaemic control and in diabetic therapy.

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	Dokumentasjonsnivå	IIa
	Grade:	Middels

Formål	Materiale og metode	Resultater	Diskusjon/kommentarer
Formålet med studien var å undersøke påvirkningen av jernmangel (ID) på HbA1c-fordelingen blant voksne uten diabetes. **Konklusjon** ID er vanlig blant kvinner og er assosiert med forandringer i HbA1c-fordeling fra <5.5 til ≥5.5%. Videre forskning trengs for å undersøke om ID er assosiert med forandringer ved høyere HbA1c-nivåer. **Land** USA **År data innsamling** 1999-2006	Deltakere inkludert (n=10,535) er voksne uten selvrapportert diabetes eller kronisk nyresykdom i National Health and Nutrition Examination Survey med alder ≥18 år og komplett blodlegeme-telling, jern-indekser og HbA1c. ID ble definert som minst to abnormaliteter inkludert free erythrocyte protoporphyrin >70 µg/dl erytrocytter, transferrin metning <16%, eller serum ferritin ≥15 µg/dl. Anemi ble definert som Hb <13.5 g/dl hos menn og <12.0 g/dl hos kvinner. Individer med selvrapportert diabetes, kronisk nyresykdom og gravide kvinner ble ekskludert. For HbA1c ble to cutpoints benyttet: </≥ 5.5% og </≥ 6.5%. Det ble kontrollert for faktorer som kunne være mulige konfundere for ID eller HbA1c: alder, rase, overvekt (især visceral adipositas). Analyser ble gjennomført ved SAS for databehandling og SUDAAN for å håndtere ulike sannsynligheter for seleksjon, planert oversampling, og det komplekse sample design til NHANES. X^2 tester, ANOVA og logistisk regresjon ble brukt. Det ble også utført flere sensitivitetsanalyser, multippel lineær regresjon og log transformasjon.	Blant kvinner (n=6,666) hadde 13.7% ID og 4.0% hadde jernmangelanemi (IDA). 316 kvinner med ID hadde HbA1c ≥5.5%, bare 32 kvinner med ID hadde HbA1c ≥6.5%. Blant menn (n=3,869) var det bare 13 som hadde ID og HbA1c ≥5.5%, og bare 1 som hadde ID og HbA1c≥6.5%. Blant kvinner, var ID assosiert med en større odds for HbA1c ≥5.5% (odds ratio 1.39 [95% KI 1.11-1.73]) etter justering for alder, rase/etnisitet, og bukomkrets men ikke med større odds for HbA1c ≥6.5% (0.79 [0.33-1.85]).	**Sjekkliste:** Var kasus-kontrollgruppene rekruttert fra sammenliknbare befolkningsgrupper? **Ja** Er gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer? **Ja** Er kasusgruppens tilstand tilstrekkelig beskrevet/diagnosen validert? **Ja** Er kontrollgruppen fri for den aktuelle tilstanden/sykdommer? Har forfatterne tatt hensyn til viktige konfunderende faktorer i design/analyse? **Ja** Er eksponering for fare/skade/tiltak målt og gradert likt i gruppene? Var den som målte eksposisjon blindet mht hvem som var kasus/kontroll? Var responsraten tilstrekkelig i begge grupper? **Styrke:** Høyt antall deltakere (n=10,535). Tatt høyde for hemoglobinopatier, som kan påvirke HbA1c. **Svakhet:** Forfatterne diskuterer begrenset mulighet til å vurdere inflammasjonsfaktoren. Ikke tatt høyde for malignitet og aplastisk anemi.

Referanse: Koga M, Saito H, Mukai M, Matsumoto S, Kasayama S. Influence of iron metabolism indices on glycated haemoglobin but not glycated albumin levels in premenopausal women. Acta diabetologica. 2010;47 Suppl 1:65-9.			**Design: Tverrsnittstudie** Dokumentasjonsnivå: IIa Grade: Middels
Formål	**Materiale og metode**	**Resultater**	**Diskusjon/kommentarer**
Å undersøke om HbA1c-nivåer blir påvirket av jernmetabolisme-indekser hos premenopausale kvinner. Det ble også undersøkt påvirkningen av jernmetabolisme-indekser på nivåer av serum glykert albumin (GA). **Konklusjon** Konkluderer med at jernmetabolisme-indekser påvirker HbA1c-nivåer, men ikke serum GA-nivåer hos premenopausale kvinner. **Land** Japan **År data innsamling** 2006	Av 285 japanske kvinner som besøkte Health Care Center ved Kinki Central Hospital ble 104 inkludert. Inkluderingskriterier var: <50 år, regelmessig menstruasjon, fastende plasma glukose (FPG) <100 mg/dl, ikke hysterektomert og ingen jerntilskudd for behandling av anemi. Hemoglobin (Hb) ≥11.4 g/dl og serum ferritin ≥15 ng/ml ble definert som normal iron state (NIS), Hb ≥11.4 g/dl og serum ferritin <15 ng/ml som jernmangeltilstand (IDS), og Hb <11,4 g/dl og serum ferritin <15 ng/ml som jernmangelanemi (IDA). Av de 104 inkluderte ble 57 deltakere diagnostisert med NIS, 30 med IDS og 17 med IDA. StatView dataprogram 5.0 ble brukt til statistiske analyser. For å korrigere for skjevfordeling, ble serum ferritin-nivåer logaritmisk transformert. Ved statistiske analyser ble Students t test brukt for å sammenligne to grupper. Single linear regresjonsanalyse og stepwise multivariat regresjonsanalyse ble gjort for å evaluere forholdet mellom HbA1c-nivåer og forskjellige variabler. P-verdi <0.05 ble regnet som statistisk signifikant.	HbA1c viste signifikant invers assosiasjon med serum jern, transferrin-metning og serum ferritin hos 104 deltakere. Multivariat regresjonsanalyse av 104 deltaker avslørte at ferritin var uavhengig negativ risiko for HbA1c og FPG var en uavhangig positiv risiko for HbA1c. Disse var også observert hos 87 premenopausale kvinner uten IDA (57 NIS og 30 IDS). Selv om **HbA1c** var inverst assosiert med serum jern (R= -0.309, P=0.0014), serum transferrin-metning (R= -0.326), P= 0.0007) og serum ferritin (log-transformert) (R= -0.0331, P= 0.0006) hos 104 deltakere, var **serum GA** [vs. serum jern, R= 0.043, P=0.6616; vs. transferrin-metning R=0.018, P=0.5860; vs. serum ferritin (log-transformert), R= -0.083, P=0.4026] og **serum FPG** [vs. serum jern, R= -0.150, p=0.1288; vs. transferrin-metning R= - 0.134, P=0.1753; vs. serum ferritin (log-trasformert), R= -0.117, P=0.2373] ikke assosiert med dem signifikant. Selv om FPG-nivåer hos individer med IDA (90.5 ± 4.0 mg/dl) og IDS (87.0 ± 4.9 mg/dl) ikke var signifikant forskjellig fra de hos individer med NIS (88.1 ± 4.7 mg/dl), var HbA1c-nivåer 5.1 ± 0.2% hos individer med IDA og 5.0 ± 0.2% hos individer med IDS, som var litt men signifikant høyere enn hos individer med NIS (4.8 ± 0.2%). Som kontrast, var serum GA-nivåer ikke forskjellig blant disse tre gruppene individer (14.7 ± 1.0% hos IDA-individer, 14.8 ± 1.1% hos IDS-individer, og 14.5 ± 1.1% hos NIS-individer).	**Sjekkliste:** Var kasus-kontrollgruppene rekruttert fra sammenliknbare befolkningsgrupper? **Ja** Er gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer? **Ja** Er kasusgruppens tilstand tilstrekkelig beskrevet/diagnosen validert? **Ja** Er kontrollgruppen fri for den aktuelle tilstanden/sykdommer? **Ja** Har forfatterne tatt hensyn til viktige konfunderende faktorer i design/analyse? **Ja** Er eksponering for fare/skade/tiltak målt og gradert likt i gruppene? **Ja** Var den som målte eksposisjon blindet mht hvem som var kasus/kontroll? Var responsraten tilstrekkelig i begge grupper? **Styrke** **Svakhet:** Forfatterne diskuterer at studien kun er utført på kvinner med normal glukose-toleranse, og ikke på individer med diabetes, da det kan være viktig i kliniske situasjoner.

Referanse: Silva JF, Pimentel AL, Camargo JL. Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia. Clinical biochemistry. 2016;49(1-2):117-20.	**Design: Tverrsnittstudie og Kasuskontroll**
	Dokumentasjonsnivå: IIa
	Grade: Middels

Formål	Materiale og metode	Resultater	Diskusjon/kommentarer
Å undersøke effekten av jernmangelanemi (IDA) på HbA1c-nivåer hos pasienter uten diabetes mellitus (DM), målt ved to vanlige metoder brukt i kliniske laboratoriers rutiner over hele verden. **Konklusjon** IDA påvirker HbA1c-nivåer og forårsaker en falsk økning i dens resultater. Selv om disse økte endringene i HbA1c-nivåer er statistisk signifikante, kan det være at de ikke er klinisk relevante når den totale variasjonen i HbA1c blir vurdert. Denne effekten er avhengig av grad av anemi og tilstedeværelse av mild anemi har sannsynligvis liten effekt på HbA1c-nivåer. Disse funnene kan kanskje være nyttig ved bruk av HbA1c til å diagnostisere DM hos pasienter med mild anemi. **Land** Brasil **År data innsamling** 2008-2012	Deltakerrekruttering fra Clinical Pathology Service ved Hospital de Clinicas de Porto Alegre i Brasil. Deltakere ble valgt ut basert på laboratoriedata og ble delt inn i to grupper; Gruppe 1 inkluderte pasienter med IDA (ferritin <15 µg/mL, hemoglobin (Hb) <13 g/dL (hvis mann) eller <12 g/dL (hvis kvinne) and mean corpuscular volume (MCV) <80 fL) og gruppe 2 inkluderte pasienter uten anemi (serum ferritin >15 µg/mL og <150 µg/mL (hvis kvinne) eller <200 µg/mL (hvis mann), og i tillegg en komplett blodlegeme-telling innenfor referanseverdier). Totalt 122 pasienter ble inkludert, 61 pasienter med IDA og 61 pasienter uten anemi. Gruppene ble matchet ved kjønn og alder. Alle andre tilstander kjent for å kunne interferere/føre til feiltolking av HbA1c-resultater ble ekskludert. For å garantere at HbA1c variabilitet kun skyldtes den interfererende faktor i analysen, ble kun individer uten DM inkludert. HbA1c-resultater ble målt ved forskjellige grader av anemi. HbA1c ble målt med både ion exchange HPLC Variant II Turbo BioRad og immunoturbidimetry (IT) Tina Quant II Roche Diagnostics for hver prøve. Programmet SPSS 19.0 ble brukt til dataanalyser. Student t test, ANOVA, Pearson og Kendall correlations ble brukt som passende. Initialt ble HbA1c-nivåer, målt ved forskjellige metoder, sammenlignet hos pasienter med og uten IDA. Etterpå, ble pasienter med anemi delt inn i tre grupper etter deres totale Hb: 1) pasienter klassifisert som å ha "mild anemi" (kvinner med Hb ≥11 g/dL og <12 g/dL og menn med Hb ≥11 g/dL og <13 g/dL); 2) pasienter med "moderat anemi" (Hb ≥8 g/dL og <11 g/dL, for begge kjønn) og 3) pasienter med "alvorlig anemi" (Hb <8 g/dL, for begge kjønn). HbA1c-resultater i begge grupper, målt ved to metoder, ble sammenlignet med HbA1c-resultater i gruppen uten anemi. For korrelasjonsanalyse ble pasientene vurdert samlet. Signifikans på 5% ble benyttet.	Det var signifikant forskjell i resultatene av HbA1c mellom pasienter målt med IDA [HPLC 5.6 ± 0.4% (38 ± 4.4 mmol/mol) og målt hos pasienter uten IDA [HPLC 5.3 ± 0.4% (34 ± 4.4 mmol/mol) and IT 5.3 ± 0.3% (34 ± 3.3 mmol/mol)], (p <0.001). Signifikante negative korrelasjoner ble observert mellom total hemoglobin (Hb), hematokrit, mean corpuscular volume (MCV) og ferritin med HbA1c-verdier målt ved IT (r = −0.557; r = − 0.539; r = −0.488; r = −0.499; p <0.01; respektivt). Disse negative korrelasjonene var svakere med HbA1c målt ved HPLC (r = −0.272; r = −0.250; r = −0.273; r = −0.229 for Hb, hematokrit, MCV and ferritin; p <0.05; respektivt). Resultater av HbA1c var høyere hos pasienter med moderat og alvorlig anemi. Men mild anemi viste ikke signifikant effekt på resultater av HbA1c, målt med begge metoder.	**Sjekkliste:** Var kasus-kontrollgruppene rekruttert fra sammenliknbare befolkningsgrupper? **Ja** Er gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer? **Ja** Er kasusgruppens tilstand tilstrekkelig beskrevet/diagnosen validert? **Ja** Er kontrollgruppen fri for den aktuelle tilstanden/sykdommer? **Ja** Har forfatterne tatt hensyn til viktige konfunderende faktorer i design/analyse? **Ja** Er eksponering for fare/skade/tiltak målt og gradert likt i gruppene? **Ja** Var den som målte eksposisjon blindet mht hvem som var kasus/kontroll? Var responsraten tilstrekkelig i begge grupper? **Styrke:** Tilstander kjent for å kunne interferere/føre til feiltolking av HbA1c ble ekskludert. HbA1c målt ved to metoder som er vanlig verden over. **Svakhet:** Få deltakere (n=122), tverrsnittstudie (mekanismen om hvordan anemi affiserer HbA1c ble ikke vurdert)

Referanse: Akkermans MD, Mieke Houdijk ECA, Bakker B, Boers AC, van der Kaay DCM, de Vries MC, et al. Iron status and its association with HbA1c levels in Dutch children with diabetes mellitus type 1. European journal of pediatrics. 2018;177(4):603-10.

Design: Tverrsnittstudie	
Dokumentasjonsnivå	IIa
Grade:	Middels

Formål	Materiale og metode	Resultater	Diskusjon/kommentarer
Studiens formål er å bestemme prevalens og type jernmangel (ID) og undersøke dens assosiasjon med HbA1c-nivåer hos pediatriske pasienter med diabetes mellitus (DM) type 1. **Konklusjon** Funksjonell, men ikke absolutt, ID var vanlig hos nederlandske pediatriske DM type 1-pasienter. HbA1c-nivåer var ikke assosiert med ID, som kan forklares ved den relativt mildt depriverte jernstatus hos pasientene. **Land** Nederland **År data innsamling** 2015-2016	Pasienter ble rekruttert ved Juliana Children's Hospital/Haga Teaching Hospital i Haag og ved Reinier de Graaf Hospital i Delft. Totalt 227 barn ble inkludert, av de i alderen 1-19 år som hadde hatt DM type 1 i minst 1 år. Eksklusjons-kriterier var kjent infeksjon siste 4 uker, medfødte malformasjoner, onkologiske tilstander, hemoglobinopatier, bruk av jerntilskudd siste 6 uker, og blodtransfusjon mottatt siste 6 måneder. Anemi ble definert som Hb-nivå >2 SD under gjennomsnittet for alders-matchede barn etter kriterier fra WHO. Absolutt ID ble definert som serum ferritin (SF) <12 µg/l hos pasienter <5 år eller SF <15 µg/l hos pasienter ≥5 år, i fravær av tegn til infeksjon og/eller akutt inflammasjon (hsCRP ≥10 mg/l). Jernmangelanemi (IDA) ble definert som absolutt ID i kombinasjon med anemi. Funksjonell ID ble definert som ZPP >61 µmol/mol heme hos pasienter <5 år eller ZPP >70 µmol/mol heme hos pasienter ≥ 5 år og/eller RDW-CV >14% eller RDW-SD >43.39 fl. SPSS 21.0 ble bruk til statistiske analyser. Det ble utført logistiske regresjonsanalyser for å undersøke mulige konfunderende faktorer: studiesenter, alder, kjønn, etnisitet, sosioøkonomisk status, sykdomsvarighet, HbA1c, cøliaki, menstruasjonssyklus, jerninntak i kosten. Det ble sammenlignet HbA1c-nivåer hos barn med nedsatt jernstatus (absolutt eller funksjonell ID med og uten anemi) med HbA1c-nivåer hos barn uten nedsatt jernstatus. Det ble også sammenlignet HbA1c-nivåer hos barn med absolutt ID før og etter behandling med jern. Det ble beregnet (alderskorrigert) korrelasjon mellom hematologiske- og jernstatus-parametre og HbA1c. Statistisk signifikans ble definert som p<0.05.	13 (5.7%) av de 227 barna hadde absolutt ID. Syv av disse 13 hadde også anemi og dermed IDA (3.1%). Av de 214 barna utem absolutt ID, hadde 100 barn (47%) funksjonell ID. Åtte av disse 100 barna hadde også anemia og dermed ACD (anaemia of chronic disease, 3.7%). Totalt hadde 113 (50%) nedsatt jernstatus, enten absolutt eller funksjonell. Femten av disse 113 barna (13%) hadde også anemi. HbA1c-nivåer (±SD, i mmol/mol (%)) hos pasienter med ulik jernstatus: **Iron status** / **Yes** / **No** / ***p* value** Normal (no absolute or functional ID) / 65 ± 17 (8.1 ± 3.7) (*n* = 103) / 65 ± 16 (8.1 ± 3.6) (*n* = 117) / 0.783 Absolute ID / 61 ± 11 (7.7 ± 3.1) (*n* = 13) / 65 ± 17 (8.1 ± 3.7) (*n* = 211) / 0.360 Functional ID / 66 ± 16 (8.2 ± 3.6) (*n* = 100) / 65 ± 17 (8.1 ± 3.7) (*n* = 104) / 0.618 Iron deficiency anemia / 66 ± 11 (8.2 ± 3.1) (*n* = 7) / 66 ± 17 (8.2 ± 3.7) (*n* = 220) / 0.947 Anemia of chronic disease / 68 ± 23 (8.4 ± 4.3) (*n* = 8) / 65 ± 16 (8.1 ± 3.6) (*n* = 201) / 0.635 Resultatene av univariate analyser viste at absolutt ID var assosiert med tilstedeværelse av menstruasjon hos jenter (p=0.003). Karakteristika hos pasienter med og uten funksjonell ID var lik (p>0.05). Multivariate analyser med justering for mulige konfunderende faktorer viste ingen statistisk signifikant avhengige determinanter av absolutt ID eller funksjonell ID. Det ble ikke observert en forskjell i HbA1c-nivåer mellom pasienter med og uten nedsatt jernstatus. Videre hadde pasienter med anemi (IDA eller ACD) like HbA1c-nivåer som pasienter med normalt Hb-nivå. Videre, eksplorative analyser viste heller ikke statistisk signifikant forskjell i HbA1c etter behandling med jern (men det foreligger ikke detaljert informasjon om diabetes-reguleringen under behandlingstiden). Korrelasjonen mellom hematologiske- og jernstatus-parametre og HbA1c vises i tabellen under: Retikulocytter var positivt og RDW negativt korrelert med HbA1c. **Parameter** / **Pearson correlation** / ***p* value** Hemoglobin / 0.046 / 0.351 Reticulocytes / 0.216 / 0.001* Red blood cell distribution width / − 0.150 / 0.028* Serum ferritin / 0.097 / 0.158 Zinceprotorphyrin / 0.050 / 0.466	**Sjekkliste:** Var kasus-kontrollgruppene rekruttert fra sammenliknbare befolkningsgrupper? **Ja** Er gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer? **Ja** Er kasusgruppens tilstand tilstrekkelig beskrevet/diagnosen validert? **Ja** Er kontrollgruppen fri for den aktuelle tilstanden/sykdommer? **Ja** Har forfatterne tatt hensyn til viktige konfunderende faktorer i design/analyse? **Ja** Er eksponering for fare/skade/tiltak målt og gradert likt i gruppene? Var den som målte eksposisjon blindet mht hvem som var kasus/kontroll? Var responsraten tilstrekkelig i begge grupper? **Styrke** **Svakhet**

Referanse: Christy AL, Manjrekar P, Babu RP, M SR, Hegde A. Elevation of HbA1C in Non-diabetic Hypothyroid Individuals: Is Anaemia the Connecting Link? -A Preliminary Study. Journal of clinical and diagnostic research : JCDR. 2013;7(11):2442-4.	**Design: Kasuskontroll**
	Dokumentasjonsnivå: IIa
	Grade: Middels

Formål	Materiale og metode	Resultater	Diskusjon/kommentarer
Formålet til studien å undersøke om forhøyet HbA1c ved hypo-tyreoidisme kan tilskrives anemi. **Konklusjon** Hypotyroide, anemiske pasienter, uten diabetes, viser forhøyde nivåer av HbA1c i prediabetes-området. Med dette bør forsiktighet vises ved bruk av HbA1c som diagnostisk verktøy for diabetes hos slike pasienter. **Land** India **År data innsamling** 2011-2012	Deltakere ble rekruttert fra Kasturba Medical College Hospital, Ambedkar circle i India. Data ble innsamlet fra 782 pasienter med alder 18 år og eldre som hadde HbA1c, perifert utstryk, hemoglobin (Hb), mean corpuscular Hb (MCH), mean corpuscular volume (MCV), mean corpuscular Hb concentration (MCHC), serum ferritin, serum TSH og plasma-glukosenivåer målt. Totalt 187 av de diagnostisert med hypotyreoidisme basert på TSH-nivåer, var ikke-diabetikere (basert på fastende plasma glukose <100mg/dl). Av disse ble 60 non-anemiske, 30 med mikrocytisk hypokrom anemi og 30 med normocytisk normokrom anemi inkludert. 63 av de anemiske ekskludert basert på eksklusjonskriterier. 120 alders-, kjønns-, plasma-glukosenivå- og anemistatus-matchede kontroller ble inkludert. Status anemi og jernmangel ble bestemt ved ferritin (<29ng/ml menn, <20ng/ml kvinner), Hb (<12g% menn, <11g% kvinner), erytrocytt-indekser og perifert utstryk. Alle individene som er inkludert er ikke-diabetikere, og de fleste av faktorene som kan interferere med glykering av Hb slik som kronisk nyresykdom, hemoglobinopatier, graviditet og hemolytisk anemi ble ekskludert, for å styrke studien. Dataene ble analysert med IBM SPSS Statistics 20. Anova med Tukey's test ble brukt for sammenligning av gruppene. Pearsons coefficient of correlation ble beregnet. Kategoriske data ble analysert ved x^2 test. OR og 95% KI ble beregnet ved logistisk regresjonsanalyse. P-verdi <0,05 ble vurdert som statistisk signifikant.	Kvinner viste større predisposisjon for å være hypotyreoid og anemisk i forhold til menn. Deltaker-karakteristika: **Type** / **Microcytic Hypochromic** / **Normocytic Normochromic** / **Non-anemic** Haemoglobin (g/dl): 9.45 ± 1.28 / 9.97 ± 0.87 / 13.8 ± 1.13 Ferritin (ng/ml): 7.72 ± 4.93 / 175.4 ± 34.41 / 208.5 ± 21.6 MCV (fL): 57.2 ± 6.11 / 79.12 ± 9.92 / 85.1 ± 4.2 MCH (pg/cell): 15.9 ± 4.7 / 31.7 ± 6.3 / 33.5 ± 2.33 Plasma Glucose (mg/dl): 84.8 ± 9.1 / 87.1 ± 6.3 / 86.3 ± 7.7 TSH (µIU/ml): 34.12 ± 13.6 / 30.32 ± 11.5 / 35.65 ± 14.9 Female: Male ratio: 28:2 / 24:6 / 45:15 HbA1c var rundt 6.57 ± 0.69 i anemiske tilfeller sammenlignet med 5.91 ± 0.41 i ikke-anemiske tilfeller. I følge tabellen under var HbA1c-nivåer lavere ved normocytisk normokrom anemi (6.32 ± 0.75) sammenlignet med mikrocytisk hypokrom anemi (6.82 ± 0.71) og forskjeller mellom deres respektive kontrollgrupper var statistisk signifikant. Fordeling av HbA1c (%) i hypotyreoide tilfeller og kontroller etter type anemi *p<0.05, **p<0.001, ***p<0.0001: **Group** / **Hypothyroid: Total** / **Hypothyroid: Female** / **Hypothyroid: Male** / **Euthyroid: Total** / **Euthyroid: Female** / **Euthyroid: Male** Microcytic hypochromic: 6.82 ± 0.71** / 6.87 ± 0.75*** / 6.55 ± 0.43* / 6.43 ± 1.07 / 6.47 ± 1.19 / 6.36 ± 0.88 Normocytic normochromic: 6.32 ± 0.75* / 6.46 ± 0.45*** / 5.93 ± 0.31* / 5.87 ± 0.46 / 6.01 ± 0.46 / 5.70 ± 0.21 Non-anemic: 5.91 ± 0.41 / 5.99 ± 0.36* / 5.80 ± 0.26 / 5.46 ± 0.62 / 5.61 ± 0.58 / 5.45 ± 0.67 Odds for at anemiske pasienter skal ha HbA1c >6.5 var 3.163 (1.426-7.016). Pasienter med tydelig hypotyreoidisme viste ikke signifikant odds ratio (0.998 [0.113-2.017]). Odds ratio for HbA1c>6.5 for anemi og grad av hypotyreoidisme *p<0.05: **Female (A1C >6.5)** / **Male(A1C >6.5)** / **Total (A1C>6.5)** **Odds ratio** / **95% C.I.** / **Odds ratio** / **95% C.I.** / **Odds ratio** / **95% C.I.** Anaemia: 3.312* / 1.118-6.987 / 3.001 / 0.987-6.512 / 3.163* / 1.426-7.016 TSH>14: 1.001 / 0.227-1.247 / 0.789 / 0.333-4.321 / 0.998 / 0.113-2.017 A1C=HbA1c	**Sjekkliste:** Var kasus-kontrollgruppene rekruttert fra sammenliknbare befolkningsgrupper? **Ja** Er gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer? **Ja** Er kasusgruppens tilstand tilstrekkelig beskrevet/diagnosen validert? **Ikke oppgitt TSH- og FT4-nivå for hypotyreose-diagnose, men kriterier for anemi-diagnosen er oppgitt.** Er kontrollgruppen fri for den aktuelle tilstanden/sykdommer? **Ja** Har forfatterne tatt hensyn til viktige konfunderende faktorer i design/analyse? **Ja** Er eksponering for fare/skade/tiltak målt og gradert likt i gruppene? **Ja** Var den som målte eksposisjon blindet mht hvem som var kasus/kontroll? Var responsraten tilstrekkelig i begge grupper? **Styrke:** De fleste tilstander som kan interferere med glykering av Hb som kronisk nyresykdom, hemoglobinopatier, graviditet og hemolytisk anemi ble ekskludert. **Svakhet:** Ikke definert nøyaktige diagnostiske kriterier for hypotyreose (kasusgruppen). Forfatterne diskuterer manglende mulighet til å måle erytrocytt life span, manglende kunnskap om behandling, og at funnene i denne studien trenger å bli validert i en større kohort.