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Self-reported food hypersensitivity in relation to biomarkers: The Fit Futures Study

A cross-sectional study

Vilde Dragland

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Preface

Autumn of 2018 I contacted Anne-Sofie Furberg after reading about Fit Futures 1 and Fit Futures 2, a study and an expansion of the Tromsø Study where Anne-Sofie were the project manager. With a curiosity for lifestyle, chronic diseases and the correlation between these I promptly asked for the opportunity to base my master thesis on this project. In cooperation with Anne-Sofie we developed several different topic questions, where one topic question in the early days stood out amongst the others with its relevancy. Upon deciding on this topic for the master thesis we contacted Martin Sørensen, a chief attending physician and specialist in general medicine, paediatric diseases and allergology, who has been a great support as a co-supervisor along with Anne-Sofie.

Food hypersensitivity is an arising topic, which the public and the press demonstrate an increased interest in. Despite being a common condition in the public, there are limited quality data concerning the burden of this disease. Hopefully more quality studies will be conducted in the following decades as an effect of the increased awareness in the public and the press. It has been especially interesting having adolescents as the study population, as there is a predominant focus on children in the existing studies – especially regarding the consequences of exclusion diets.

I want to thank my supervisor Anne-Sofie for letting my thesis be a part of this grand study, and for always encouraging and supporting me throughout this process. I also want to say my gratefulness to Martin Sørensen, who have been a great resource and guidance with his expertise in food hypersensitivity. A big thank you to Dina Berg Stensen, who have always been available at short notice, and have helped me tremendously with all statistics and SPSS - giving the most helpful and describing answers one could ask for.

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Vilde Dragland

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Abstract

Background: Food hypersensitivity is recognized as a rather common condition, that can occur at any age. There is limited high-quality data on the burden of this condition, especially after child age. The aim of this thesis has been to explore whether levels of biomarkers in blood differ between adolescents with self-reported hypersensitivity against certain food and the control group in a general youth population.

Method: This project is based on data from the Tromsø Study Fit Futures 2. The study population includes 376 females and 307 males (age 17-21) in upper secondary school from the neighbouring municipalities Tromsø and Balsfjord, North Norway. Data on self-hypersensitivity against foods was assessed by a web-based questionnaire and levels of Hb, Fe, Ferritin, Calcium and Vitamin D were measured.

Results: There was a statistically significant difference between mean Hb-levels in participants with any kind of food reaction ($p < 0.05$), and food reactions to wheat ($p < 0.001$), nuts ($p < 0.05$) and peanuts ($p < 0.001$) compared to participants with no food reactions; the subjects with food reactions having a lower mean value. Amongst adolescents with a reported food reaction to wheat, there were also a statistically significant lower level of Ferritin and Calcium values (all $p < 0.05$). Aside from these there were no significant differences in mean/median biomarker values for Hb, Fe, Ferritin, Calcium or Vitamin D when comparing subjects with and without self-reported food reactions. Self-reported reaction to wheat was also associated with having Calcium levels below reference level ($p < 0.05$). Except for this, there were no associations between having a food reaction and having biomarker levels below reference levels or in the lower quartile.

Conclusion: This study suggests that there is a slight difference in biomarker levels when comparing a youth population with self-reported food reactions to a control group, especially in subjects reporting wheat hypersensitivity. More detailed research is needed on this subject to conclude with how and to which extent this affects the nutritional status of these adolescents.

Key words

Food hypersensitivity; food intolerance; food allergy; biomarkers

Nomenclature

IgE	Immunoglobulin E
Fe	Iron
Hb	Haemoglobin

Abbreviations

TFF1	The Tromsø Study Fit Futures 1
TFF2	The Tromsø Study Fit Futures 2
UNN	University hospital of North Norway
CMA	Cow's milk allergy
GI	Gastrointestinal
LNP	Lactase non-persistence
CME	Cow's milk exclusion
GFD	Gluten-free diet(s)
NCGS	Non-celiac gluten sensitivity
SD	Standard deviation
IQR	Interquartile range
REK	Regional Committee for Medical Health and Research Ethics
BMI	Body mass index

1 Introduction

Food hypersensitivity is an "umbrella" term for any adverse and abnormal reaction after exposure to a given food. One broadly differentiates between allergic and non-allergic food hypersensitivity, also referred to as food allergy and food intolerance (1, 2).

The estimated prevalence of food hypersensitivity remains uncertain, as epidemiologic data are largely lacking and inconsistent (3). There are no uniform criteria for diagnosing food hypersensitivity (4), resulting in a great diversity of study methodology, structures and interpretations of the diagnostic criteria, complicating the assessment of the true prevalence (2). It is generally assumed that prevalence based on questionnaires and self-reporting are immensely overestimating the true prevalence of food hypersensitivity (5). However, it is generally recognized that perceived adverse reactions to one or more foods are common (3).

The current main treatment for food hypersensitivity is avoidance of the allergen causing the adverse reactions (5). In recognizing perceived food hypersensitivity as a common condition, one can conclude that a vast portion of the population totally or partially exclude one or more food from their diet. Whether these measures influence the nutritional status of these individuals, and if so to which degree, have not been thoroughly investigated in all age groups. As these preventive actions against food reactions are increasingly prevalent, this trend may have an effect on the nutritional state and health of the general population.

The aim of this study is to investigate whether there is an association between self-reported hypersensitivity and levels of biomarkers in an adolescent population. Our specific hypothesis is that levels of biomarkers in blood differ in adolescents with self-reported hypersensitivity compared to their peers. The analysis in this study is based on the Tromsø Study Fit Futures 2 (TFF2).

Background

1.1 Definitions of food allergy and food intolerance

As mentioned, food hypersensitivity is defined as any adverse reaction after exposure to a given food in a dose normally tolerated, and is categorized into food allergy and food intolerance; the latter group often referred to as non-allergic food hypersensitivity (1, 2, 5).

The two categories are separated by key pathophysiological differences, as well as variation in clinical presentation and severity (3).

Food allergy is defined as an adverse immune response that arises reproducibly when the individual is exposed to a specific food allergen (2, 6). Allergies are broadly divided into IgE-mediated and non-IgE-mediated allergies, of which the IgE-mediated can be detected by skin prick test or by measuring serum IgE (2). A food allergen is as a component in food (typically a protein) that allergen-specific immune cells recognize and react against, causing the adverse immune response. The most common food allergens stem from cow's milk, eggs, peanuts, tree nuts, wheat, soy, fish and shellfish, and the symptoms of exposure can manifest itself in many different organs and vary from an innocent itch or urticaria to anaphylactic shock and death (2, 3, 6). Measures recommended to avoid severe outcomes of allergic reactions include absolute exclusion of the given food causing the allergy, and having emergency adrenaline treatment available in case of exposure to allergen; the former is recognized as the current main treatment for food allergy. A few patients are offered immunotherapy, with the goal of developing tolerance through gradually, controlled exposure for the specific allergen (3, 5).

Food intolerance is defined as a non-immunological adverse reaction after exposure to a given food in a dose normally tolerated (2, 6). Intolerances are associated with less severe symptoms compared to allergic reactions, and there is a greater variety in clinical presentation. Due to this, and the fact that there is also a great diversity of mechanisms behind the adverse reactions, food intolerances are complicated to both understand and diagnose. Many of the mechanisms behind certain food intolerances are currently not adequately described to fully understand (3). Common for both food allergies and food intolerances are that the symptoms are reproducible by exposure to the given food, and the only "cure" is to avoid the food responsible for the adverse reactions (2, 5).

1.2 Prevalence of food hypersensitivity

Epidemiologic data on food allergies and food intolerances are as mentioned lacking, and the true prevalence of these have not been established due to several complicating factors (3). Misclassification, inconsistency, lack of simple diagnostic tests, biased participation and no standardization of criteria are just a few of the described complicating factors (7, 8).

There have been conducted community-based studies in the UK (9), Holland (10), USA (11), Sweden (12) and Australia (13), exploring the frequency of perceived adverse reactions to

food. The prevalence of food hypersensitivity reported in the respective studies are 20%, 12%, 16%, 25% and 17% (9-13). A German systematic review from more recent times (2016) estimated the prevalence of self-reported food hypersensitivity in Europe to range from 5.7% to 61.6%. The same review also reports that physician-diagnosed hypersensitivity has an estimated prevalence ranging between 0.2-4.2%, and double-blind proven immediate-reactions an estimated prevalence ranging between 0.0-2.2% (14). Several other studies report similar statistics; with a broad range estimate and a notable variation between self-reported, physician-diagnosed, and confirmed cases through oral food challenges (5). What causes this obvious gap between perceived and true prevalence remains indecipherable (15).

Prevalence of food allergy

The gold standard of diagnosing food allergy as well as food intolerance is double-blind placebo-controlled oral food challenges; which most epidemiological studies on food hypersensitivity do not practice (8). Therefore, the true prevalence of food allergies overall, as well as IgE-mediated and non-IgE-mediated allergies individually, remain ambiguous and vary greatly between different studies (3). Food allergy is however generally acknowledged as less common, and estimates suggest it has a lower community prevalence than food intolerance (1, 15). In some countries, it is estimated that the true prevalence of IgE-mediated food allergies may be as high as 4-7% in preschool children, and closer to 1-2% in the adult population. There is a clear reduction in prevalence with age, due to the fact that a high percentage of children with allergy will develop a tolerance against the given allergen as they grow older. This is especially true with allergies against cow's milk and egg, while less likely to happen in children with nut allergy (3).

Prevalence of food intolerance

Food intolerance is estimated to be as prevalent as 20% in a general population, however there are several limitations related to these estimates (1). As mentioned, food intolerances have a greater variety in mechanisms causing the adverse reactions, and there exists a notable shortage of knowledge about these mechanisms. As a result of this there is also a lack of precise and accurate diagnostic tests available for food intolerances, which makes it a challenge to assess whether a self-reported food intolerance represents a true food intolerance (1, 3).

Increase in prevalence

Several studies indicate that there might be a true rise in prevalence of food allergies the last 10-20 years. It is however a challenge in assessing change in incidence and prevalence of food allergy over time, due to inconsistency in both study design and definitions of food allergy (2, 8, 16). An increase in prevalence may be affected by other variations over time, such as an increase in research funding, increased interest and awareness by the press and public and different diagnostic tools. It remains uncertain how much of the measured increase in prevalence that actually represents a true increase (7, 16).

Another factor in the apparent increase in prevalence, is that studies with self-reporting are prone to overestimation (3). In an older household survey from the United Kingdom 1 of 5 in the study population reported that they had experienced adverse reactions to ≥1 food products. Double-blind placebo-controlled food challenges where performed in the study population, which concluded with a prevalence of 2% having proven true adverse reactions to food (9). Similar studies have been conducted in Germany, acknowledging further that the percentage of self-reported food reactions are higher than the true adverse reactions identified by food challenges in the same population (17). A more recent Swedish study revealed that 4.8% of children at age 12 reported food allergies against milk, egg, cod and/or wheat; 1.4% of these where diagnosed with food allergy after clinical evaluation, and 0.6% had a proven food allergy after double-blind placebo-controlled food challenges (18).

In summary, there are many compelling studies that suggest increasing prevalence of food allergies, however solid evidences are lacking (7, 8, 16). One can however note that there is a high percentage in the population with self-reported hypersensitivity against one or more food products (9-13), and based on this, one can assume it is likely that a high percentage of the population partially or totally exclude one or more food from their diet as well.

1.3 Milk allergy and milk intolerance

Cow's milk allergy (CMA) and cow's milk intolerance are different diagnosis; CMA is defined as having an adverse immune response triggered by cow's milk protein, and milk intolerance (also known as lactose intolerance) is defined as a non-allergic adverse reaction caused by deficiency of the enzyme lactase (19-21).

CMA has a prevalence ranging between 2-5% in infants and young children, and is acknowledged as the most common food allergy in children/infants < 3 years – having a peak in prevalence in the first year of life (19, 20, 22). Primary lactose intolerance on the other hand, is more prevalent after childhood (≥ 5 years), due to a decline in lactase expression – with approximately 70% of the world population suffering from so-called lactase non-persistence (LNP) (23, 24). The peak onset of lactase non-persistence occurring in teenagers and young adults (21). Lactose intolerance in children < 5 years are mainly transient, and one differs between secondary lactose intolerance (due to underlying gut conditions such as gastroenteritis or Crohn's), developmental lactase deficiency in premature infants (usually a transient lactose intolerance, due to maturational delay) and congenital lactase deficiency (21, 24-26). The latter is an autosomal recessive disorder known as alactasia, which is a rare and severe condition where lactase activity is completely absent or very low (21, 27).

The clinical presentation of CMA most frequently involves the skin and GI tract, but may also involve the respiratory tract. GI symptoms are often nonspecific and variable, and include oral and perioral swelling, dysphagia, early satiety, nausea, vomiting, dyspepsia, diarrhoea to weight loss, constipation, abdominal pain, and rectal bleeding (19, 20). When it comes to lactose intolerance the clinical presentation varies between infants and older children/teenagers/young adults; diarrhoea being more common in infants, and symptoms such as abdominal pain, bloating, abdominal distension, flatulence and low-grade diarrhoea being more common in older children/teenagers/young adults (21).

1.4 Egg allergy

Egg allergy is acknowledged as the second most common allergy in young children and infants, after milk protein allergy, affecting 0.5-2.5% of young children (28, 29). The hen egg white contains most of the known allergenic proteins, and the most allergenic protein (ovomucoid) is resistant to heat and digestive enzyme degeneration (30, 31). Due to this there is a great variation in clinical presentation, where most egg-allergic individuals are only allergic to raw or partially cooked egg, while the minority are allergic to all forms of egg (raw, cooked and baked) (32). Typical symptoms of egg allergy after exposure to egg include urticaria, itching, vomiting and angioedema, and it is reported for triggering 7-12% of paediatric anaphylactic cases (29, 33). Egg allergy has a good prognosis, with the majority of children developing tolerance over time (34).

1.5 Gluten-related disorders

The spectrum of hypersensitivity to gluten includes wheat allergy, celiac disease and non-celiac gluten sensitivity (NCGS) (35). As far as we know there are no studies showing the overall prevalence of gluten-related disorders, and both the prevalence of wheat allergy and NCGS remain ambiguous and not fully explored – both being relatively new diagnoses (36).

Wheat allergy

Wheat allergy can be classified based on the route of exposure, where ingesting wheat can cause food allergy manifesting itself in the skin, GI tract or the respiratory tract (wheat-dependent exercise-induced anaphylaxis) (37, 38). Children have a higher prevalence of wheat allergy compared to adults, as the majority outgrow their allergy before adolescence (39).

Celiac disease

Celiac disease occurs in genetically predisposed individuals, and is a chronic T-cell mediated autoimmune enteropathy in the small intestine, triggered by exposure to dietary gluten (37, 40). Positive serology and obvious celiac histopathology are the basis of diagnosis (40). The prevalence of celiac disease autoimmunity (positive serology) ranges between 0.2-8.5%, while the prevalence of celiac disease based on intestinal biopsy findings ranges between 0.2-2.4% (41).

Non-celiac gluten sensitivity

NCGS is the most recent inclusion in the spectrum of gluten-related disorders, and naturally also the least explored; its pathogenesis and pathophysiological aspects remaining fairly unclear (35, 42). There is a lack of diagnostic markers for NCGS, and as a result the prevalence of NCGS relies on self-reporting, making the true prevalence of the condition unidentified (42, 43). A few studies conducted reported an estimated self-reported NCGS prevalence ranging from 0.6% to 13% (43-50).

1.6 Nut allergy

Nut allergy is often referred to as tree nut allergy, and include nuts like chestnuts, hazelnuts, acorns, almonds, pistachios, cashew nuts, pecans walnuts, brazil nuts, pine nuts and macadamia nuts (51). There is incomplete knowledge of prevalence, as most studies are based

on self-reporting, leading to an overestimation (2). One systematic review that included self-reported, test results and oral food challenges observed a prevalence of self-reported tree nut allergy up to 7.3%, while the prevalence of tree nut allergy using objective oral food challenges ranged from 0.1% to 4.3% (52). It is associated with severe symptoms, accounting for 18-40% of anaphylaxis, and is seldom outgrown (53, 54).

1.7 Peanut allergy

Though peanut is often referred to as a nut, it is in fact categorized as a legume; being more related to chickpeas, lentils and beans. (51). Peanut allergy has become more prevalent in western countries the last decade, being as prevalent as 1.4-3.0% (22, 55). The allergy is developed in the first years of life, and is usually lifelong. Compared to other food allergies, it is associated with more severe symptoms and outcomes, being the main cause of anaphylaxis and death due to food allergy (55, 56).

1.8 Nutritional adequacy in subjects with food hypersensitivity

As mentioned, the most common food allergens are cow's milk, eggs, nuts, wheat, soy and sea food (2, 3). Furthermore, it has become a growing trend to eliminate wheat from the diet due to a perception that the gut is hypersensitive to wheat products, or that elimination of wheat is beneficial for the health (57). All food mentioned above have many important nutrients and trace elements; especially dairy products and wheat have significant roles in covering the body's need for carbohydrates, fat and fatty acids, vitamins and trace elements (58-61). Based on the Directorate of Health in Norway the general Norwegian population already have a diet with insufficient amounts of coarse grains, dietary fibres, vitamin D and folate. Certain groups of the population also lack iron and iodine in their diet (58).

Exclusion diets where one eliminates important food such as cow's milk or wheat, is associated with increased risk of nutritional consequences. Especially children in development are at risk, as an incomplete diet lacking nutrition can cause greater adverse effects in children compared to adults (59). Several studies indicate that children with one or more food allergies have reduced nutritional status compared to children without food allergies (62, 63). Another study suggests there is a higher risk of calcium and vitamin D deficiency in children with food allergies; however, there are other studies with conflicting results (64, 65).

1.8.1 Milk and dairy products

Nutritional content

Milk and dairy products are one of the greatest contributor to fat in a regular Norwegian diet; contributing with 45% of saturated fat, as well as 27% of total fat (58). More than 60% of calcium and iodine in a Norwegian diet stem from dairy products, and patients with cow's milk allergy often require calcium supplements to reach a sufficient calcium intake (59, 60). Additionally dairy products are also an important source of protein, vitamin A and vitamin B12 (60). In Norway some milk and dairy products are supplemented with vitamin D; a strategic attempt to raise the unsatisfying vitamin D status amongst Norwegians, especially in the elderly, the immigrants and the parts of the population experiencing Polar Night in the winter months (58, 61).

Effects of milk restricted diets

In the past decade, there have been a decline in consumption of cow's milk, as well as an increase of lactose-free milk consumption; one study reporting GI symptoms as the main cause of choosing lactose-free milk over regular cow's milk (66). The main disadvantageous of avoiding milk and dairy products are reduced Calcium intake and Vitamin D deficiency; causing an increased risk of rickets in children and osteomalacia in adults, and an increased risk for osteoporosis and fractures (21, 61, 67, 68).

A Norwegian study from 2000 suggested that children (31-37 months) following a strict cow's milk exclusion (CME) diet had significantly lower intake of energy, fat, protein, calcium, riboflavin (vitamin B_{12}) and niacin compared to children with an unrestricted diet. Even after applying milk substitution to the CME diet, the children did not meet the recommended nutrient intake for calcium and riboflavin (62). A study on Swedish children and adolescents, observed an association between LNP subjects and a reduced intake of milk and Calcium compared to subjects who tolerated lactose (69). Similar results were observed in a study of children with CMA, showing a reduced intake of Calcium, as well as more frequently insufficient levels of Vitamin A and D compared to the control group (70). Several studies indicate that children on CME diets are more prone to fussy eating and a less varied diet overall even in long-term, which may also result in inadequate nutrient intake (71-73).

1.8.2 Wheat

Wheat and grain products are important sources to dietary fibre, iron, vitamin B (thiamine, niacin, riboflavin) and trace elements (74). Individuals on gluten-free diets (GFD) are at risk

of having inadequate intake of Iron, Folate, Calcium, Selenium, Zinc, Niacin, Thiamine, Riboflavin, Vitamin D, Vitamin A and Vitamin B_{12} (75-81). Several gluten-free substitutions contain very little dietary fibre and other nutrition; it is recommended that individuals following a strict GFD make an additional effort to secure an adequate nutritional status (74). Furthermore, studies suggest there is an increased risk of weight gain and overweight when following GFD. This is related to the fact that many gluten-free substitutions have a high calorie content compared to the gluten product it imitates (82). Gluten-free substitutions also often contain higher amounts of carbohydrate, fat (particularly saturated fat), combined with a reduced amount of proteins and a higher glycaemic index. All characteristics mentioned above is associated with a negative health impact (83).

The last two decades there have been a notable increase of individuals following a GFD, based on perceived gluten sensitivity; resulting in a higher number following GFD than the estimated prevalence of celiac disease in a general population (35, 57). However, it is important to note that a strict GFD is demanding to follow (84). Consequently, the adherence to the GFD is reportedly lower than the prevalence of both self-reported and proven gluten sensitivity (43, 84). Based on these data one may conclude that there is a substantial percent of the population partially or totally excluding gluten-containing products from their diet. One may also conclude that there is an increased number of individuals occasionally or frequently substituting wheat products with less nutritional gluten-free substitutes, due to the increased global market of gluten-free products (35).

1.8.3 Egg, nuts and peanuts

Hen eggs are known as a nutritious food, being a good source for proteins of high quality, vitamins (A, B_2, B_6, B_{12}, D, E, K), minerals and healthy profile of fatty acids and lipids (85, 86).

Nuts are also considered as healthy and nutrient rich, containing healthy monosaturated and polyunsaturated fatty acid profiles, fibres, Vitamin E, K and B_1, minerals such as magnesium and potassium, carotenoids, and antioxidants (87). There are several health benefits linked to eating nuts on a regular basis, such as decreasing triglycerides, cholesterol and fasting blood glucose, reduction of oxidative stress, inflammation, visceral adiposity and cardiovascular disease risk (87-90).

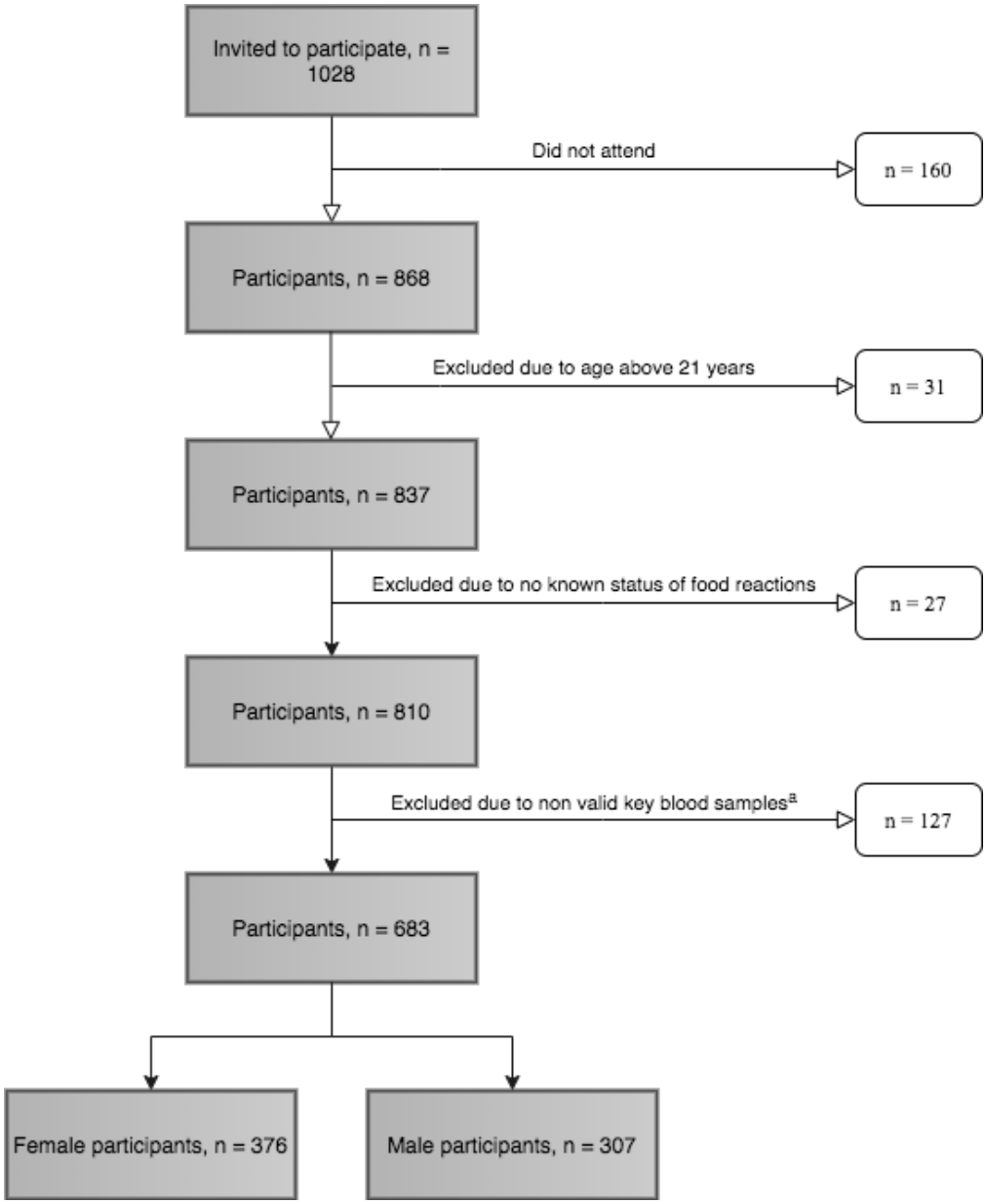
2 Materials and methods

2.1 Population and study design

The Tromsø Study Fit Futures 1 and the follow-up study Fit Futures 2 (TFF1 and TFF2) are cross-sectional studies on the health and lifestyles of adolescents in upper-secondary school in the Norwegian municipalities Tromsø and Balsfjord. This project includes data from TFF2 exclusively. The youth surveys were conducted at a research lab at the University Hospital of North Norway (UNN) during school hours. TFF1 were conducted in 2010-2011; all first-year students at the 8 upper-secondary schools in Tromsø and Balsfjord were invited to participate, of which 92.8% (n = 1038) attended (91). The school year of 2012-2013 the second wave of the study were conducted. All third-year students at upper secondary school and all participants from TFF1 (including those who did not attend school this school-year) were invited to participate in TFF2. A total of 1028 students where invited, of which 868 attended (71.9%).

The survey consisted of an interview, a web-based questionnaire about general lifestyle, health and disease, clinical examinations (including height and weight measurement) and blood sampling. The biomarkers Hb, Fe, Ferritin, Calcium and Vitamin D were analysed. In addition, all participants reported their age, sex, general diet, snuff consumption, smoking habits, alcohol consumption, physical activity, whether they had been diagnosed with asthma, allergic rhinitis and/or eczema. All participants with unknown value for self-reported food reactions, age above 21 years, missing data for the key blood biomarkers (Hb, Fe, Ferritin, Calcium, Vitamin D) in TFF2 were excluded in this project. The final study population includes 683 participants, of which 376 were female and 307 were male.

Fig 1:
Study population. The Tromsø Study; Fit Futures 2.



[a] Key blood samples: Haemoglobin, Fe, Ferritin, Calcium, Vitamin D.

2.2 Methods

2.2.1 Assessment of self-reported hypersensitivity

Information on self-reported hypersensitivity against food was collected in the web-based questionnaire, using yes-no questions. All participants were given the introductory question to self-reported food hypersensitivity; "In the past 12 months, have you reacted against anything in the food?". The participants reporting a food reaction would get follow-up questions for specific food items; in example "In the past 12 months, have you reacted against any of these

food items; Milk protein?". The follow-up questions included in this project were milk protein, milk lactose, egg, wheat or other seeds, peanut, hazelnut, almond, walnut or pecan nut, cashew nut or pistachio nut, and brazil nut. The food reactions not included in the present analysis had too few subjects reporting a reaction against the given food. Reactions against milk protein and milk lactose were assessed collectively as food reactions against milk. All categories of nuts (not including peanut, being a legume) were also assessed collectively.

2.2.2 Assessment of biomarkers in blood

Non-fasting blood samples were drawn from an antecubital vein by trained research nurses. The Department of Laboratory Medicine at UNN Tromsø analysed Fe, Ferritin and Calcium in serum and Hb in EDTA blood samples. Serum vitamin-D was analysed at the Haukeland University Hospital, the Hormone Laboratory, according to method described previously (92).

2.2.3 Statistical analysis

The statistical analyses for this thesis were done using IBM SPSS® Statistics version 26. Characteristics of the study population were described using summary statistics, and were sex stratified, as there are differences in reference levels between the sexes. The continuous variables were presented in means and standard deviation (normal distribution) or median and interquartile range (IQR) (non-normal distribution), while categorical variables were presented in number of subjects and percentage. Comparisons of the continuous variables were evaluated using Student's T-test (normally distributed data) or Mann-Whitney U test (non-normally distributed data), while comparisons of the categorical variables were evaluated using Pearson's Chi-Square test. Statistical significance levels for these analyses were set to $p < 0.05$.

2.2.4 Ethics

All participants in TFF2 gave a written informed consent to be part of the survey. The Fit Futures study has been approved by the Regional Committee for Medical Health and Research Ethics (REK), the Norwegian Data Protection Authority and the Norwegian Directorate of Health. This master project was approved by REK Nord (reference 2019: 68485).

3 Results

Characteristics

Selected characteristics of the study sample are shown in Table 1. Of the 868 participants in TFF2, 683 adolescents (17 to 21 years) met our inclusion criteria; of which 376 were female and 307 were male. The mean age was 18.3 years for the total population, as well as for both females and males seperately. The prevalence of atopic conditions (atopic eczema, asthma, allergic rhinitis) ranged between 10.6% to 19.1% in females (atopic eczema being most prevalent), and 11.1% to 13.7% in males (asthma being the most prevalent).

Upon questions about general diet, 13.0% of the females and 10.4% of the males reported to rarely or never drink milk and/or liquid dairy products, while around 2/3 of females and over ¾ of males reported eating cheese weekly. Over half of both men and women reported taking vitamin supply sometimes or on a daily basis. The majority of both females and males reported eating fat fish (58.8% and 56.7% respectively) and lean fish (63.0% and 63.8% respectively) less than once weekly. A higher proportion of females reported a daily consumption of both fruit (44.4%) and vegetables (41.8%) compared to males (29.3% and 29.6%, respectively). However, the majority of both males and females reported eating fruit and vegetables weekly.

Table 1

Characteristics of the study population by sex. The Tromsø Study; Fit Futures 2. (n=683).

	Female N (%) n=376	**Male** N (%) n=307
Age, years	18.3 (0.7)	18.3 (0.6)
Height, cm	166.0 (6.6)	179.5 (6.6)
Weight, kg	63.3 (11.4)	75.2 (14.2)
BMI, kg/m^2		
Underweight (<18.5)	18 (4.8)	24 (7.8)
Normal (18.5-24.9)	286 (76.1)	199 (64.8)
Overweight (25-29.9)	45 (12.0)	61 (19.9)
Severely overweight (>30)	27 (7.2)	23 (7.5)
Atopic eczema		
Yes	72 (19.1)	35 (11.4)
No	260 (69.1)	235 (76.5)
Don’t know/unknown status	44 (11.7)	37 (12.0)
Asthma		
Yes	51 (13.6)	42 (13.7)
No	306 (81.4)	248 (80.8)

Don’t know/unknown status	19 (5.1)	17 (5.5)
Allergic rhinitis		
Yes	40 (10.6)	34 (11.1)
No	305 (81.1)	237 (77.2)
Don’t know/unknown status	31 (8.2)	36 (11.8)
Smoking[a]		
Yes	79 (21.0)	81 (26.4)
No	296 (78.7)	226 (73.6)
Unknown status	1 (0.3)	0 (0.0)
Snuff[a]		
Yes	140 (37.2)	120 (39.1)
No	236 (62.8)	187 (60.9)
Alcohol use		
More than 4 times a month	18 (4.8)	16 (5.2)
2-4 times a month	189 (50.3)	158 (51.5)
Once a month or less	140 (37.2)	105 (34.2)
Never	29 (7.7)	28 (9.1)
Recreational physical activity[b]		
Low level	54 (14.4)	82 (26.2)
Medium level	157 (41.8)	65 (21.2)
High level	163 (43.4)	160 (52.1)
Unknown status	2 (0.5)	0 (0.0)
Dietary habits		
Fat fish intake		
Less than once weekly	221 (58.8)	174 (56.7)
Weekly	153 (40.7)	129 (42.0)
Unknown status	2 (0.5)	4 (1.3)
Lean fish intake		
Less than once weekly	237 (63.0)	196 (63.8)
Weekly	135 (35.9)	106 (34.5)
Unknown status	4 (1.1)	5 (1.6)
Fruit intake		
Rarely/never	27 (7.2)	54 (17.6)
Weekly	181 (48.1)	162 (52.8)
Daily	167 (44.4)	90 (29.3)
Unknown	1 (0.3)	1 (0.3)
Vegetable intake		
Rarely/never	26 (6.9)	25 (8.1)
Weekly	192 (51.1)	188 (61.2)
Daily	157 (41.8)	91 (29.6)
Unknown status	1 (0.3)	3 (1.0)
Cheese intake		
Rarely/never	18 (4.8)	5 (1.6)
Monthly	64 (17.0)	28 (9.1)
Weekly	255 (67.8)	236 (76.9)

Daily	38 (10.1)	36 (11.7)
Unknown status	0 (0.0)	2 (0.7)
Milk and liquid dairy products[c] intake		
Rarely/never	49 (13.0)	32 (10.4)
Weekly or daily	327 (87.0)	275 (89.6)
Vitamin supplement		
Yes	207 (55.1)	164 (53.4)
No	168 (44.7)	143 (46.6)
Unknown status	1 (0.3)	0 (0.0)

Values are means (SD) or number of subjects (%).
BMI = body mass index; SD = standard deviation;
[a] Smoking and snuff: Yes = sometimes or daily; No = Never or in the past but not currently.
[b] Recreational physical activity: Low level = reading, watching TV, or other sedentary activity; Medium level =Walking, cycling, or other forms of exercise at least 4 hours a week; High level = Participation in recreational sports, heavy outdoor activities with minimum duration of 4 hours a week, or participation in heavy training or sports competitions regularly several times a week.
[c] Milk and dairy products: Whole milk, semi-skimmed milk, skimmed milk, extra semi-skimmed milk, kefir, yoghurt, fat-reduced yoghurt and kultura.

Prevalence of food reactions

Of the 683 adolescents in this sample, 17.4% (119/683) reported to have had a reaction against food in the last 12 months. Of the five types of food reaction analysed in this project, the most prevalent type of food reaction was to milk protein and/or milk lactose (7%), thereafter to wheat and other seeds (4.0%), nuts (2.3%), egg (1.9%) and lastly to peanuts (1.5%). Of the total 48 participants reporting a reaction to milk, the most prevalently reported was against milk lactose (54.2% (26/48)), followed by reacting to both milk protein and milk lactose (31.3% (15/48)), and the least common being against milk protein exclusively (14.6% (7/48)).

There was a higher proportion of females reporting food reactions the last 12 months (20.5%) compared to males (13.7%). Females also had a higher prevalence of self-reported reactions to milk (8.5%), wheat (6.1%), nuts (3.2%) and peanuts (2.5%), than the males (milk; 5.2%, wheat; 1.3%, nuts; 1.3%, peanuts; 0%). Only in regards to self-reported reaction to egg did the males have a slightly higher prevalence than the females (2.0% versus 1.9%). Both in males and females the most common food reaction reported was to milk (5.2% and 8.5% respectively).

Association between food reactions and biomarker levels

Participants with self-reported food reactions the last 12 months had a statistically significantly lower mean Hb levels compared to participants with no self-reported food reactions ($p < 0.05$) (Table 2a). There were also significantly lower mean Hb levels in participants with self-reported hypersensitivity to wheat ($p < 0.001$), nuts ($p < 0.05$) and peanuts ($p < 0.001$) compared with participants with no self-reported reaction to these foods (Table 2d to Table 2f). Mean Hb levels also differs in males with self-reported hypersensitivity to egg compared to males with no hypersensitivity to egg ($p < 0.05$) (Table 2c).

Table 2a

Mean (standard deviation) or Median (interquartile range) of biomarkers in adolescents by **self-reported food reaction**. The Tromsø Study; Fit Futures 2. (n=683).

	Total population n = 683			**Female** n = 376			**Male** n =307		
	Any food reaction n = 119	**No food reaction** n = 564	**p**[a]	**Any food reaction** n = 77	**No food reaction** n = 299	**p**[a]	**Any food reaction** n = 42	**No food reaction** n = 265	**p**[a]
Haemoglobin, g/dL	13.4 (1.4)	13.7 (1.4)	0.04	12.6 (0.9)	12.7 (0.9)	0.60	14.8 (0.8)	14.8 (0.9)	0.89
Fe, µmol/L (median, IQR)	19.0 (7.5)	18.3 (7.5)	0.33	18.3 (7.6)	17.1 (7.5)	0.22	20.4 (7.4)	19.6 (7.3)	0.53
Ferritin[b], µg/L	52.0 (42.0)	53.0 (56.7)	0.75	36.0 (36.0)	33.0 (37.0)	0.20	87.0 (61.0)	78.0 (56.0)	0.39
Calcium, mmol/L	2.4 (0.1)	2.4 (0.1)	0.10	2.3 (0.1)	2.3 (0.1)	0.38	2.4 (0.1)	2.4 (0.1)	0.84
Vitamin D[b], nmol/L (median, IQR)	42.6 (33.4)	41.1 (34.5)	0.90	45.9 (36.9)	49.4 (33.4)	0.62	30.6 (38.0)	33.2 (27.7)	0.61

Values are means (SD) if not otherwise stated.
IQR = interquartile range.
[a] T-tests for normally distributed data and Mann-Whitney U test for non-normally distributed data.
[b] Non-normally distributed data, numbers are median (IQR).

Table 2b

Mean (standard deviation) or Median (interquartile range) of biomarkers in adolescents by **self-reported food reaction against milk protein and lactose**. The Tromsø Study; Fit Futures 2. (n=683).

	Total population n = 683			**Female** n = 376			**Male** n =307		
	Food reaction against milk n = 48	**No food reaction** n = 635	**p**[a]	**Food reaction against milk** n = 32	**No food reaction** n = 344	**p**[a]	**Food reaction against milk** n = 16	**No food reaction** n = 291	**p**[a]

Haemoglobin, g/dL	13.5 (1.4)	13.6 (1.4)	0.48	12.7 (0.7)	12.7 (0.9)	0.93	15.1 (0.1)	14.8 (0.9)	0.13
Fe, µmol/L	19.7 (8.5)	18.3 (7.4)	0.23	19.7 (8.8)	17.1 (7.4)	0.06	19.6 (8.1)	19.8 (7.3)	0.92
Ferritin[b], µg/L (median, IQR)	44.0 (42.0)	54.0 (55.0)	0.46	35.0 (38.0)	33.0 (37.0)	0.75	76.0 (66.0)	78.0 (56.0)	0.99
Calcium, mmol/L	2.4 (0.1)	2.4 (0.1)	0.73	2.4 (0.1)	2.3 (0.1)	0.65	2.4 (0.1)	2.4 (0.1)	0.87
Vitamin D[b], nmol/L (median, IQR)	38.8 (26.5)	41.9 (34.6)	0.50	43.6 (25.3)	49.3 (35.2)	0.26	31.9 (31.2)	32.7 (28.3)	0.60

Values are means (SD) if not otherwise stated.
IQR = interquartile range.
[a] T-tests for normally distributed data and Mann-Whitney U test for non-normally distributed data.
[b] Non-normally distributed data, numbers are median (IQR).

Table 2c

Mean (standard deviation) or Median (interquartile range) of biomarkers in adolescents by **self-reported food reaction against egg**. The Tromsø Study; Fit Futures 2. (n=683).

	Total population n = 683			**Female** n = 376			**Male** n =307		
	Food reaction against egg n = 13	**No food reaction** n = 670	**p[a]**	**Food reaction against egg** n = 7	**No food reaction** n = 369	**p[a]**	**Food reaction against egg** n = 6	**No food reaction** n = 301	**p[a]**
Haemoglobin, g/dL	13.4 (1.0)	13.6 (1.4)	0.56	12.6 (0.5)	12.7 (0.9)	0.77	14.4 (0.4)	14.8 (0.9)	0.03
Fe, µmol/L	21.9 (6.7)	18.3 (7.5)	0.09	22.0 (8.4)	17.2 (7.5)	0.10	21.8 (4.3)	19.7 (7.3)	0.48
Ferritin[b], µg/L (median, IQR)	42.0 (74.0)	53.5 (54.0)	0.97	33.0 (19.0)	34. 0 (36.0)	0.59	106.0 (66.0)	78.0 (56.0)	0.35
Calcium, mmol/L	2.4 (0.1)	2.4 (0.1)	0.97	2.3 (0.1)	2.3 (0.1)	0.77	2.4 (0.1)	2.4 (0.1)	0.71
Vitamin D[b], nmol/L (median, IQR)	37.4 (32.7)	41.3 (34.5)	0.69	37.4 (47.3)	48.6 (33.8)	0.64	43.0 (30.8)	32.5 (28.6)	0.23

Values are means (SD) if not otherwise stated.
IQR = interquartile range.
[a] T-tests for normally distributed data and Mann-Whitney U test for non-normally distributed data.
[b] Non-normally distributed data, numbers are median (IQR).

Table 2d

Mean (standard deviation) or Median (interquartile range) of biomarkers in adolescents by **self-reported food reaction against wheat and other seeds**. The Tromsø Study; Fit Futures 2. (n=683).

	Total population	**Female**	**Male**

	n = 683			n = 376			n =307		
	Food reaction against wheat n = 27	**No food reaction** n = 656	**p[a]**	**Food reaction against wheat** n = 23	**No food reaction** n = 353	**p[a]**	**Food reaction against wheat** n = 4	**No food reaction** n = 303	**p[a]**
Haemoglobin, g/dL	12.8 (1.1)	13.7 (1.4)	< .001	12.4 (0.7)	12.7 (0.9)	0.16	14.8 (0.7)	14.8 (0.9)	0.96
Fe, µmol/L	19.6 (7.4)	18.4 (7.5)	0.42	18.6 (7.3)	17.2 (7.6)	0.40	25.0 (6.2)	19.7 (7.3)	0.15
Ferritin[b], µg/L (median, IQR)	34.0 (32.0)	55.0 (55.0)	0.01	33.0 (21.0)	34.0 (37.0)	0.64	99.0 (63.0)	78.0 (56.0)	0.74
Calcium, mmol/L	2.3 (0.1)	2.4 (0.1)	0.01	2.3 (0.8)	2.3 (0.8)	0.18	2.4 (0.1)	2.4 (0.1)	0.40
Vitamin D[b], nmol/L (median, IQR)	43.9 (33.8)	41.1 (34.3)	0.18	44.7 (40.1)	48.7 (34.2)	0.91	39.5 (30.8)	32.5 (28.6)	0.46

Values are means (SD) if not otherwise stated.
IQR = interquartile range.
[a] T-tests for normally distributed data and Mann-Whitney U test for non-normally distributed data.
[b] Non-normally distributed data, numbers are median (IQR).

Table 2e

Mean (standard deviation) or Median (interquartile range) of biomarkers in adolescents by **self-reported food reaction against hazelnut, almond, walnut, pecan nut, cashew nut, pistachio nut and/or brazil nut**. The Tromsø Study; Fit Futures 2. (n=683).

	Total population n = 683			**Female** n = 376			**Male** n =307		
	Food reaction against nuts n = 16	**No food reaction** n = 667	**p[a]**	**Food reaction against nuts** n = 12	**No food reaction** n = 364	**p[a]**	**Food reaction against nuts** n = 4	**No food reaction** n = 303	**p[a]**
Haemoglobin, g/dL	12.8 (1.3)	13.7 (1.4)	0.01	12.4 (1.1)	12.7 (0.9)	0.21	14.0 (1.1)	14.8 (0.8)	0.06
Fe, µmol/L	17.1 (9.3)	18.4 (7.5)	0.49	17.0 (10.3)	17.3 (7.5)	0.89	17.5 (6.6)	19.8 (7.3)	0.54
Ferritin[b], µg/L (median, IQR)	44.5 (53.0)	53.0 (54.0)	0.24	28.0 (55.0)	34.0 (35.0)	0.50	71.0 (61.0)	78.0 (56.0)	0.98
Calcium, mmol/L	2.4 (0.1)	2.4 (0.1)	0.23	2.3 (0.1)	2.3 (0.1)	0.18	2.4 (0.1)	2.4 (0.1)	0.22
Vitamin D[b], nmol/L (median, IQR)	49.1 (43.0)	41.1 (34.1)	0.18	60.2 (33.9)	48.2 (33.9)	0.21	28.9 (32.9)	32.5 (28.5)	0.68

Values are means (SD) if not otherwise stated.
IQR = interquartile range.
[a] T-tests for normally distributed data and Mann-Whitney U test for non-normally distributed data.
[b] Non-normally distributed data, numbers are median (IQR).

Table 2f

Mean (standard deviation) or Median (interquartile range) of biomarkers in adolescents by **self-reported food reaction against peanuts**. The Tromsø Study; Fit Futures 2. (n=683).

	Total population n = 683			**Female** n = 376			**Male** n =307		
	Food reaction against peanuts n = 10	**No food reaction** n = 673	**p[a]**	**Food reaction against peanuts** n = 10	**No food reaction** n = 366	**p[a]**	**Food reaction against peanuts** n = 0	**No food reaction** n = 307	**p[a]**
Haemoglobin, g/dL	12.7 (0.7)	13.7 (1.4)	< .001	12.7 (0.7)	12.7 (0.9)	1.00	-	14.8 (0.8)	-
Fe, µmol/L	20.7 (10.0)	18.4 (7.5)	0.33	20.7 (10.0)	17.2 (7.5)	0.15	-	19.7 (7.3)	-
Ferritin[b], µg/L (median, IQR)	44.5 (41.0)	53.0 (54.0)	0.35	44.5 (41.0)	33.5 (35.0)	0.40	-	78.0 (56.0)	-
Calcium, mmol/L	2.4 (0.0)	2.4 (0.1)	0.11	2.4 (0.0)	2.3 (0.1)	0.69	-	2.4 (0.1)	-
Vitamin D[b], nmol/L (median, IQR)	45.0 (41.4)	41.1 (34.4)	0.43	45.0 (41.4)	48.7 (33.7)	0.91	-	32.5 (28.5)	-

Values are means (SD) if not otherwise stated.
IQR = interquartile range.
[a] T-tests for normally distributed data and Mann-Whitney U test for non-normally distributed data.
[b] Non-normally distributed data, numbers are median (IQR).

Among adolescents with a reported food reaction to wheat there was (as mentioned above) a lower mean Hb level, as well as a lower mean/median Ferritin and Calcium levels ($p < 0.05$), compared to adolescents with no reported reaction to wheat. Aside from the differences in biomarker values mentioned, there were no other significant difference in serum levels amongst the participants with self-reported (specific) food reaction compared to no self-reported (specific) food reaction.

Table 3a

Number of subjects (%) with biomarker levels below and above normal reference level (93). The Tromsø Study; Fit Futures 2. (n=683).

	Any food reaction n = 119	**No food reaction** n = 564	**p[a]**
Fe < 9 µmol/L	7 (5.9)	46 (8.2)	0.400
Fe ≥ 9 µmol/L	112 (94.1)	518 (91.8)	

Calcium < 2.18 mmol/L	1 (0.8)	2 (0.3)	0.467
Calcium ≥ 2.18 mmol/L	118 (99.2)	562 (99.6)	
Vitamin D < 50 nmol/L	76 (63.9)	348 (61.7)	0.658
Vitamin D ≥ 50 nmol/L	43 (36.1)	216 (38.3)	
	Food reaction against milk n = 48	**No food reaction** n = 635	**p**[a]
Fe < 9 µmol/L	4 (8.3)	49 (7.7)	0.878
Fe ≥ 9 µmol/L	44 (91.7)	586 (92.3)	
Calcium < 2.18 mmol/L	-	3 (0.5)	0.633
Calcium ≥ 2.18 mmol/L	48 (100)	632 (99.5)	
Vitamin D < 50 nmol/L	35 (72.9)	389 (61.3)	0.109
Vitamin D ≥ 50 nmol/L	13 (27.1)	246 (38.7)	
	Food reaction against egg n = 13	**No food reaction** n = 670	**p**[a]
Fe < 9 µmol/L	-	53 (79.1)	0.291
Fe ≥ 9 µmol/L	13 (100)	617 (92.1)	
Calcium < 2.18 mmol/L	-	3 (0.4)	0.809
Calcium ≥ 2.18 mmol/L	13 (100)	667 (99.6)	
Vitamin D < 50 nmol/L	8 (61.5)	416 (62.1)	0.968
Vitamin D ≥ 50 nmol/L	5 (38.5)	254 (37.9)	
	Food reaction against wheat n = 27	**No food reaction** n = 656	**p**[a]
Fe < 9 µmol/L	2 (7.4)	51 (77.7)	0.944
Fe ≥ 9 µmol/L	25 (92.6)	605 (92.2)	
Calcium < 2.18 mmol/L	1 (3.7)	2 (0.3)	0.009
Calcium ≥ 2.18 mmol/L	26 (96.3)	654	
Vitamin D < 50 nmol/L	18 (66.7)	406 (61.9)	0.616
Vitamin D ≥ 50 nmol/L	9 (33.3)	250 (38.1)	
	Food reaction against nuts[b] n = 16	**No food reaction** n = 667	**p**[a]
Fe < 9 µmol/L	1 (6.2)	52 (7.8)	0.819
Fe ≥ 9 µmol/L	15 (93.8)	615 (92.2)	
Calcium < 2.18 mmol/L	-	3 (0.4)	0.788
Calcium ≥ 2.18 mmol/L	16 (100)	664 (99.6)	
Vitamin D < 50 nmol/L	8 (50)	416 (62.4)	0.314
Vitamin D ≥ 50 nmol/L	8 (50)	251 (37.6)	
	Food reaction against peanuts n = 10	**No food reaction** n = 673	**p**[a]
Fe < 9 µmol/L	-	53 (7.9)	0.355
Fe ≥ 9 µmol/L	10 (100)	620 (92.1)	
Calcium < 2.18 mmol/L	-	3 (0.4)	0.832
Calcium ≥ 2.18 mmol/L	10 (100)	670 (99.6)	
Vitamin D < 50 nmol/L	7 (70.0)	417 (62.0)	0.603
Vitamin D ≥ 50 nmol/L	3 (30.0)	256 (38.0)	

[a] Chi-square test

[b] Nuts; hazelnut, almond, walnut, pecan nut, cashew nut, pistachio nut and/or brazil nut

Table 3b

Number of females (%) with biomarker levels below and above normal reference level (93). The Tromsø Study; Fit Futures 2. (n=376).

	Food reaction n = 77	**No food reaction** n = 299	**p**[a]
Haemoglobin < 11.7 g/dL	7 (9.1)	27 (9.0)	0.987
Haemoglobin ≥ 11.7 g/dL	70 (90.9)	272 (91.0)	
Ferritin < 15µg/L	10 (13.0)	49 (16.4)	0.464
Ferritin ≥15µg/L	67 (87.0)	250 (83.6)	
	Food reaction against milk n = 32	**No food reaction** n = 344	**p**[a]
Haemoglobin < 11.7 g/dL	2 (6.3)	32 (9.3)	0.565
Haemoglobin ≥ 11.7 g/dL	30 (93.8)	312 (90.7)	
Ferritin < 15µg/L	4 (12.5)	55 (16.0)	0.604
Ferritin ≥15µg/L	28 (87.5)	289 (84.0)	
	Food reaction against egg n = 7	**No food reaction** n = 369	**p**[a]
Haemoglobin < 11.7 g/dL	-	34 (9.2)	0.400
Haemoglobin ≥ 11.7 g/dL	7 (100)	335 (90.8)	
Ferritin < 15µg/L	-	59 (16.0)	0.249
Ferritin ≥15µg/L	7 (100)	310 (84.0)	
	Food reaction against wheat n = 23	**No food reaction** n = 353	**p**[a]
Haemoglobin < 11.7 g/dL	2 (8.7)	32 (9.0)	0.952
Haemoglobin ≥ 11.7 g/dL	21 (91.3)	321 (90.9)	
Ferritin < 15µg/L	2 (8.7)	57 (16.1)	0.341
Ferritin ≥15µg/L	21 (91.3)	296 (83.9)	
	Food reaction against nuts[b] n = 12	**No food reaction** n = 364	**p**[a]
Haemoglobin < 11.7 g/dL	2 (16.7)	32 (8.8)	0.349
Haemoglobin ≥ 11.7 g/dL	10 (83.3)	332 (91.2)	
Ferritin < 15µg/L	3 (25.0)	56 (15.4)	0.368
Ferritin ≥15µg/L	9 (75.0)	308 (84.6)	
	Food reaction against peanuts n = 10	**No food reaction** n = 366	**p**[a]
Haemoglobin < 11.7 g/dL	-	34 (9.3)	0.312
Haemoglobin ≥ 11.7 g/dL	10 (100)	332 (90.7)	
Ferritin < 15µg/L	1 (10.0)	58 (15.8)	0.616
Ferritin ≥15µg/L	9 (90.0)	308 (84.2)	

[a] Chi-square test
[b] Nuts; hazelnut, almond, walnut, pecan nut, cashew nut, pistachio nut and/or brazil nut

Table 3c

Number of males (%) with biomarker levels below and above normal reference level (93). The Tromsø Study; Fit Futures 2. (n=307).

	Food reaction n = 42	**No food reaction** n = 265	**p[a]**
Haemoglobin < 13.4 g/dL	1 (2.4)	16 (6.0)	0.336
Haemoglobin ≥ 13.4 g/dL	41 (97.6)	249 (94.0)	
Ferritin < 22 µg/L	1 (2.4)	5 (1.9)	0.830
Ferritin ≥22 µg/L	41 (97.6)	260 (98.1)	
	Food reaction against milk n = 16	**No food reaction** n = 291	**p[a]**
Haemoglobin < 13.4 g/dL	-	17 (5.8)	0.320
Haemoglobin ≥ 13.4 g/dL	16 (100)	274 (94.2)	
Ferritin < 22 µg/L	-	6 (2.1)	0.562
Ferritin ≥22 µg/L	16 (100)	285 (97.9)	
	Food reaction against egg n = 6	**No food reaction** n = 301	**p[a]**
Haemoglobin < 13.4 g/dL	-	17 (5.6)	0.549
Haemoglobin ≥ 13.4 g/dL	6 (100)	284 (94.4)	
Ferritin < 22 µg/L	-	6 (2.0)	0.727
Ferritin ≥22 µg/L	6 (100)	295 (98.0)	
	Food reaction against wheat n = 4	**No food reaction** n = 303	**p[a]**
Haemoglobin < 13.4 g/dL	-	17 (5.6)	0.626
Haemoglobin ≥ 13.4 g/dL	4 (100)	286 (94.4)	
Ferritin < 22 µg/L	-	6 (2.0)	0.776
Ferritin ≥22 µg/L	4 (100)	297 (98.0)	
	Food reaction against nuts[b] n = 4	**No food reaction** n = 303	**p[a]**
Haemoglobin < 13.4 g/dL	1 (25.0)	16 (5.3)	0.087
Haemoglobin ≥ 13.4 g/dL	3 (75.0)	287 (94.7)	
Ferritin < 22 µg/L	-	6 (2.0)	0.776
Ferritin ≥22 µg/L	4 (100)	297 (98.0)	

[a] Chi-square test
[b] Nuts; hazelnut, almond, walnut, pecan nut, cashew nut, pistachio nut and/or brazil nut

Table 3a to 3c compare participants with and participants without the specific self-reported food reactions, and levels below and above reference values of the key biomarkers. There was an association between having reported a reaction to wheat and a having calcium levels below reference value ($p < 0.05$). Apart from this finding, there was no observed association between self-reported food reaction and biomarker levels below reference values.

4 Discussion

In this thesis, we have described several self-reported food reactions in a general adolescent population, and studied associations with biomarker levels of Hb, Fe, Ferritin, Calcium and Vitamin D. To our knowledge this is the first report looking at various types of self-reported food hypersensitivity, and their associations with circulating levels of different biomarkers in an adolescent population (17-21 years). This study population of 683 subjects had an overall self-reported food hypersensitivity prevalence of 17.4% (119/683); and a higher proportion of females (77/376) reporting food hypersensitivity compared to males (42/307). We have observed a difference in the mean levels of Hb, when comparing participants with no food reaction the last 12 months with participants with any self-reported food reaction, self-reported food reaction to wheat, nuts and peanuts; the participants with food reaction having a lower mean Hb value. There is also demonstrated a lower mean/median value of Calcium and Ferritin in subjects with self-reported hypersensitivity towards wheat. Males who reported a food reaction to egg had a significantly lower mean Hb value, compared to males with no food reaction to egg. There is also observed an association between having reported a food reaction to wheat and having Calcium levels below normal reference values.

Prevalence of self-reported hypersensitivities

The prevalence of self-reported food hypersensitivity in our population (17.4%) did match the prevalence reported in several other studies (ranging between 12-25%) (9-13). The most common type of milk hypersensitivity reported in TFF2 was towards milk lactose, which corresponds to research showing that lactose intolerance prevalence peaks in adolescence, compared to milk protein allergy (which is commonly outgrown) (23, 24). Additionally, our study shows that a reaction towards milk is the most commonly reported reaction in both sexes, which may correlate to both milk allergy (although commonly outgrown) being the most common type of food allergy in children (19, 20, 22), and that an estimated 70% of the general adult population have LNP (23, 24). Self-reported reactions towards egg in our population (1.9%) correspond with the prevalence of egg allergy in young children (0.5-2.5%) found in other studies (28, 29). However, one would assume there would be a slightly lower prevalence in an adolescent population, as the majority of children outgrow their egg allergy (34).

As mentioned earlier, self-reported adverse reactions to food are prone to overestimating the true prevalence of food allergy and food intolerances (3, 5, 15). When measuring the

prevalence of adverse reactions towards wheat in our population, one did not differentiate between whether the reaction was linked to wheat allergy, celiac disease or NCGS. This complicates the interpretation of the prevalence in our population (4.0%), when comparing it to the prevalence in other studies. However, one should note that research on the prevalence of gluten-related disorders, as well as wheat allergy and NCGS are largely lacking, and that only celiac disease has been thoroughly researched (36). There is also a limited knowledge about the prevalence of tree nut allergy, one study reporting a range between 0.1-4.3% (when using oral food challenges) (52), which our observed prevalence (2.3%) corresponds with. Lastly, the observed prevalence of self-reported peanut allergy (1.5%) correlates with other research, reporting a prevalence of 1.4-3.0% (22, 55).

Associations between self-reported food reactions and biomarkers

There is limited research on food hypersensitivity and possible nutritional consequences (assessed by biomarker levels) in an adolescent population, as most studies about food allergies and intolerances have a predominant focus on infants and young children. One should also note that several types of food hypersensitivities are relatively recently added classifications of food hypersensitivities (such as wheat allergy and NCGS), these subgroups are not currently adequately researched or understood (36).

In our population, we found a significant lower mean Hb level in participants with any food reactions, compared to participants with reported food reactions to any food, as well as to wheat, nuts and peanuts respectively. The participants with reported reaction to wheat also had a significantly lower mean/median Ferritin and Calcium, as well as having a higher risk of having Calcium levels below reference values. Several studies report that patients on a gluten-free diet show inadequate intakes of Iron, Folate, Calcium, Selenium, Zinc, Niacin, Thiamine, Riboflavin, Vitamin D, A and B_{12} (75-81), which corresponds to our findings of significantly lower mean/median value of Ferritin and Calcium, and inadequate Calcium levels. However, we could not find any reports on wheat hypersensitivity being associated with lower Hb values.

In regard to participants with reported hypersensitivity towards nuts and peanuts having a lower mean Hb value, there is currently limited research on the nutritional consequences of having hypersensitivity towards these foods. Additionally, there is also lacking reports on nutritional consequences in participants with a general self-reported food hypersensitivity. Our finding of a lower mean Hb value in participants with any reported food hypersensitivity,

does not tell which of the food hypersensitivities which contribute to this, and to which degree. Also, all findings of participants with food reaction(s) having a lower mean Hb value, does not imply causality and we cannot conclude whether this is due to nutritional consequences of possible elimination diet or other factors. TFF2 data do not show correspondingly lower mean level of Ferritin (except in participants with self-reported wheat hypersensitivity), and the mechanisms for the lower Hb value in participants with food hypersensitivities remain elusive.

Strengths and limitations

The strengths of this study include having a high attendance rate (84.4%) and population-based design, and therefore a reduced risk of selection bias. The study population consists of third-year students at upper-secondary school both from rural and urban districts, and one can assume that the results are representative for other adolescent populations in Norway.

There are several limitations to our study. In evaluating the presence of food hypersensitivity, the participants answered a web-based multiple-choice questionnaire, themselves interpreting the questions. Self-interpretation increases the risk of information bias in our study, and this information bias would most likely overestimate the true prevalence of food hypersensitivities, as is reported in several other studies based on self-reported food reactions (5, 15, 17). However, we base our hypothesis on the assumption that the participants having an experience they subjectively interpret as food reaction, will to some degree exclude the given food from their diet. Although there is a high probability that the prevalence reported in our study overestimates the true prevalence of food hypersensitivities, this should not necessarily affect the outcome of nutritional status.

Another limitation is however that we can only assume that the participants reporting food reaction(s) are also excluding the given food from their diet. Only in regard to milk and dairy products have there been additional questions on the general consumption; all participants answered a question about their milk and liquid dairy product intake, of which 13.0% of the females and 10.4% of the males reported to rarely or never drink milk or dairy products. Comparing this to the proportion reporting milk hypersensitivity (8.5% of females, 5.2% of males), one can assume that there is a high probability that the participants reporting food reaction to milk also reported excluding milk from the diet. One should however note that the question about milk consumption is divided into rarely/never (with no specific definition of rarely) or weekly to daily (ranging between one glass weekly to 24 glasses daily), this due to

how the questions and multiple choices in the questionnaire where articulated. Due to this wide range, one cannot conclude that those answering weekly/daily intake of milk actually have a recommended intake of milk (three portions a day in Norway), and therefore ensures the daily recommendations for calcium and iodine through milk, compared to the participants answering a rarely/never.

The analysis of mean biomarker level in relation to self-reported food reactions was not adjusted for possible confounding factors. Information on lifestyle, health, drug use and diseases in TFF2 may be included in future analysis. For example, the participants also answered a question regarding vitamin supplementation, of which 55.1% of the females and 53.4% of the males answered to take vitamin supplementation sometimes to daily. However, type of vitamin supplementation and dosage were not specified, and therefore it would be hard to judge whether this could have an impact on the key biomarker levels.

In further research one should divide the various hypersensitivities into subgroups of food reaction severity and related degree of food avoidance, to properly recognize the effects of partial and total exclusion diets individually. Further examination of the general diet in subjects experiencing food reactions could also be of interest, to investigate whether adolescents on exclusion diets are more or less aware of their general diet choices compared to the control group, and whether they make significantly different diet choices to replace the nutrients they may be lacking. There should also be a more comprehensive investigation of the nutritional status, with a more extensive list of biomarkers, alternatively with other measurements in addition to this, that might give an indication of the true nutritional status of the subject.

5 Conclusion

The results from this study suggest that there might be slight difference in biomarker levels when comparing adolescents with self-reported food reaction to their healthy peers, especially in subjects reporting a food reaction to wheat. There was a significantly lower Hb level in subjects reporting any kind of food reaction, and in subjects with self-reported food reaction to wheat, nuts and peanuts compared to the control group. Subjects reporting a reaction to wheat also had significantly lower Ferritin and Calcium levels, as well as having a higher risk of having Calcium levels below reference levels. Further research is however needed, to be able to conclude with how and to which degree having food reactions and following exclusion diets affects the general health; these should include an evaluation of the extent to which the adolescents are actually excluding the given food from their diet, and a further assessment of nutritional status through a more extensive list of biomarker levels or other measurements.

6 References

1. Tuck CJ, Biesiekierski JR, Schmid-Grendelmeier P, Pohl D. Food Intolerances. Nutrients. 2019;11(7):1684.

2. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. Nutr Res. 2011;31(1):61-75.

3. Turnbull JL, Adams HN, Gorard DA. Review article: the diagnosis and management of food allergy and food intolerances. Aliment Pharmacol Ther. 2015;41(1):3-25.

4. Chafen JJ, Newberry SJ, Riedl MA, Bravata DM, Maglione M, Suttorp MJ, et al. Diagnosing and managing common food allergies: a systematic review. Jama. 2010;303(18):1848-56.

5. Madsen C. Prevalence of food allergy: an overview. Proc Nutr Soc. 2005;64(4):413-7.

6. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy. 2001;56(9):813-24.

7. Sicherer SH. Epidemiology of food allergy. J Allergy Clin Immunol. 2011;127(3):594-602.

8. Prescott SL, Pawankar R, Allen KJ, Campbell DE, Sinn J, Fiocchi A, et al. A global survey of changing patterns of food allergy burden in children. World Allergy Organ J. 2013;6(1):21.

9. Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. The Lancet. 1994;343(8906):1127-30.

10. Jansen JJ, Kardinaal AF, Huijbers G, Vlieg-Boerstra BJ, Martens BP, Ockhuizen T. Prevalence of food allergy and intolerance in the adult Dutch population. J Allergy Clin Immunol. 1994;93(2):446-56.

11. Altman DR, Chiaramonte LT. Public perception of food allergy. Journal of Allergy and Clinical Immunology. 1996;97(6):1247-51.

12. Björnsson E, Janson C, Plaschke P, Norrman E, Sjöberg O. Prevalence of Sensitization to Food Allergens in Adult Swedes. Annals of Allergy, Asthma & Immunology. 1996;77(4):327-32.

13. Woods R, Abramson M, Raven J, Bailey M, Weiner J, Walters E. Reported food intolerance and respiratory symptoms in young adults. European Respiratory Journal. 1998;11(1):151-5.

14. Grabenhenrich LB. [The epidemiology of food allergy in Europe]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2016;59(6):745-54.

15. Woods RK, Stoney RM, Raven J, Walters EH, Abramson M, Thien FC. Reported adverse food reactions overestimate true food allergy in the community. Eur J Clin Nutr. 2002;56(1):31-6.

16. Loh W, Tang MLK. The Epidemiology of Food Allergy in the Global Context. Int J Environ Res Public Health. 2018;15(9).

17. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, et al. Prevalence of adverse reactions to food in Germany – a population study. Allergy. 2004;59(3):338-45.

18. Winberg A, West CE, Strinnholm Å, Nordström L, Hedman L, Rönmark E. Assessment of Allergy to Milk, Egg, Cod, and Wheat in Swedish Schoolchildren: A Population Based Cohort Study. PLOS ONE. 2015;10(7):e0131804.

19. Pensabene L, Salvatore S, D'Auria E, Parisi F, Concolino D, Borrelli O, et al. Cow's Milk Protein Allergy in Infancy: A Risk Factor for Functional Gastrointestinal Disorders in Children? Nutrients. 2018;10(11).

20. Kansu A, Yüce A, Dalgıç B, Şekerel BE, Çullu-Çokuğraş F, Çokuğraş H. Consensus statement on diagnosis, treatment and follow-up of cow's milk protein allergy among infants and children in Turkey. Turk J Pediatr. 2016;58(1):1-11.

21. Heine RG, AlRefaee F, Bachina P, De Leon JC, Geng L, Gong S, et al. Lactose intolerance and gastrointestinal cow's milk allergy in infants and children - common misconceptions revisited. World Allergy Organ J. 2017;10(1):41.

22. Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. Allergy. 2014;69(1):62-75.

23. Wahlqvist ML. Lactose nutrition in lactase nonpersisters. Asia Pac J Clin Nutr. 2015;24 Suppl 1:S21-5.

24. Lebenthal E, Antonowicz I, Shwachman H. Correlation of lactase activity, lactose tolerance and milk consumption in different age groups. Am J Clin Nutr. 1975;28(6):595-600.

25. Welsh JD, Poley JR, Bhatia M, Stevenson DE. Intestinal disaccharidase activities in relation to age, race, and mucosal damage. Gastroenterology. 1978;75(5):847-55.

26. Antonowicz I, Lebenthal E. Developmental pattern of small intestinal enterokinase and disaccharidase activities in the human fetus. Gastroenterology. 1977;72(6):1299-303.

27. Diekmann L, Pfeiffer K, Naim HY. Congenital lactose intolerance is triggered by severe mutations on both alleles of the lactase gene. BMC Gastroenterol. 2015;15:36.

28. Caubet JC, Wang J. Current understanding of egg allergy. Pediatr Clin North Am. 2011;58(2):427-43, xi.

29. Dhanapala P, De Silva C, Doran T, Suphioglu C. Cracking the egg: An insight into egg hypersensitivity. Mol Immunol. 2015;66(2):375-83.

30. Bleumink E, Young E. Studies on the atopic allergen in hen's egg. II. Further characterization of the skin-reactive fraction in egg-white; immuno-electrophoretic studies. Int Arch Allergy Appl Immunol. 1971;40(1):72-88.

31. Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. J Immunol. 1997;159(4):2026-32.

32. Leonard SA, Nowak-Węgrzyn A. Re-defining food allergy phenotypes and management paradigm: is it time for individualized egg allergy management? Clin Exp Allergy. 2011;41(5):609-11.

33. Tan JW, Joshi P. Egg allergy: an update. J Paediatr Child Health. 2014;50(1):11-5.

34. Tey D, Heine RG. Egg allergy in childhood: an update. Curr Opin Allergy Clin Immunol. 2009;9(3):244-50.

35. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, et al. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med. 2012;10:13.

36. Ashtari S, Pourhoseingholi MA, Rostami K, Aghdaei HA, Rostami-Nejad M, Busani L, et al. Prevalence of gluten-related disorders in Asia-Pacific region: a systematic review. J Gastrointestin Liver Dis. 2019;28(1):95-105.

37. Elli L, Branchi F, Tomba C, Villalta D, Norsa L, Ferretti F, et al. Diagnosis of gluten related disorders: Celiac disease, wheat allergy and non-celiac gluten sensitivity. World J Gastroenterol. 2015;21(23):7110-9.

38. Cianferoni A. Wheat allergy: diagnosis and management. J Asthma Allergy. 2016;9:13-25.

39. Keet CA, Matsui EC, Dhillon G, Lenehan P, Paterakis M, Wood RA. The natural history of wheat allergy. Ann Allergy Asthma Immunol. 2009;102(5):410-5.

40. Kowalski K, Mulak A, Jasińska M, Paradowski L. Diagnostic challenges in celiac disease. Adv Clin Exp Med. 2017;26(4):729-37.

41. Levinson-Castiel R, Eliakim R, Shinar E, Perets TT, Layfer O, Levhar N, et al. Rising prevalence of celiac disease is not universal and repeated testing is needed for population screening. United European Gastroenterol J. 2019;7(3):412-8.

42. Elli L, Roncoroni L, Bardella MT. Non-celiac gluten sensitivity: Time for sifting the grain. World J Gastroenterol. 2015;21(27):8221-6.

43. Carroccio A, Giambalvo O, Blasca F, Iacobucci R, D'Alcamo A, Mansueto P. Self-Reported Non-Celiac Wheat Sensitivity in High School Students: Demographic and Clinical Characteristics. Nutrients. 2017;9(7).

44. Tanpowpong P, Ingham TR, Lampshire PK, Kirchberg FF, Epton MJ, Crane J, et al. Coeliac disease and gluten avoidance in New Zealand children. Arch Dis Child. 2012;97(1):12-6.

45. DiGiacomo DV, Tennyson CA, Green PH, Demmer RT. Prevalence of gluten-free diet adherence among individuals without celiac disease in the USA: results from the Continuous National Health and Nutrition Examination Survey 2009-2010. Scand J Gastroenterol. 2013;48(8):921-5.

46. Aziz I, Lewis NR, Hadjivassiliou M, Winfield SN, Rugg N, Kelsall A, et al. A UK study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care. Eur J Gastroenterol Hepatol. 2014;26(1):33-9.

47. Volta U, Bardella MT, Calabrò A, Troncone R, Corazza GR. An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity. BMC Med. 2014;12:85.

48. Golley S, Corsini N, Topping D, Morell M, Mohr P. Motivations for avoiding wheat consumption in Australia: results from a population survey. Public Health Nutr. 2015;18(3):490-9.

49. Ontiveros N, López-Gallardo JA, Vergara-Jiménez MJ, Cabrera-Chávez F. Self-Reported Prevalence of Symptomatic Adverse Reactions to Gluten and Adherence to Gluten-Free Diet in an Adult Mexican Population. Nutrients. 2015;7(7):6000-15.

50. van Gils T, Nijeboer P, CE IJ, Sanders DS, Mulder CJ, Bouma G. Prevalence and Characterization of Self-Reported Gluten Sensitivity in The Netherlands. Nutrients. 2016;8(11).

51. Lomas JM, Järvinen KM. Managing nut-induced anaphylaxis: challenges and solutions. J Asthma Allergy. 2015;8:115-23.

52. Zuidmeer L, Goldhahn K, Rona RJ, Gislason D, Madsen C, Summers C, et al. The prevalence of plant food allergies: a systematic review. J Allergy Clin Immunol. 2008;121(5):1210-8.e4.

53. Weinberger T, Sicherer S. Current perspectives on tree nut allergy: a review. J Asthma Allergy. 2018;11:41-51.

54. Fleischer DM, Conover-Walker MK, Matsui EC, Wood RA. The natural history of tree nut allergy. J Allergy Clin Immunol. 2005;116(5):1087-93.

55. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med. 2015;372(9):803-13.

56. Hourihane JO, Kilburn SA, Dean P, Warner JO. Clinical characteristics of peanut allergy. Clin Exp Allergy. 1997;27(6):634-9.

57. Aziz I. The Global Phenomenon of Self-Reported Wheat Sensitivity. Am J Gastroenterol. 2018;113(7):945-8.

58. Helsedirektoratet 2017: 12. Utviklingen i norsk kosthold2017. Available from: https://www.helsedirektoratet.no/rapporter/utviklingen-i-norsk-kosthold.

59. Helsedirektoratet 2018. Kosthold ved sykdommer og tilstander2018. Available from: https://www.helsedirektoratet.no/tema/kosthold-og-ernaering/kosthold-ved-sykdommer-og-tilstander.

60. Helsedirektoratet: Nasjonalt Råd for ernæring 2017: 05. Kostråd om fett - en oppdatering og vurdering av kunnskapsgrunnlaget. 2017.

61. Helsedirektoratet: Nasjonalt Råd for ernæring 2018: 11. Vitamin D i Norge: Behov for tiltak for å sikre god vitamin D-status? 2018.

62. Henriksen C, Eggesbø M, Halvorsen R, Botten G. Nutrient intake among two-year-old children on cows' milk-restricted diets. Acta Paediatrica. 2000;89(3):272-8.

63. Flammarion S, Santos C, Guimber D, Jouannic L, Thumerelle C, Gottrand F, et al. Diet and nutritional status of children with food allergies. Pediatric Allergy and Immunology. 2011;22(2):161-5.

64. Sova C, Feuling MB, Baumler M, Gleason L, Tam JS, Zafra H, et al. Systematic Review of Nutrient Intake and Growth in Children with Multiple IgE-Mediated Food Allergies. Nutrition in Clinical Practice. 2013;28(6):669-75.

65. Maslin K, Oliver EM, Scally KS, Atkinson J, Foote K, Venter C, et al. Nutritional adequacy of a cows' milk exclusion diet in infancy. Clin Transl Allergy. 2016;6:20.

66. Zingone F, Bucci C, Iovino P, Ciacci C. Consumption of milk and dairy products: Facts and figures. Nutrition. 2017;33:322-5.

67. Fox AT, Du Toit G, Lang A, Lack G. Food allergy as a risk factor for nutritional rickets. Pediatr Allergy Immunol. 2004;15(6):566-9.

68. Doulgeraki AE, Manousakis EM, Papadopoulos NG. Bone health assessment of food allergic children on restrictive diets: a practical guide. J Pediatr Endocrinol Metab. 2017;30(2):133-9.

69. Almon R, Sjöström M, Nilsson TK. Lactase non-persistence as a determinant of milk avoidance and calcium intake in children and adolescents. J Nutr Sci. 2013;2:e26.

70. Boaventura RM, Mendonça RB, Fonseca FA, Mallozi M, Souza FS, Sarni ROS. Nutritional status and food intake of children with cow's milk allergy. Allergol Immunopathol (Madr). 2019;47(6):544-50.

71. Maslin K, Dean T, Arshad SH, Venter C. Dietary variety and food group consumption in children consuming a cows' milk exclusion diet. Pediatr Allergy Immunol. 2016;27(5):471-7.

72. Maslin K, Dean T, Arshad SH, Venter C. Fussy eating and feeding difficulties in infants and toddlers consuming a cows' milk exclusion diet. Pediatr Allergy Immunol. 2015;26(6):503-8.

73. Maslin K, Grundy J, Glasbey G, Dean T, Arshad SH, Grimshaw K, et al. Cows' milk exclusion diet during infancy: Is there a long-term effect on children's eating behaviour and food preferences? Pediatr Allergy Immunol. 2016;27(2):141-6.

74. NAAF - Norges Astma og Allergiforbund 2006: 10. Cøliaki og hveteallergi2006. Available from: https://www.naaf.no/fokusomrader/allergi-og-overfolsomhet/mat-og-matoverfolsomhet/hveteallergi/.

75. Thompson T, Dennis M, Higgins LA, Lee AR, Sharrett MK. Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? J Hum Nutr Diet. 2005;18(3):163-9.

76. Hallert C, Grant C, Grehn S, Grännö C, Hultén S, Midhagen G, et al. Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. Aliment Pharmacol Ther. 2002;16(7):1333-9.

77. Shepherd SJ, Gibson PR. Nutritional inadequacies of the gluten-free diet in both recently-diagnosed and long-term patients with coeliac disease. J Hum Nutr Diet. 2013;26(4):349-58.

78. Ohlund K, Olsson C, Hernell O, Ohlund I. Dietary shortcomings in children on a gluten-free diet. J Hum Nutr Diet. 2010;23(3):294-300.

79. Wild D, Robins GG, Burley VJ, Howdle PD. Evidence of high sugar intake, and low fibre and mineral intake, in the gluten-free diet. Alimentary Pharmacology & Therapeutics. 2010;32(4):573-81.

80. Babio N, Alcázar M, Castillejo G, Recasens M, Martínez-Cerezo F, Gutiérrez-Pensado V, et al. Patients With Celiac Disease Reported Higher Consumption of Added Sugar and Total Fat Than Healthy Individuals. Journal of Pediatric Gastroenterology and Nutrition. 2017;64(1):63-9.

81. NCF - Norsk Cøliakiforening. Mer om cøliaki.

82. Theethira TG, Dennis M. Celiac disease and the gluten-free diet: consequences and recommendations for improvement. Dig Dis. 2015;33(2):175-82.

83. Penagini F, Dilillo D, Meneghin F, Mameli C, Fabiano V, Zuccotti GV. Gluten-Free Diet in Children: An Approach to a Nutritionally Adequate and Balanced Diet. Nutrients. 2013;5(11):4553-65.

84. Silvester JA, Weiten D, Graff LA, Walker JR, Duerksen DR. Is it gluten-free? Relationship between self-reported gluten-free diet adherence and knowledge of gluten content of foods. Nutrition. 2016;32(7-8):777-83.

85. López Sobaler AM, Aparicio Vizuete A, Ortega RM. [Role of the egg in the diet of athletes and physically active people]. Nutr Hosp. 2017;34(Suppl 4):31-5.

86. Zdrojewicz Z, Herman M, Starostecka E. Hen's egg as a source of valuable biologically active substances. Postepy Hig Med Dosw (Online). 2016;70(0):751-9.

87. de Souza RGM, Schincaglia RM, Pimentel GD, Mota JF. Nuts and Human Health Outcomes: A Systematic Review. Nutrients. 2017;9(12).

88. Blanco Mejia S, Kendall CW, Viguiliouk E, Augustin LS, Ha V, Cozma AI, et al. Effect of tree nuts on metabolic syndrome criteria: a systematic review and meta-analysis of randomised controlled trials. BMJ Open. 2014;4(7):e004660.

89. Del Gobbo LC, Falk MC, Feldman R, Lewis K, Mozaffarian D. Effects of tree nuts on blood lipids, apolipoproteins, and blood pressure: systematic review, meta-analysis, and dose-response of 61 controlled intervention trials. Am J Clin Nutr. 2015;102(6):1347-56.

90. Morgillo S, Hill AM, Coates AM. The Effects of Nut Consumption on Vascular Function. Nutrients. 2019;11(1).

91. Winther A, Dennison E, Ahmed LA, Furberg AS, Grimnes G, Jorde R, et al. The Tromsø Study: Fit Futures: a study of Norwegian adolescents' lifestyle and bone health. Arch Osteoporos. 2014;9:185.

92. Oberg J, Jorde R, Almås B, Emaus N, Grimnes G. Vitamin D deficiency and lifestyle risk factors in a Norwegian adolescent population. Scand J Public Health. 2014;42(7):593-602.

93. UNN. Laboratoriehåndbok: Universitetssykehuset Nord-Norge HF; 2018 [Available from: https://labhandbok.unn.no/.

GRADE 1

<table>
<tr><td colspan="3">Reference: Prescott SL, Pawankar R, Allen KJ, Campbell DE, Sinn J, Fiocchi A, et al. A global survey of changing patterns of food allergy burden in children. World Allergy Organ J. 2013;6(1):21.</td><td>Design: Cross-sectional study
GRADE: ⊕⊕⊕</td></tr>
<tr><th>Objective</th><th>Methods and material</th><th>Results</th><th>Discussion/comments</th></tr>
<tr><td>Collect existing data on the global patterns and prevalence of food allergy, and the quality of evidence available.

Conclusion

Food allergy is a significant paediatric health issue, where 1 in 10 children have challenge-proven IgE-mediated allergy. The prevalence is likely to increase the coming decade. The survey also reveals a scarcity of quality data in several regions.

Country

89 various countries

Year of data collection

September 2012 to March 2013</td><td>This survey was a collaborative project between the World Allergy Organization (WAO) and the Worldwide Universities Network (WUN). A web-based questionnaire was developed in February-June 2012, and disseminated September 2012 to the 93 peak national/regional member societies of WAO (data was also collected from neighbouring non-WAO member countries). Each country provided data on the overall prevalence of food allergy in their country, most common clinical presentations of food allergy, the 5 most common food allergens for different age groups, any change in food allergy the last 10 years – including the level of evidence, the source and the age group most affected. 89 countries completed the survey; 12 in Western Europe, 5 Nordic countries, 17 in Central/Eastern Europe, 18 in Asia and Oceania, 15 in the Americas, 10 in the Middle East and 12 in Africa.

The 89 countries were categorized by the best level of evidence available for each country, with the highest level where oral food challenges (OFC) and the lowest were self-reporting. In each case, there was performed a literature search by investigators to confirm the cited data source, and to look for additional evidence.</td><td>51 of 89 countries had no food allergy prevalence data of any kind. 9 of 89 countries had accurate food allergy data based on OFC. Infants and preschool children (<5 years) had a prevalence ranging from 1% in Thailand to 10% in Australia. School-aged children (> 5 years) with OFC-proven food allergy were lower in all regions, ranging from <1% in Turkey to 2,5% in the UK. However, there were very few studies using OFC in this age group. A German Study that included children 0-17 years (mean age 9,2 years) found OFC-proven food allergy prevalence to be 4,2%, with higher rates in younger children. The majority of the data collected were based on self-reporting or parent-reporting. This results in higher rates of food allergy, compared to reports based on OFC or specific IgE-confirmed food allergy. The prevalence of self-reported food allergy ranged from less than 5% to 19%. Countries that both provided OFC data and self-reported/parent-reported food allergy, show evidence that there is likely an over-estimation of food allergies.

Regions that are currently lacking data on food allergy prevalence include Central and South America, Africa, Eastern Europe and the Middle East.</td><td>This study provides a global view of the trends for food allergy currently, including the quality of evidence available and areas where research is lacking.

Most regions lack accurate or present-day prevalence, and even in high prevalence regions there is a general scarcity of quality data. The majority of prevalence estimates are based on parent- or self-reporting, increasing the risk of information bias, and few cases are objectively confirmed through the gold standard of oral food challenge (OFC). Many of the studies using OFC are prone to selection bias due to poor participation rates. However, it is noted that some studies adjust for participation bias.

Some studies are more well designed than others, and there is a big heterogeneity in study designs. It is acknowledged that prevalence based on parent- or self-reporting overestimates the true prevalence, when comparing it to prevalence based on OFC, and they therefore differ between self-reported and confirmed prevalence.

The findings in this study are consistent with other studies. However, the most acknowledged finding is that there is a general global lack of quality data on food allergy prevalence.</td></tr>
</table>

GRADE 2

<table>
<tr><td colspan="3">Reference: Maslin K, Oliver EM, Scally KS, Atkinson J, Foote K, Venter C, et al. Nutritional adequacy of a cows' milk exclusion diet in infancy. Clin Transl Allergy. 2016;6:20.</td><td>Design: Case-control study
GRADE: ⊕⊕⊕</td></tr>
<tr><th>Objective</th><th>Methods and material</th><th>Results</th><th>Discussion/comments</th></tr>
<tr><td>To compare the nutritional intake of a group of infants consuming a cows' milk free diet to a matched control group of infants consuming an unrestricted diet over a period of 6 months.

Conclusion

This study suggests that although infants consuming a milk-free diet have a nutritional intake that is significantly different to matched controls who are eating an unrestricted diet, this difference is not constant and it is not seen for all nutrients.

Country

England

Year of data collection

2006 to 2008</td><td>Participants in this study are a subgroup of the Prevalence of Infant Food Allergy study, a prospective food allergy birth cohort study from the South of England. The diets of 39 infants (13 milk-free and 26 controls) were assessed, through the parents keeping a prospective food diary, reporting every 4 weeks until the age of one. A specialist allergy dietitian advised the parents of infants with suspected milk allergy, to strictly and completely avoid cow's milk, and other mammalian milk products. These infants were not excluding any other foods from their diet (e.g. soya). Each infant following a milk exclusion diet who had returned at least 3 weeks of quantitative diet data covering a period of 12 weeks had their dietary intake data analysed. Each milk-free infant was matched to two control infants, according to age, number of food diaries available and breastfeeding status, thus forming a nested matched case–control study. Dietary analysis was performed with the dietary analysis package 'CompEatPro' (Nutrition Systems, 2008). Mean daily values for nutrient intake were calculated by the dietary analysis package, imported into Statistical Package for the Social Sciences version 18 (SPSS Inc) and compared to UK Recommended Nutrient Intakes (RNI)</td><td>13 of in total 74 infants on a milk free diet met the inclusion criteria. Each milk-free infant was matched to 2 control infants, resulting in dietary analysis of 13 milk-free and 26 control infants.

All infants had mean intakes in excess of the requirements for energy and the recommended intakes for protein, calcium, iron, selenium, zinc, vitamins A, C, D and E. The mean daily intake differed significantly between the groups across the whole time period for selenium ($p = 0.003$) and vitamin C ($p = 0.01$), and selenium were at all time-points higher in the infants following a milk free diet. Observed vitamin C intake was at all-time points higher for infants following an unrestricted diet ($p=0.001$). Differences were also found between the two study groups at differing time periods for protein, calcium, iron and vitamin E (all $p<0.05$)</td><td>This case-control study suggests that infants consuming a milk-free diet have a significantly different nutritional intake compared to the control group. However, the difference is not constant over the 6 months the study was conducted, and is not seen for all nutrients.

It is highlighted that there exists a selection bias, as the majority of infants in this study were born to well-educated mothers, who may be more likely to follow recommended feeding advice than less well-educated mothers. The parents also received guidance by a specialist allergy dietitian, and previous research suggests that that infants consuming exclusion diets who had not received nutritional advice were likely to have diets deficient in vitamin D and calcium compared to those who had received nutritional advice. The authors therefore emphasize that these findings cannot be extrapolated to infants not receiving individualized diet advice.

The authors also identify several cofounders, such as the milk reactive infants eating soya products as dairy alternative, that are high in selenium and low in vitamin C.</td></tr>
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GRADE 3

Reference: Almon R, Sjöström M, Nilsson TK. Lactase non-persistence as a determinant of milk avoidance and calcium intake in children and adolescents. J Nutr Sci. 2013;2:e26.	**Design:** Case-control study **GRADE:** ⊕⊕⊕

Objective	Methods and material	Results	Discussion/comments
To observe whether there is a difference in regards to milk avoidance, calcium intake, vitamin D intake and anthropometric features related to obesity in lactase non-persistent (LNP) children and adolescents compared to lactase persistent (LP) subjects. **Conclusion** Milk avoidance was significantly more common in LNP children and adolescents. Additionally, energy intake from milk and calcium intake was lower in LNP. LNP status did not affect vitamin D intake or anthropometric features. **Country** Sweden **Year of data collection** 1998-1999	Swedish children (n=298, mean age: 9.6) and adolescents (n=386, mean age: 15.6) that were part of the European Youth Heart Study, who had been randomly sampled through a multiphase sampling procedure (overall participation rate in Sweden: 50%). The consumption of milk was evaluated by an interviewer-mediated 24h recall, and a food record collected the day before served as checklist for this data, where portion sizes was estimated by using a food atlas. Dietary data were processed by StorMats (version 4.02; Rudans Lättdata) and analysed using the Swedish National Food database (version 99.1). The genetic analysis was performed by isolating genomic DNA from the EDTA whole blood samples with the QIAamp DNA Blood Mini Kit spin procedure. The DNA fragment spanning the -13910-C/T polymorphic site was genotyped by pyrosequencing, using a PSQ96 SNP reagent Kit and a PSQ 96MA system (Pyrosequencing AB) PSQ96MA 2.0.1 software.	The genotype LCT-13910 CC (associated with LNP), was found in 94 subjects. 39 subjects in total reported milk avoidance, adolescents reporting it more commonly than children (n=34): the OR for subjects with LNP compared with LP subjects was 3.2 (95% CI 1.5, 7.3, P = 0.003), with sex and LCT-13910 C > T genotype as covariates in the model. Summarized, the main findings was that LNP subjects had a lower milk consumption, a lower daily energy intake based on these products, and a lower calcium intake. There were no difference in total energy intake, vitamin D intake or anthropometric features. It is however noted that vitamin D intake were below the recommendations issued by the Swedish National Food Agency in both LP and LNP subjects.	This study compares how LNP and LP status affects milk consumption, calcium intake, vitamin D intake, energy intake and anthropometric features. Results suggest LNP subjects had a lower milk consumption, a lower daily energy intake based on these products, and a lower calcium intake. The main limitations are the sample size and the age of onset of LP that can vary regionally and with ethnicity, this reduces the precision of the results. Additionally, almost all children and a majority of the adolescents consumed milk to some degree, despite having LNP, and some LP subjects avoided milk completely due to other health reasons or preferences. The authors does not note that participation rate in all Sweden for the European Youth Heart Study as a whole, was 50%, increasing the risk of selection bias.

GRADE 4

Reference: Hallert C, Grant C, Grehn S, Grännö C, Hultén S, Midhagen G, et al. Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. Aliment Pharmacol Ther. 2002;16(7):1333-9.	**Design:** Case-control study **GRADE:** ⊕⊕

Objective	Methods and material	Results	Discussion/comments
To assess the vitamin nutrition status of a series of coeliac patients living on a gluten-free diet for 10 years. **Conclusion** The findings suggest that subjects on a gluten-free diet for several years have a higher risk of developing a poor vitamin status. **Country** Sweden **Year of data collection** 1984-1996	Patients from a coeliac cohort (n=47) diagnosed at 6 gastroenterology units in Sweden (between 1884-88) were invited to this study if they were characterized with a proven healed intestinal mucosa 8-12 years after diagnosis and start of gluten-free diet. This resulted in a sample size of 30 subjects. Patients unwilling to participate or who used folate supplementation were excluded. Blood samples such as folate, vitamin B_{12}, pyridoxal 5′-phosphate and homocysteine were drawn at routine laboratory investigations. Normative total plasma homocysteine values, as determined by high-performance liquid chromatography, were obtained from a general Nordic population sample aged 43–64 years (n=592). Compliance with a gluten-free diet was evaluated by histological evidence of remission. 25 of the 30 subjects showed normal histology, and 5 showed borderline histology. The patients also submitted a 4-day food record from a holiday using household measures, which were used as dietary history. The patents were also measured in height and weight, and filled out a form covering current medications, physical activity and smoking habits. This was used to calculate basal metabolic rate, and to register energy intake.	30 subjects (18 females, 12 males) aged between 45-64 years, were included in this study. The control group consisted of a general Nordic population sample aged 43–64 years (n=504, 50% females). Male coeliac patients had a mean homocysteine concentration of 13.6 µmol/L compared to 11.2 µmol/L in the male control group, and female coeliac patients had a mean value of 10.8 µmol/L compared to 9.9 µmol/L in female controls. Low pyridoxal 5′-phosphate levels were seen in 11 subjects (37%; 95% CI, 20–54), low plasma folate in 6 subjects (20%; 95% CI, 6–34) and low plasma vitamin B-12 in none of the subjects. The total plasma homocysteine level had a negative correlation with pyridoxal 5′-phosphate ($r=-0.50$) ($P<0.01$), folate ($r=-0.46$) ($P<0.01$) and vitamin B-12 ($r=-0.01$). Multivariate analysis showed that 33% of the variation in the total plasma homocysteine concentration could be explained by the plasma pyridoxal 5′-phosphate and folate levels (F ratio=5.87) ($P<0.008$).In a total of 14 subjects (47%; 95% CI, 29–65) there were observed a low pyridoxal 5′-phosphate level (n=8), a low folate level (n=3) or both (n=3). The mean intake of vitamin B_{12} were lower in coeliac patients compared to the control group ($P<0.05$). There were poor correlations between vitamin intakes and plasma levels ($r<0.18$). Summarized, there were sign of poor vitamin status in 56% of the coeliac patients.	This study suggests that subjects on a gluten-free diet for several years have a higher risk of developing a vitamin deficiencies, notably folate deficiency. A new finding in this study is a raised plasma homocysteine level in coeliac patients, which is linked to increased risk of cardiovascular disease, as well as being an indication of poor vitamin status. Further research on this is needed. The authors note that the 4-day food records may be subject to bias, such as the subjects changing dietary habits or underreporting. Furthermore, it is noted that vitamin losses through industrial and household food processing are currently unknown, as is the vitamin bioavailability in humans. Strengths of the study include the 10-year follow-up, and the use of intestinal biopsy to assess adherence to the gluten-free diet. The coeliac cohort had an unremarkable number of concomitant diseases similar to others, and should not have affected the total homocysteine levels. Limitations that the authors note are possible dietary changes during the years, and that the findings are limited to a Nordic diet, as this was the diet consumed by the subjects. Further research is needed to conclude with the full effect of a strict gluten-free diet on the vitamin status.

GRADE 5

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<tr><td colspan="3">Reference: Wild D, Robins GG, Burley VJ, Howdle PD. Evidence of high sugar intake, and low fibre and mineral intake, in the gluten-free diet. Alimentary Pharmacology & Therapeutics. 2010;32(4):573-81.</td><td>Design: Case-control study
GRADE: ⊕⊕</td></tr>
<tr><th>Objective</th><th>Methods and material</th><th>Results</th><th>Discussion/comments</th></tr>
<tr><td>To assess the nutritional composition of a gluten-free diet (GFD), compared to a non-GFD in a representative non-celiac population.

Conclusion
This study suggests an adequate energy intake in subjects on GFD, with a higher proportion of energy coming from carbohydrates. Females on a GFD had a lower intake of magnesium, iron, zinc, manganese, selenium and folate compared to a local control population. A higher percentage of males on a GFD fail to rech recommended intakes of magnesium and selenium.

Country
The UK
Year of data collection
January 2007 – May 2008</td><td>Participants were patients with diagnosed celiac disease, that had been on a GFD for 6 months or more prior to the project. Dietary compliance was evaluated through a dietitian review and self-reporting. The subjects completed a 5-day food diary (including 3 weekdays and 2 days at the weekend), where the EPIC validated food diary was used. There were given instructions at the time of recruitment and a follow-up phone call by the dietitian. Participants' heights and weights were measured, and used to calculate BMI and end energy requirements. Routine blood samples for micronutrient levels were drawn. A basal metabolic rate was calculated, but since the participants did not report their physical activity level, it was only assumed that all subjects were sedentary. There was no direct significance on the interpretation of the data contained within the food diaries, due to the missing data regarding physical activity levels – this was only used for and affected the assessment of under- and over-reporting in the food diaries.

Nutrient data from 256 females and 195 males from the National Diet and Nutrition Survey (NDNS) selected from Northern region (2000-2001, age ranging between 19 to 64) was utilized as the control population. Additionally, the UK Women's Cohort Study (UKWCS) were used as a control group in some cases.</td><td>100 of the 139 recruited returned a food diary, 7 of these were excluded as they did either contained significant amounts of gluten (1 male, 2 females) or were not detailed enough and therefore could not be analysed (3 males, 1 female). The 93 diaries (62 females, 31 males) that met the inclusion criteria had a mean age of 53 (21-79) for females and 56 (18-74) for males.

Females on a GFD consumed a significantly ($P<0.05$) higher amounts of macronutrients compared to an age and gender specific local population (carbohydrates covering a higher proportion of the energy intake). Compared to the UKWCS women on a GFD had significantly ($P<0.05$) lower fibre intake, but no significant difference in macronutrients intake. Males on GFD consumed higher amounts of energy and a lower amount of fibre compared to the NDNS control group.

Females on GFD consumed higher amount of calcium and magnesium compared to the UKWCS subjects, but a lower intake of magnesium, iron, zinc, manganese, selenium and folate ($P<0.05$) compared to the NDNS controls. No overall difference was seen between the female groups in regards of meeting dietary reference levels for nutrient intake, except for a lower percentage of females on GFD meeting the reference level for selenium (11% vs 35%) and magnesium (31% vs 71%) compared to the control group. There were no comparable group for the males in regards of micronutrient intake. It is however noted that a certain percentage of males on GFD do not meet the recommended intake for magnesium (23%) and selenium (6%).</td><td>This study suggests that females on a GFD have a higher intake of energy (which is also seen in the male population on GFD), calcium and magnesium ($P<0.05$) compared to a local population, but a lower intake of fibre, magnesium, iron, zinc, manganese, selenium and folate ($P<0.05$) with no significant difference in energy intake, compared to the UKWCS controls. This suggests that females on GFD make less nutrient dense energy food choices in their diet.

The strengths highlighted by the authors is that all subjects had been on a GFD for 6 months or more (median time was around 7 years for males, 9.5 years for females), that the nutritional composition of gluten-free foods were verified by manufacturers, and the food intake were compared with comparable populations.

There are several limitations that the authors underline. Including that it is a relatively small sample size, that one of the control groups (the UKWCS) is a health-conscious cohort of middle aged women, and that both control groups had an underrepresentation of younger subjects. It is also noted that the use of self-reported 5-day food diary contain several limitations. Self-reporting generally is prone to information bias, and may cause over- and underreporting. It is also suggested that a food diary less than a week is too short to be able to evaluate intakes of vitamin, minerals, and trace elements.</td></tr>
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