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Dietary intake and blood lipid profile in six-year-old Icelandic children 2001-2002 and 2011-2012

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HÁSKÓLI ÍSLANDS

Mataræði og blóðfitur sex ára barna á Íslandi 2001-2002 og 2011-2012

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Bakgrunnur og markmið: Næringarástand og mataræði barna er talið geta haft langtímaáhrif á vöxt, þroska og heilsu síðar á lífsleiðinni. Þekking á fæðuvali og næringargildi fæðu mismunandi hópa nýtist meðal annars til að móta stefnu stjórnvalda í manneldismálum og til að móta áherslur aðgerða sem miða að því að bæta fæðuval og hafa þannig áhrif á almannaheilsu. Markmið þessa verkefnis var að bera saman fæðuval, næringargildi og blóðfitur sex ára íslenskra barna sem rannsökuð voru með tíu ára millibili, 2001-2002 og 2011-2012. Markmiðið var einnig að kanna tengsl milli neyslu á orkugefandi næringarefnum og blóðfita meðal sex ára barna.

Aðferðir: Tvær landskannanir á mataræði sex ára barna voru framkvæmdar á árunum 2001-2002 og 2011-2012. Fullnægjandi þriggja daga fæðuskráning fékkst frá 131 einstaklingi (72%) 2001-2002 og 162 einstaklingum (83%) 2011-2012. Samanburður var gerður á neyslu matvæla úr völdum fæðuflokkum, hlutfallslegri skiptingu orkuefnanna auk neyslu vítamína og steinefna var milli kannanna. Gæði heildar mataræðis var metið út frá því hversu vel fæðuvalið samræmdist opinberum ráðleggingum um fæðuval (healthy eating index, HEI; stig gefin frá 0-8). Blóðsýni voru tekin úr 137 (76%) og 145 einstaklingum (74%), hver um sig í áðurnefndri röð. Heildar kólesteról, HDL og þríglýseríð voru mæld í blóði þátttakenda og LDL reiknað.

Niðurstöður: Ef borin er saman neysla sex ára barna 2001-2002 og 2011-2012 sást aukning í heildarneyslu grænmetis og ávaxta (63%), kjöts og kjötvara (26%), osta (41%), morgunkorns (61%) og svaladrykkja (12%). Neysla á mjólk og mjólkurvörum dróst saman um 19% og neysla á kökum, kartöflum og gosi var marktækt minni 2011-2012 heldur en 2001-2002. Miðgildi HEI stiga (mælikvarði á gæði heildarmataræðis) var marktækt hærra 2011-2012 í samanburði við 2001-2002 (2 vs. 3 $p<0,0001$). Enginn munur sást á heildar orkuinntöku eða framlagi heildar fitu, kolvetna og próteina til heildar orku inntöku (E%) milli rannsókna. Hinsvegar vegar urðu breytingar í fitugæðum þar sem neysla á mettuðum fitusýrum og trans fitusýrum reyndist lægri á árunum 2011-2012 (13,3 E% og 0,8 E%) en 2001-2002 (miðað við (14,7 E% og 1,4 E%), $p<0,0001$). Neysla á ómettuðum fitusýrum jókst að sama skapi. Neysla á viðbættum sykri lækkaði um 1,6 E% ($p=0,0003$) á milli kannana og á sama tíma jókst neysla á fæðutrefjum (13 g/dag 2011-2012 miðað við 11 g/dag 2001-2002, p=<0,0001).

Heildar styrkur kólesteróls í blóði og styrkur LDL voru marktækt lægri árin 2011-2012 samanborið við 2001-2002 (4,6 mmól / L miðað við 4,4 mmól / L, $p = 0,003$ og 2,8 mmól / L á móti 2,5 mmól / L, $p = <0, 0001$, hver um sig í áðurnefndri röð). Línuleg aðfallsgreining gaf til kynna að með hverri E% aukningu á neyslu mettaðrar fitu hækkaði LDL kólesteról um 0,03 mmól/L (p=0,04).

Ályktanir: Niðurstöður sýna að ýmsar jákvæðar breytingar hafa orðið á mataræði sex ára barna á Íslandi á frá árunum 2001-2002 til 2011-2012. Þó er enn langt í land með að mataræði barna sé í samræmi við ráðleggingar. Sú breyting sem varð á fituneyslu barnanna á tímabilinu (meiri neysla ómettaðra fitusýra á kostnað mettaðra fitusýra og trans fitusýra) gæti átt þátt í lækkun LDL kólesteróls í blóði sex ára barna.

ABSTRACT

Background and objectives: It has been suggested that nutrition and diet in childhood has long-term effects on growth, development and health later in life. Knowledge of dietary intake of different groups within a population is important for public health, as it can be used for policy making and as a foundation for strategies aimed at improving dietary intake and thus influencing public health. The aims of the thesis were to compare dietary intake, nutrition and blood lipids in six-year-old Icelandic children from two national dietary surveys conducted 10 years apart, in 2001-2002 and 2011-2012. The secondary aim was to assess the association between the contribution of energygiving nutrients and blood lipids among six-year-old children.

Methods: Two national cohort studies were conducted on six-year-olds in 2001-2002 and 2011-2012. Complete three-day food records were returned by 131 subjects (72%) in the 2001-2002 study and 162 subjects (83%) in the 2011-2012 study. The two studies were compared with regards to consumption of specific food groups, contribution of energy providing nutrients and intake of vitamins and minerals. Total diet quality was assessed by adherence to public food-based dietary guidelines (healthy eating index, HEI; points from 0-8). Blood samples were collected from 137 (76%) and 145 subjects (74%), respectively. Serum total cholesterol, HDL and triglycerides were measured and LDL calculated.

Results: When comparing dietary intake in six-year-old children in 2001-2002 and 2011-2012 an increase in total consumption of fruits and vegetables (63%), meat and meat products (26%), cheeses (41%), breakfast cereals (61%) and cool drinks (12%) was observed. Consumption of milk and milk products decreased by 19% and consumption of cakes, potatoes and soda was significantly lower in 2011-2012 compared to 2001-2002. The median HEI score (an indicator of total diet quality) was significantly higher in 2011-2012 compared to 2001-2002 (2 vs. 3 $p<0.0001$). There was no difference in total energy intake or contribution of total fat, carbohydrates and proteins to total energy intake (E%), between the two studies.

However, changes in fat quality were observed where intake of saturated fatty acids and trans fatty acids were lower in 2011-2012 (13.3 E% and 0.8 E%) compared to 2001-2002 (14.7 E% and 1.4 E%, $p= <0.0001$). At the same time, intake of unsaturated fatty acids increased. Intake of added sugar decreased by 1.6 E% (p=0.0003) between the two studies and higher intake of dietary fibre was observed 2011-2012 compared with 2001-2002 (13.2 g/day compared to 11.1 g/day, p=<0.0001). Total cholesterol and LDL concentrations in blood serum were significantly lower in 2011-2012 compared with 2001-2002 (4.6 mmol/L vs. 4.4 mmol/L, p=0.003 and 2.8 mmol/L vs. 2.5 mmol/L, p=<0.0001, respectively). In a multiple linear regression model, one E% increase in SFA intake was related to 0.03 mmol/L increase in LDL cholesterol (p=0.04).

Conclusion: The results show several improvements in dietary habits of six-year-old children in Iceland from 2001-2002 to 2011-2012, while it is still a long way to go for the diet among children to be in adherence to food-based dietary guidelines. The change in intake of dietary fat during the period (increased intake of unsaturated fatty acids at the expense of saturated fatty acids and trans fatty acids) might have contributed to improved blood lipid profile among six-year-old children.

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ABBREVIATIONS

AR	average requirement
E%	percent of total energy
CHD	coronary heart disease
CVD	cardiovascular diseases
FBDG	food-based dietary guidelines
HDL	high-density lipoprotein
HEI	healthy eating index
ICEFOOD	an Icelandic nutrient calculation program
ISGEM	the Icelandic National Food Composition Database
LDL	low-density lipoprotein
LI	lower level of intake
MUFA	monounsaturated fatty acid
N-3 FA	omega-3 fatty acids
NNR	nordic nutrition recommendations
PUFA	polyunsaturated fatty acid
RDI	recommended daily intake
SAS	an integrated system of software products
SFA	saturated fatty acids
SD	standard deviation
TAG	triacylglyceride
TC	total cholesterol
VLDL	very-low-density lipoprotein

1. INTRODUCTION

Diet is one of the major determinants of health and well-being [1-6]. As dietary habits tend to track from childhood to adulthood actions aiming at improving dietary intake of children are of great importance.

Knowledge about current dietary intake of a population, or a group within a population, is important in order to be able to develop actions and strategies aimed at improving dietary intake. Furthermore, monitoring trends in children's dietary intake is of value when it comes to evaluating the effects of public strategies and actions. The Unit for Nutrition Research has for the last two decades, assessed dietary intake of children of different ages. Results from these studies have been used to develop and revise public recommendations [7-12]. However, trends in dietary intake of children using repeated measurements in the same age group had not been conduced, in a similar way, as for the adult population.

National dietary surveys have been conducted in Iceland since 1939. The most recent national dietary survey from 2010-2011 including adults (18-80 years old) showed that changes towards healthier dietary habits have occurred in the past 10 years in Iceland. These changes include 43% decrease in the intake of trans fatty acids, from 1.4 E% to 0.8 E% ($p= <0.001$), and a significant drop in the intake of saturated fatty acids [13]. It is predicted that similar changes in dietary intake might have occurred among children as for the adult population.

The relationship between energy-contributing nutrients and blood lipid profile is rather well established in children [14-16]. However, national dietary studies in children are rarely accompanied with lipid profile, or other clinical assessment, and have therefore limited ability to estimate if changes in dietary habits resulted in health effects on the population level.

The aims of the thesis were to investigate the trends in dietary intake of six-year-old children from two national dietary surveys conducted 10 years apart, in 2001-2002 and 2011-2012 and to compare the children's nutrient intake and blood lipid profile. The secondary aim was to assess the association between the contribution of energy-giving nutrients and lipid profile in the former and the latter study.

This thesis is part of a larger study and based on two longitudinal studies on infant and child nutrition [9-10, 17-19]. The focus of this thesis is on cardiovascular risk factors, specifically blood lipid profile in relation to diet and nutrient intake. The thesis is based on a review of the literature and the manuscript „Lower intake of saturated and trans fatty acids is associated with improved lipid profile in a six-year-old population: Two national cohort studies conducted 10 years apart" by Hafdis Helgadottir, Ingibjörg Gunnarsdottir, Thorhallur I. Halldorsson, Gestur I Palsson and Inga Thorsdottir. Results related to the aims of the thesis that could not be fitted into the above-mentioned manuscript are presented in the results section of this thesis.

2. REVIEW OF LITERATURE

Knowledge from different sources is needed to set dietary recommendations and to monitor in what direction the diet of a population, or group within a population, is moving (Figure 1).

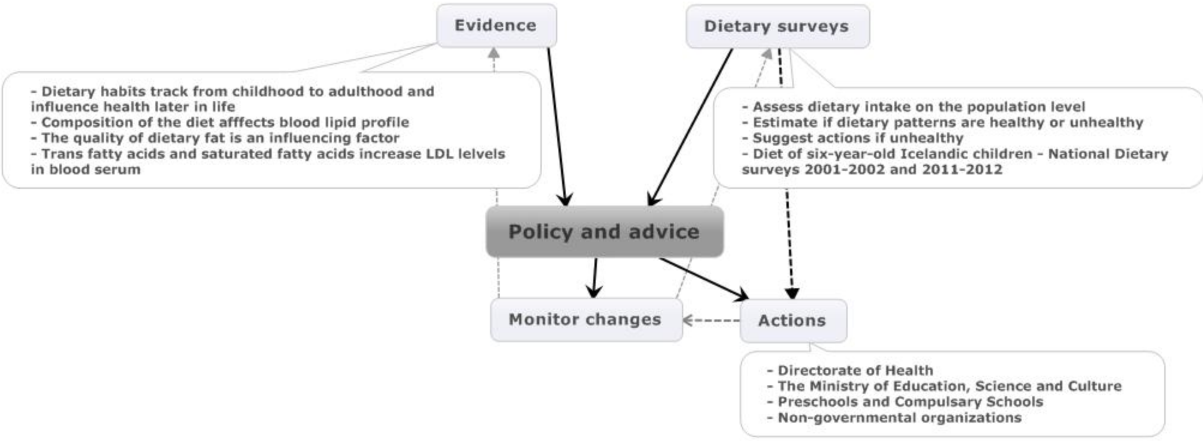

Figure 1 Interactions between the evidence, dietary surveys, policy and advice making and monitoring diet on a population level.

2.1 Cardiovascular health and nutrition

2.1.1 Cholesterol and blood lipids

Dietary fat provides the human body with energy in a packed form and provides essential fatty acids and fat-soluble vitamins. Cholesterol is a lipid and is an important structural component of the cell membrane and plays a role as a precursor for the synthesis of steroids, e.g. steroid hormones, bile salts and vitamin D [20]. Triglycerides, cholesterol, fat-soluble vitamins and phospholipids, from dietary fat, travel from the digestive system to the lymphatic system surrounded by a lipoprotein coat, forming a chylomicron. The chylomicrons travel the lymphatic system to the blood stream. From there they are carried to several tissues, including the liver. In the liver the triglycerides, phospholipids and cholesterol are repacked into lipoproteins, with amphiphilic proteins and lipids on the surface, and circulate the body through the bloodstream. There are different types of lipoproteins which contain apolipoproteins that have specific receptors on the cell membrane which indicates their destination. Lipoproteins are classified by density; very-low-density lipoproteins (VLDL) are the smallest and carry triglycerides from the liver to adipose tissue. Low-density lipoproteins (LDL) carry cholesterol from the liver to cells of the body. When cholesterol is abundant, LDL receptor synthesis is blocked, making celluptake of cholesterol from LDL impossible. In return LDL molecules are oxidized and taken up by macrophages. LDL ingestion stimulates secretion of mediators that trigger multiple inflammatory and proliferative cascades, some of which can lead to atherosclerosis. LDL is often referred to as the „bad cholesterol" lipoprotein. High-density lipoproteins (HDL) collect free cholesterol from the body's tissues, and bring it back to the liver, where the cholesterol is recycled. HDL is often referred to as the „good cholesterol" lipoprotein. High concentrations of LDL and low concentrations of HDL are strongly associated with coronary heart disease (CHD) [21].

Optimal concentration of LDL in blood serum in adults is <2.6 mmol/L [21]. High levels of LDL are 4.1-4.9 mmol/L and very high are ≥4.9 mmol/L. In children and adolescents the optimal level of LDL is <2.9 mmol/L, borderline high 2.9-3.3 mmol/L and high levels are above 3.4 mmol/L [22]. LDL is calculated from serum total cholesterol, triglycerides and HDL concentrations expressed in mmol/L with the Friedwald formula [23]: $C_{LDL} = C_{TC} - C_{HDL} - (TAG/5)$ which is valid if TAG concentrations do not exceed 4.52 mmol/L [24].

2.1.2. The evidence

A systematic review assessed the effect of reduction or modification of dietary fat for preventing cardiovascular diseases (CVD) [25]. Reviewed studies suggested a small but potentially important reduction in CVD on modification of dietary fat, but not reduction in total fat alone. The protective effects were higher if the duration of the modification lasted for at least two years. In the NNR systematic review by Schwab et al. (2013) the evidence of total cholesterol and LDL levels being reduced when saturated fatty acids (SFA) are substituted with monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) were *convincing* [26]. On the other hand the evidence of HDL levels being increased when SFA are substituted with MUFA or PUFA were *limited or no conclusion*. Evidence suggests that there are no clear health benefits of replacing SFA with carbohydrates, despite small improvements in weight and body mass index [25], most likely depending on the quality of the carbohydrates the SFA are substituted for [27]. Some studies suggest that increased fibre intake, at the expense of SFA, decreases serum cholesterol levels in adults and children [28-31]. Epidemiological studies and randomized control intervention found a relationship between intake of long chain n-3 PUFA's, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and reduced risk of cardiovascular diseases and reduces the risk of coronary death [32].

In the past years, studies have focused to a greater extent on the whole diet, showing that a diet composed of unsaturated fats, whole grains as the main carbohydrate type, fruits and vegetables and omega-3 fatty acids, can contribute to maintaining a optimal blood lipid profile and thus a protection against coronary heart disease [28, 33]. These foods are the major components of the Mediterranean diet, which is the most known and well-studied dietary pattern [34]. Some evidence suggest that the Mediterranean diet has beneficial effect on waist circumference, HDL cholesterol levels, triglyceride levels, blood pressure and glucose metabolism in a general population, compared to other diets [34], and has been suggested to have more favourable improvements in CVD risk factors and vascular inflammatory factors among overweight/obese individuals, compared to low fat diets [35].

2.1.3 Proposed mechanism

It has been suggested that consumption of processed trans fatty acids increases LDL and lowers HDL [28]. Trans fatty acid intake increases plasma activity of cholesteryl ester transfer protein, which is the main enzyme for the transfer of cholesterol esters from HDL to LDL and there for might be the reason for a decrease in HDL levels and increase in LDL levels [36]. When compared to consumption of SFA or unsaturated fatty acids, trans fatty acids seem to increase levels of triglycerides and reduce the particle size of LDL cholesterol, which may both increase the risk of developing CHD. Intake of trans fatty acids has also been associated with increased production of inflammatory factors. All of these factors contribute to higher risk of cardiovascular disease (CVD).

Studies indicate that decreasing the proportion of SFA in the diet depends on what the SFA are substituted with [37]. By replacing 5% of total energy intake of SFA with PUFA, LDL levels decreased by 0.29 mmol/L. By replacing with fibres, the concentration decreased by 0.18 mmol/L and 0.22 mmol/L, by replacing with MUFAs. The biological mechanisms causing an increase in LDL levels, as result of SFA intake, are suggested to be a decrease in hepatic LDL receptor activity and protein and mRNA abundance which affects the removal of lipoproteins in the plasma [38]. That leads to an increase in LDL cholesterol production rate. In contrast, intake of PUFAs seems to increase LDL receptor levels and thus a decrease in LDL cholesterol production rate.

Studies indicate that the biological effects of n-3 fatty acids related to CVD risks include; preventing arrhythmias, decreasing platelet aggregation, lowering plasma triglycerides, increasing HDL cholesterol and LDL particle size, decreasing blood pressure, reversing cholesterol accumulation from atheromatous plaques and decreasing inflammation [32].

The effects of substituting carbohydrates with SFA seem to depend on the dietary fibre content and glycemic index of the carbohydrates consumed. It has been suggested that fibre intake leads to a reduced glycemic response and therefore decreased insulin stimulation of cholesterol synthesis in the liver [39].

2.1.4 Childhood nutrition and risk factors for CVD

The five largest studies on nutrition and cardiovascular (CVD) risk factors in children and young adults include the Bogalusa Heart Study, the Cardiovascular Risk in Young Finns Study, Special Turku Coronary Risk Factor Intervention Project for babies (STRIP), Muscatine study and Dietary Intervention Study in Children (DISC) [40].

The Muscatine Study, a longitudinal study from 1970, indicated that elevated levels of cholesterol in childhood tracked through adulthood [41]. Correlation coefficient of LDL levels in childhood (7-8 years of age) with adult LDL levels (20-25 years of age) was 0.65 for girls and 0.56 for boys. The risk for adult levels higher than 90th percentile was higher when childhood levels were greater than 50th percentile of total cholesterol. The risk of children having elevated levels in adulthood, who had cholesterol levels at the 90th percentile, was 24-32%.

The Bogalusa Heart Study is a longitudinal study and the longest and detailed study of children in the world, focusing on the nature of CVD from 1972. Results from the Bogalusa Heart Study indicated that elevated levels of LDL, above 75th percentile remained elevated 12 years later and was the best predictor of follow up lipid levels [42]. It also indicated that subjects in the Bogalusa Heart Study with LDL cholesterol concentrations above 3.35 mmol/L in childhood had significantly higher prevalence of adult dyslipidemia, compared with subjects with LDL cholesterol concentration below 2.84 mmol/L in childhood [43].

In the Cardiovascular Risk in Young Finns Study, an observational study that was started in 1978, participants (3-18 years of age), were monitored for more than 20 years. It showed that childhood diet was a significant determinant of preferences 21 years later and there were associations between fat intake in childhood and quality of diet in adulthood, with regards to cardiovascular health of the diet [3].

The DISC study, a prospective randomized intervention study among children aged 8-10 years with hypercholesterolemia, was started in 1987 with a total follow up time of seven years [44]. Results indicated that the intervention group, which were promoted to follow a diet where total fat was 28% of total energy intake and SFA less than 8%, had greater reduction in total fat (4.9 E% vs. 3.4 E%) and SFA intake (2.3 E% vs. 1.4 E%) than the control group seven years after the intervention and significantly lower LDL levels (0.09 mmol/L) three years after the intervention. The levels were also lower at the last follow up, but not significantly.

The STRIP study, an intervention study that was started in 1990, included a repeated dietary counselling (at least annually) from infancy to 19 years of age based on the Nordic Nutrition Recommendations [2]. The children in the intervention group had lower SFA intake than the control group (-2.1 E% in boys and -1.9 E% in girls) and decreased LDL concentration compared to the control group (-0.18 mmol/L in boys and -0.10 mmol/L in girls). The effect of the intervention on other blood lipids were only significant in boys and were more favourable compared to the control group. Other studies have found similar trends, that intake of SFA is associated with cholesterol levels in children [14, 45].

The relationship between energy-contributing nutrients and blood lipid profile is rather well studied in children [14-16, 46-49]. High intake of SFA has been correlated with high total cholesterol and LDL levels [14, 16, 46]. Some studies have been focusing on the relationship between total diet quality and metabolic disease risk in children and adolescents, such as obesity, blood lipids, hypertension, insulin levels and waist circumference [1, 50-55]. Nearly all of the studies found a positive association between unhealthy dietary patterns, or western dietary patterns (patterns retrieved from cluster analysis), and metabolic risk factors among children and adolescents [1, 51-55]. The unhealthy dietary pattern was characterized by food high in fat, especially SFA, sweetened food products and low in grains, fish, fruits and vegetables. According to the Cardiovascular Risk in Young Finns study there were some indications, from a longitudinal analysis with repeated measurements, that a health conscious eating pattern was associated with a better lipid profile, compared to a traditional eating pattern from childhood to adult [1].

2.2 Assessing dietary intake on the population level

Dietary intake is a major risk factor for chronic diseases and an increasing challenge for public health [56]. Diet and exercise throughout life, at population level, can reduce the risk of a worldwide epidemic of chronic disease [57].

2.2.1 National dietary surveys

A National dietary survey is a good tool for observing dietary habits and assessing nutritional status of a population [58-59]. National nutritional surveys produce valuable information on the degree of existing nutritional problems, which can be used to distribute resources to those groups in need and to formulate policies to improve the overall nutrition in a population. The purpose of a national dietary survey is to assess the diet of a population. It involves assessing the intake of food and nutrients in a representative sample of a population. If the diet is characterised by an unhealthy pattern it is possible to act on it before it becomes a major problem. To know if the following actions are successful, the assessment of the diet needs to recur and dietary trends be monitored. The knowledge can form a basis for actions to promote healthy eating habits and thus the prevention of diet-related diseases. The dietary survey also creates a professional foundation to evaluate the changes of composition of nutrients in foods, e.g. vitamin A and D in Icelandic cod liver oil and iodized salt in some countries.

2.2.2 National dietary surveys in Iceland - adults

National dietary surveys of The Icelandic Nutrition Council have been conducted since 1939 where diet of adults was assessed. In 1990 the survey showed some positive and negative results. Consumption of fruits and vegetables was too low, compared to dietary recommendations and the diet of Icelanders was characterised by high proportion of dietary fat, 41 E%. Fish consumption and milk and milk products consumption was high. Added sugar contributed appropriately to total energy intake, 8.4% on average [60]. In 2002, another dietary survey was conducted [12]. There had been large changes in the diet of the Icelandic population. The main findings were a great reduction in total fat intake, from 41 E% to 35 E%, and a reduction in saturated fatty acids (SFA) and trans fatty acids from 19 E% to 16 E%. However, intake of added sugar intake had increased, especially among young people, 15-19 years of age, from 14% of total energy intake to 21%. There were also changes in the consumption of food from specific food groups, e.g. a decrease in consumption of milk and fish and an increase in consumption of e.g. sugared sweetened beverages, water, fruits and vegetables and bread.

According to the most recent National dietary survey in Iceland conducted in 2010-2011, dietary habits of adults in Iceland had changed since 2002 [13]. The trend was towards healthier dietary habits. The main results, compared to a former dietary survey in 2002 [12], were decreased intake of SFA, from 16 E% to 15 E% and added sugar intake, from 10 E% to 9 E%, while intake of fruits and vegetables and whole grain bread and cod liver oil had increased.

2.2.3. Dietary surveys in Iceland - children

Different surveys in children have been conducted in Iceland since 1992-1993 [61] and different age groups have been targeted through the years. The most recent national dietary survey on children's diet was conducted in 2011-2012 and six-year-old children were the target group studied. Results from the study are used in the present thesis. Two longitudinal studies on infant's nutrition [17, 19, 62], with follow up studies at the age of six years [11, 63-65] have been conducted and cross-sectional studies on dietary intake in 10-15 year olds [61], two-year-olds [10], 9 and 15 year olds [66], seven-year-olds [67-68] and 3 and 5 year olds [69].

According to aforementioned studies, the diet of Icelandic children is generally characterized by sufficient intake of vitamins and minerals compared with recommended daily intake, except for vitamin D. Consumption of milk and milk products seems to be relatively high and intake of added sugar and SFA is too high in all age groups. On average consumption of fruits and vegetables and fibre is low among Icelandic children and adolescents. Fish consumption was low in some of the studies, especially in older children and adolescents. The studies show similar dietary patterns but it seems that dietary habits of children have improved since 1992 and as the children get older the diet quality gets worse, which is reflected in more consumption of foods poor in nutrients.

The aforementioned longitudinal studies, on infant's nutrition and health with a follow up at the age of six years have been useful in developing nutrition recommendations for infants [9-11] and evaluating the effects of changed recommendations and actions in the primary health care [19].

In older children aforementioned studies have lead to an awakening in the Icelandic society and actions have been taken. In 2004, a project called "*Everything affects us, especially ourselves!"* was established and directed by the Public Health Institute of Iceland, currently answering to the Directorate of Health [70]. The aims of the project were to promote health and welfare of children and their families in Iceland and prevent diseases among children and youth. The project was carried out in cooperation with municipalities and health clinics in Iceland until 2010. It involved, for example, campaigns on healthy food choices and exercise, e.g. more fruit and vegetable consumption. Public Health Institute of Iceland also published brochures and documents on the subject, such as infant's nutrition recommendations, children's nutrition recommendations, manual for preschool kitchens and manual for school canteens [71-74].

In 2007, the Directorate of Health started developing a project to provide schools in Iceland with a holistic policy, on prevention and health promotion issues. The main subjects are exercise, nutrition, mental health and lifestyle and the policy is called "*Heilsueflandi skóli".* At first the project was implemented in one upper secondary school, today all of the regular secondary schools in Iceland have implemented the policy and many of the compulsory schools. Because of the health-promoting policy, some positive changes have been reported regarding healthier food options at the schools and shops in the schools' surrounding.

2.3 Nutrition Recommendations

Many organisations are involved in ensuring health and welfare of the people in the world like WHO and Food and Agriculture Organization (FAO) which have established nutritional requirements and recommendations worldwide. It also includes European Food Safety Authority (EFSA), Institute of Medicine (IOM), the American Heart Association (AHA) and so forth.

The Nordic Council of Ministers is responsible for the Nordic Nutrition Recommendations [75]. The recommendations are aimed at healthy individuals with a variety of levels of exercise and not to be used for evaluating intake at individual level. The main objective of the Nordic Nutrition Recommendations is, “on the basis of state of the art of knowledge, to ensure a diet that provides energy and nutrients for optimal growth, development, function and health during the whole life” [75]. In 2006 updated nutrition recommendations and food-based dietary guidelines were published by The Public Health Institute of Iceland [76]. The recommendations were based on the Nordic Nutrition Recommendations [75]. However, in the Icelandic recommendations, results from Icelandic dietary surveys as well as cultural factors were taken into consideration.

Nutrition recommendations are divided into two types of recommendations. Firstly, nutrient recommendations which are based on physiological data on the requirements of different nutrients, i.e. recommended daily intake of various vitamins and minerals [75]. Values for evaluating the adequacy of intake of vitamins and minerals are average requirements (AR), lower level of intake (LI) for certain vitamins and recommended intake (RI). The AR is used to evaluate the risk for insufficient intake of nutrients in a certain group of individuals. The number of individuals that has intake below the AR indicates those having an increased risk of inadequate intake. According to NNR the RI is “the amount of nutrient that according to present knowledge can meet the known requirement and maintain good nutritional status among practically healthy individuals” [75].

Secondly, recommendations on energy distribution, i.e. desired proportion of the energy providing nutrients, carbohydrates, protein and fat, where quality of the nutrients is highly emphasised. The proportion of energy providing nutrients is expressed as percentages of total energy intake. The reference value for energy requirements is designed for an individual of average height, of ideal weight and who exercises moderately every day.

2.3.1. Food-based dietary guidelines

For the past decades, research has focused on nutrients or single foods, but they are not consumed separately but in many different combinations that include other nutrients, additives, contaminants and unknown compounds. Its interactions can lead to complex synergistic effects on the body‘s metabolism. The overall dietary pattern reflects the types and the amount of foods that are consumed in reality. The analysis of dietary food patterns has emerged as a useful tool for clarification of relationships between diet and health [77-79]. The overall dietary pattern may affect health more than individual foods and nutrients do. Findings from whole diet research have lead to formation of food-based dietary guidelines.

Food-based dietary guidelines (FBDG) is advice provided at the food level [75]. The FBDG are a useful tool for professionals to use to inform and educate groups and individuals about healthy diet and a tool for the individual consumer to acquire an overall healthy diet.

In 1986, the National Nutrition Council published Dietary Goals for Icelanders and subsequently approval of a National Food and Nutrition Policy in 1989 by the executive and parliamentary branch of the Icelandic government [80]. The results from the National dietary survey in 1979 influenced the policy and goal establishing. The National policy is based on the dietary goals where great emphasis was on varied diet and reduction of SFA, mainly from milk and dairy products, butter, lamb and margarine. The food policy and the dietary goals have surely influenced nutrition education and awareness in the population.

In the report for the national dietary survey in Iceland in 2002, new revised food-based dietary guidelines, by the Icelandic Nutrition Council, were published. The revised FBDG took into account results from the most recent studies on health and nutrition along with results from the national dietary survey in Iceland [12]. That included even more emphasis on fruits, vegetables and fish consumption. The message to the public stated that consumption of SFA should be more moderate and substituted with soft margarine and oils.

The NNR serve as a source for food-based dietary guidelines in the Nordic countries. In Iceland both recommendations on nutrients and FBDG have been revised from the former version of nutritional goals and the recommended daily intake [76]. Together with the Nordic Nutrition Recommendations, the FBDG also take into account new research in the field of nutrition and health in other countries as well as research on diet among adults and children in Iceland [7, 11-12, 75]. The Public Health Institute of Iceland has developed FBDG recommended for adults and children from two years of age [81]. The FBDG are based on eight recommendations: consume fruits and vegetables every day or ≥500 g/day of fruits, vegetables and pure juices; fish or other seafood two times per week or more (300 g/week at least); choose whole grain bread and other fibre-rich cereals (≥ 2·5 g/MJ of fibre per day); ≥ 5 g/day fish liver oil or other vitamin D source; two portions of low fat and low in sugar milk and dairy products/daily; oils and soft fat instead of hard fat (≤ 10 E% SFA, including trans fatty acids);

less than 6 g/day of salt for women and 7 g/day for men; and choose water to satisfy your thirst (≤10 E% from added sugar).

2.3.2 Healthy eating index

A healthy eating index (HEI) was established in the US in 1995 to develop an index of overall diet quality on population level [82]. It has been used to monitor dietary changes over time for US individuals from 2 years of age and older. Since 1995 some adjustments have been made to the healthy eating index due to changes in food-based dietary guidelines [83].

Most studies on diet as a whole, that is the view of diet as a combination of multiple dietary factors as a single exposure, can mainly be divided into two methods determining the dietary patterns [84]. Firstly, analysing diet scores that estimate compliance with dietary guidelines. Secondly, data-driven methods that use cluster analysis to develop dietary patterns.

In the last decade, studies on children’s nutrition have been focusing more on analysing whole dietary patterns [85]. Some of the reviewed studies researched associations between index or pattern scores and nutrient intake, nutritional biomarkers and anthropometry while others analysed dietary patterns and tried to establish associations with child, family or socio-demographic factors and health and development. Some studies on children’s nutrition indicated a positive association between higher healthy index scores and higher nutrient intake [85]. None of the aforementioned studies found associations between dietary patterns or index scores and blood lipids.

2.4 Summary

Dietary habits and blood lipid profile in childhood seem to track to adulthood. Increased levels of total cholesterol and LDL and decreased levels of HDL are major risk factors for cardiovascular diseases. Composition of energy providing nutrients in a diet is associated with blood lipid levels in both adults and children. The evidence suggests that by substituting MUFA or PUFA for SFA, a reduction in total cholesterol and LDL levels will occur.

Repeated monitoring of dietary intake of adults in Iceland showed that positive changes in dietary habits in Iceland have occurred. A possible explanation of this is the actions followed by the surveys, including public dietary goals, food and nutrition policies and public health campaigns by the Public Health Institute of Iceland and Directorate of Health.

A national dietary survey on the diet of six-year-old Icelandic children was conducted in 2001-2002. The study was a cross-sectional study and provided important information on dietary habits of Icelandic children at that time. The results indicated inadequate adherence to food-based dietary guidelines among six-year-old children and that improvements were needed. These results, amongst results from dietary surveys in different age groups, lead to actions promoting healthier dietary habits and health of Icelandic children. In 2011-2012, another cross-sectional study on diet of six-year-olds was conducted to monitor trends in dietary intake of children. By monitoring the diet of children, the effectiveness of aforementioned actions can be measured.

3. METHODS

The main methods used in the thesis are described in the „subjects and methods“ section in the Manuscript (chapter 4). More detailed information about the methods can be found in these published papers [9-11, 17]. Additional methods and author‘s contribution are presented here.

3.1 Three-day weighed food records

When choosing a method in dietary surveys on a population level there are some things that need to be taken into considerations. Available methods are weighed food records, diet history, 24hr recall and food frequency questionnaire. In the present study, three-day weighed food records were used. In dietary surveys with children, three-day weighed food records that can assess energy and nutrient intake are obtained with help from each child‘s parents or caretakers, because children at this age have low literacy levels, limited cognitive abilities and difficulties in estimating portion size [86]. Weighed food records have been reported as an accurate method for estimating energy intake in children compared to standard measure, doubly labelled water [86-87]. Weighed food records provide specific information regarding type of food, food source, food processing method, food preparation and other details when describing the foods and the amount [58]. The disadvantages are that this method requires literacy and therefore is dependant on the subject’s parents or caretakers and requires a high level of motivation and can lead to a poor response rate. In the present study it was advised to keep the record continuously for three days, record each food item separately and give precise information about the type of food, cooking procedure and time of serving, and to weigh and register all leftovers. Recipes for composite dishes should also be recorded. When researching relationships between diet and various biochemical values or for assessing individual adherence to FBDG, multiple days of intake must be collected [58].

3.2. Author’s contribution

Data collection

The preparation work for the study had already been done when I entered the study. That included contacting parents of the subjects, providing them with information and materials to keep the child’s food records for three days. Anthropometric measures were also done before I entered the study.

I participated in the recording of the food diaries, entering the weighed food records into a calculating program, ICEFOOD, designed for the national dietary survey of the Icelandic

Nutrition Council [13].The calculated results from ICEFOOD, which included three days of food records for each of the participant, were manually scanned for abnormality. An average of common food and food categories, nutrients, heavy metals, caffeine and sweeteners was calculated for 162 participants for further analysis.

Analyzing and presenting the data

The average daily intake for each subject was calculated by using Microsoft Office Excel 2007. The data from the six-year-old study in 2001-2002 and present study was imported to SAS version 9.2 and SAS Enterprise Guide (SAS Institute Inc., Cary, NC, USA) for further statistical analyses. All of the data was trimmed and organised. Data from the present study was from an updated version of ICEFOOD and thus comparable food categories were made, when building a combined data set.

In the present thesis the food-based dietary guidelines (FBDG) were adjusted for six-year-olds according to Kristjansdottir et. al [67]. The FBDG were used to define a healthy eating index (HEI) which consisted of points from zero to eight, by adherence to each of the FBDG. Eight points gave a 100% score. The eight factors used to determine total diet quality in the present study were the following: ≥400 g/day of fruits, vegetables and pure juices; ≥ 34 g/day fish or other seafood; 5 g/day fish liver oil (as the main source of vitamin D in the population studied); two portions milk and dairy products/daily; ≤0.5 g /1000 kJ [75], corresponding to approximately 3.2 g salt daily (according to average intake of energy of about 1500 kcal per day, in both studies 2001-2002 and 2011-2012); ≥ 2.5 g/MJ of fibre per day; ≤10 E% SFA (including trans fatty acids) and ≤10 E% from added sugar.

When analysing the relationship between blood lipids and energy providing nutrients and diet quality the cohorts were combined for more statistical power, the trends in both studies were similar. Author’s contributions to presenting the data were following:

- Gunnarsdottir I, Helgadottir H, Thorisdottir B, Thorsdottir I. Diet of six-year-old Icelandic children - National Dietary Survey 2011-2012. Laeknabladid. 2013; 99(1): 17-23.
- Helgadottir H, Gunnarsdottir I, Halldorsson T, Palsson GI, Thorsdottir I. Lower intake of saturated and trans fatty acids is associated with improved lipid profile in a six-year-old population: Two national cohort studies conducted 10 years apart. Manuscript submitted in September 2013.

I performed statistical analysis of the data in SAS, for both of the above mentioned manuscripts, where the results were described in both tables and figures. I wrote the first draft of the paper in which I am the first author, with guidance from the supervisor and co-authors. I also reviewed the manuscript by Gunnarsdottir et al. and approved the final manuscript as submitted.

4. MANUSCRIPT

Manuscript, September 20th 2013

Lower intake of saturated and trans fatty acids is associated with improved lipid profile in a six-year-old population: Two national cohort studies conducted 10 years apart

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Key words: Children, blood lipids, dietary survey, diet, dietary fat

Abstract

Objective: To evaluate nutrient intake and blood lipid profile in six-year-old children from two national dietary surveys conducted 10 years apart.

Design: Two national cohort studies conducted on 6-year-olds in 2001-2002 and 2011-2012. Dietary intake was assessed by three-day weighed food records and used to determine nutrient intake. Serum total cholesterol, HDL and triacylglycerol were measured and LDL calculated.

Setting: Iceland.

Subjects: Six-year-old children (n=293).

Results: No difference was observed in total energy intake or the contribution of total fat, carbohydrates or protein to total energy intake (E%) between the studies. However, average intake of saturated fatty acids (SFA) and trans fatty acids, was lower in 2011-2012 than 2001-2002 (13·3E% vs. 14·7E%, p= 0·0001, and 0·8E% vs. 1·4E%, p= <0·0001, respectively), replaced by higher intake of unsaturated fatty acids. Intake of added sugar decreased by 1·6 E% (p=0·0003) and higher intake of dietary fibre (g/d) was seen in the 2011-2012 compared with 2001-2002 (13·2 g/d vs. 11·1 g/d, p=<0·0001). Total cholesterol and LDL concentrations were significantly lower in 2011-2012 compared with 2001-2002 (4·6 mmol/L vs. 4·4 mmol/L, p=0·003 and 2·8 mmol/L vs. 2·5 mmol/L, p=<0·0001, respectively). In a multiple linear regression model, one E% increase in SFA intake was related to 0·03 mmol/L increase in LDL cholesterol (p=0·04).

Conclusion: Lower intake of saturated fat and trans fatty acids, replaced by unsaturated fat, might have contributed to improved lipid profile on the population level. Carbohydrate quality has also improved in the in the population studied.

Introduction

Dietary habits and nutrient intake tend to track from childhood to adulthood and have been suggested to influence health later in life [(1-4)]. Monitoring trends in children's dietary intake is of great importance for public health, as it can be used as basis in the development of strategies aiming at improving dietary habits and thereby health. Recent national dietary survey from 2010-2011 including adults (18-80 years old) showed that changes towards healthier dietary habits have occurred the past 10 years in Iceland, including 43% decrease in intake of trans fatty acids, from 1·4E% to 0·8E% (p= <0·001), and a significant drop in the intake of saturated fat [(5)]. No information on health measurements other than weight and height were available in the survey. Our hypothesis is that similar changes in dietary habits might have occurred among children as for the adult population [(6-7)], which might have contributed to more favourable lipid profile [(8-10)]. The relationship between energy contributing nutrients and blood lipid profile is rather well established in children [(8-10)]. However, national dietary studies in children are rarely accompanied with lipid profile, or other clinical assessment, and have therefore limited ability to estimate if changes in dietary habits resulted in health effects on population level.

The aim of the study was to compare nutrient intake and blood lipid profile in six-year-old children from two national dietary surveys conducted 10 years apart, in 2001-2002 and 2011-2012. This report presents comparison of two national dietary surveys, including measurements of blood lipid profile. A secondary aim was to assess the association between the contribution of energy giving nutrients and lipid profile.

Subjects and methods

Subjects

Subjects were randomly selected and recruited into population based longitudinal cohort studies conducted with a 10 year interval (subjects in the former cohort born in 1995-6 and subjects in the latter cohort born in 2005) [11-15]. Methods in the two studies have been previously published in detail [11, 13-16]. A follow up of the children born 1995-1996 was conducted in 2001-2002 (n=180) around their sixth birthday [17-18]. Follow up of the children in the latter study at six years of age was conducted in 2011-2012 (n=196) [19]. In the present study data from the follow up studies, when the children were six years of age, is used.

Informed written consent from the parents was obtained, and all individual information was processed with strict confidentiality. The studies were approved by the Icelandic Bioethics Committee, the Icelandic Data Protection Authority, and the Local Ethical Committee at Landspitali-The National University Hospital of Iceland.

Weighed food records

Three-day weighed food records were used to assess diet and nutrient intake and for determination of diet quality. The records were obtained around the children’s sixth birthday (average age 72·3 ± 1·6 months in 2001-2002 and 73·4 ± 3·2 months in 2011-2012). The parents/carers were given detailed written and oral instructions and were provided with accurate electronic scales (PHILIPS HR 2385, Koninklijke Philips Electronics N.V, Wien, Austria). They were advised to keep the record continuously for three days, record each food item separately and give precise information about the type of food, cooking procedure and time of serving, and to weigh and register all leftovers. All data was entered into a calculating program, ICEFOOD, designed for the national dietary survey in Iceland [5, 20]. Nutrient losses due to food preparation were included in the calculations. Consumption of particular food and food categories were estimated to grams per day and nutrients were estimated from information about chemical content from food codes and recipes. In the former study the program included 452 food codes or recipes from the Icelandic Nutrition Council, based on 394 food items from the National Nutrition Database, ISGEM. In the latter study the program included 607 food codes or recipes based and 514 food items [5].

Serum lipids

Fasting blood samples were taken from the children‘s antecubital fossa. The children were appointed to blood sampling after the collection of food records in collaboration with the children‘s parents. All blood samples were analysed for serum total cholesterol (TC), HDL and triacylglycerol (TAG). TC and TAG were analysed using an enzymatic colorimetric test (Cholesterol CHOD-PAP, Roche Diagnostics, Mannheim, Germany). HDL was measured using the same method after precipitation and centrifugation. LDL was calculated from the serum TC, TAG and HDL concentrations expressed in mmol/L using Friedewald formula (21) which is considered valid if TAG concentrations do not exceed 4·52 mmol/L (22).

Anthropometrics

Height and weight of study participants were measured at Landspitali – Children’s Hospital. Subjects wore light weight clothing and no shoes. Height was measured to the nearest 0·1 cm, using ulmer stadiometer, Busse design (Nersinger Straβe 18, 89275 Elchingen, Germany), and weight was measured to the nearest 0·05 kg using a Taniter BWB-620 electronic scale (2625 South Clearbrook Drive, Arlington Height, Illinois 60005, USA) in 2001-2002 and Marel Model M1100-C2 Weighing Instrument (Marel hf, Austurhraun 9 210 Gardabaer, Iceland) in 2011-2012.

Statistical analysis

Descriptive analyses (mean and standard deviation) were used for describing the characteristics of study participants. A linear regression model with a 95% confidence interval was used for the comparison between studies. For TC, LDL, HDL and TAG a multivariate linear regression was fitted on contribution of energy providing nutrients to estimate the association. The associations were adjusted for gender, energy intake, study and body mass index (BMI) at 6 years. The examined macronutrients were total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), carbohydrates, added sugar and dietary fibre. The statistical analyses were performed using SAS version 9.2 and SAS Enterprise Guide (SAS Institute Inc., Cary, NC, USA).

Results

Study group

Complete three day food records were returned by 131 subjects (72%) in the 2001-2002 study and 162 subjects (83%) in the 2011-2012 study. Blood samples were collected from 137 (76%) and 145 subjects (74%), respectively. The mean weight and length at birth and at 12 months, dietary intake in infancy, socio-demographic factors (parent's age and education), as well as parental BMI of the children included in this current analysis did not differ from the children in the original studies. This suggests that the random selection of participants resulted in a representative sample from the source population.

The characteristics of the subjects and their parents are presented in Table 1. In the 2011-2012 study the participants were one month older on average than in the former study, which might explain the small difference observed in height between the two cohorts.

Table 1 Characteristics of the subjects and their parents.

	2001-2002 cohort (*n* 137)		2011-2012 cohort (*n* 145)			
	Mean	SD	Mean	SD	Δ[1]	CI 95%[2]
Mother's age (years)	35·7	5·4	36·3	4·9	0·6	-0·7, 2·0
Father's age (years)	37·7	5·9	38·9	5·9	1·2	-0·4, 2·8
Mother's BMI (kg/m2)	25·5	4·4	24·8	4·9	-0·6	-2·0, 0·7
Father's BMI (kg/m2)	26·5	3·2	26·2	3·2	-0·3	-1·4, 0·8
Mother's ≥ 12 y of schooling [*n* (%)]	70 (74)[3]		121 (81)[3]		0·7[4]	1·3, 0·2
Father's ≥ 12 y of schooling [*n* (%)]	76 (80)[3]		111 (76)[3]		1·4[4]	0·8, 2·5
Age (months)	72·3	1·6	73·4	3·2	1·1	0·5, 1·7
Weight (kg)	23·0	3·4	23·0	3·7	-0·05	-0·9, 0·8
Height (cm)	119·0	4·4	120·0	4·9	1·0	-0·03, 2·1
BMI (kg/m^2)	16·1	2·3	15·9	1·8	-0·2	-0·6, 0·3

[1]Δ, Mean difference.

[2] 95% confidence limit

[3] Frequency [n (%)]

[4] Odds ratio

Energy and nutrient intake of 6-year-old-children 2001-2002 and 2011-2012

The mean intake of energy and contribution of energy providing nutrients in the two cohorts are presented in Table 2.

Table 2 Mean energy intake and contribution of energy giving nutrients to total energy intake in the 2001-2001 cohort compared with the 2011-2012 cohort.

	2001-2002 cohort (*n* 131)		2011-2012 cohort (*n* 162)			
	Mean	SD	Mean	SD	Δ[1]	CI 95%[2]
Energy (kcal/d)	1494	308	1543	324	48·8	-24·5, 122·2
Protein (E%)	15·6	2·8	15·4	2·9	-0·3	-0·9, 0·4
Total fat (E%)	33·1	5·4	32·2	4·9	-0·9	-2·1, 0·3
SFA (E%)	14·7	3·1	13·3	2·7	-1·3	-2·0, -0·7
Trans fat (E%)	1·4	0·5	0·8	0·3	-0·7	-0·7, -0·6
MUFA (E%)	9·5	1·7	10·1	1·8	0·6	0·2, 1·0
PUFA (E%)	3·8	1·2	4·7	1·5	0·9	0·6, 1·2
Omega-3 (E%)	0·9	0·4	1·2	0·6	0·3	0·2, 0·4
Omega-6 (E%)	2·9	0·9	3·4	1·2	0·5	0·3, 0·8
Omega-6/Omega-3	3·6	1·3	3·2	1·2	-0·4	-0·6, -0·1
Carbohydrate (E%)	50·8	5·3	50·3	5·5	-0·5	-1·8, 0·7
Fibre (g)	11·1	3·2	13·2	4·0	2·1	1·3, 3·0
Fibre (g/MJ)	1·8	0·5	2·1	0·5	0·3	0·1, 0·4
Added sugar (E%)	12·7	4·3	11·2	4·5	-1·6	-2·6, -0·5

[1] Δ, Mean differences.

[2] 95% confidence limit

No difference was observed in total energy intake or the contribution of total fat, carbohydrates or protein to total energy intake (E%) between the studies. However, average intake of saturated fatty acids (SFA) and trans fatty acids, was lower in 2011-2012 than 2001-2002 (13·3E% vs. 14·7E%, *p*= 0·0001, and 0·8E% vs. 1·4E%, *p*= <0·0001, respectively), replaced by higher intake of unsaturated fatty acids. Although the contribution of carbohydrates to total energy intake was not different between the two studies, intake of added sugar decreased by 1·6 E% (*p*=0·0003) and higher intake of dietary fibre (g/d) was seen in the study 2011-2012 compared with the study 2001-2002 (13·2 g/d vs. 11·1 g/d, *p*=<0·0001).

Blood lipids and its association with the contribution of energy giving nutrients

Children‘s blood lipid profile in both studies are shown in Table 3. Total cholesterol was 0·2 mmol/L lower in 2011-2012 than in 2001-2002 (*p*=0·003) and LDL was 0·3 mmol/L lower (*p*=<0·0001). There was a trend towards a higher HDL concentration in the 2011-2012 study compared with the 2001-2002 study, of borderline significance (*p*=0·06). No difference in TAG concentration was observed between the two cohorts.

Table 3. Mean serum lipid concentration in the 2001-2002 cohort and 2011-2012 cohort.

	2001-2002 cohort (*n* 137)		2011-2012 cohort (*n* 145)			
	Mean	SD	Mean	SD	Δ[1]	CI 95%[2]
TC (mmol/L)	4·6	0·6	4·4	0·6	-0·2	-0·4, -0·07
LDL (mmol/L)	2·8	0·5	2·5	0·6	-0·3	-0·4, -0·1
HDL (mmol/L)	1·5	0·3	1·6	0·3	0·07	-0·003, 0·2
TAG (mmol/L)	0·6	0·2	0·6	0·2	0·02	-0·04, 0·08

TC, total cholesterol.

LDL, low density lipoprotein.

HDL, high density lipoprotein.

TAG, triacylgliceride.

[1] Δ, Mean differences.

[2] 95% confidence limit

The relationship between blood lipids and contribution of energy providing nutrients was examined with multivariate linear regression. The trend was similar in both studies and thus the cohorts were combined for more statistical power. As shown in Table 4, a one percent increase in the contribution of saturated fat to total energy intake was associated with 0·03 mmol/L (p=0·04) higher LDL cholesterol concentration in the merged dataset, adjusted for potential confounding factors.

Table 4 Linear regression analysis for TC[1] (mmol/L) and LDL[2] (mmol/L) and contribution of energy providing nutrients for the combined cohorts.

	TC[1]				LDL[2]			
	Unadjusted		Adjusted*		Unadjusted		Adjusted*	
	Δ[3]	CI 95%[4]	Δ[3]	CI 95%[4]	Δ[3]	CI 95%[4]	Δ[3]	CI 95%[4]
Total Fat (E%)	0·02	0·006, 0·03	0·02	0·004, 0·03	0·02	0·004, 0·03	0·02	0·003, 0·03
SFA (E%)	0·04	0·01, 0·06	0·03	0·002, 0·05	0·03	0·01, 0·06	0·03	0·002, 0·05
MUFA (E%)	0·01	-0·03, 0·06	0·02	-0·02, 0·07	0·01	-0·02, 0·05	0·03	-0·01, 0·07
PUFA (E%)	0·02	-0·03, 0·07	0·04	-0·01, 0·09	-0·0005	-0·05, 0·05	0·03	-0·02, 0·07
Protein (E%)	0·006	-0·02, 0·03	0·01	-0·02, 0·04	0·009	-0·01, 0·03	0·01	-0·01, 0·04
Carbohydrates (E%)	-0·02	-0·03, -0·003	-0·02	-0·03, -0·005	-0·01	-0·03, -0·002	-0·02	-0·03, -0·005
Added sugar (E%)	-0·003	-0·02, 0·01	-0·009	-0·03, 0·008	-0·007	-0·02, 0·008	-0·02	-0·03, 0·0005
Fibre (g)	-0·01	-0·03, 0·01	-0·01	-0·04, 0·01	-0·01	-0·03, 0·005	-0·007	-0·03, 0·02

[1]TC, total cholesterol.

[2]LDL, low density lipoprotein.

[3]Δ, Mean differences as estimated using multivariate linear regression.

[4] 95% confidence limit.

*Adjusted for gender, energy intake, BMI and study.

Figure 1 shows the relationship between SFA intake and LDL levels. Multivariate linear regression, adjusted for gender, energy intake, BMI and study, showed that LDL levels were significantly higher when intake was between 15-17E% compared to less than 11% of total energy (Δ=0·3 *p*=0·003).

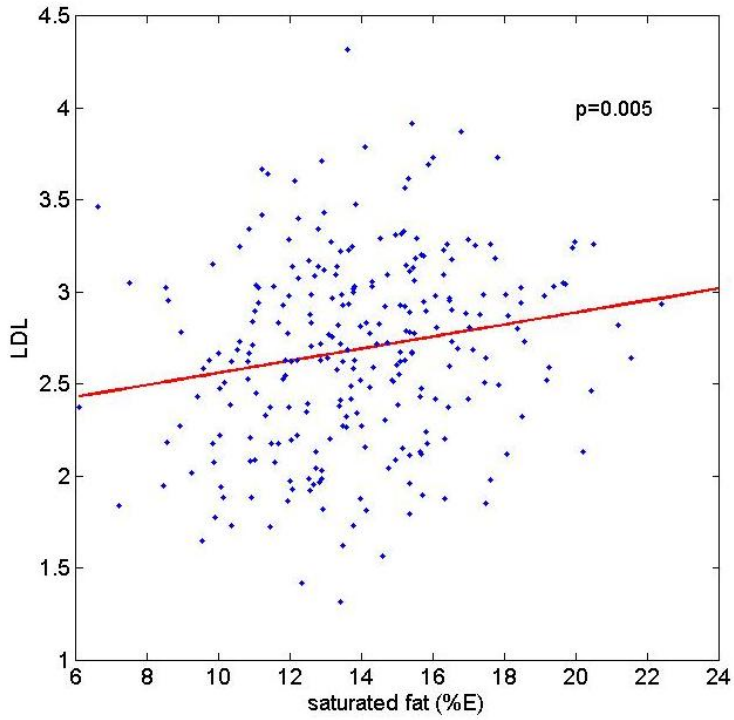

Figure 1 The relationship between SFA intake and LDL levels.

Discussion

The present study shows that the diet quality of 6-year-old children in the population studied has improved in the past 10 years. Intake of saturated fat and trans fatty acids has decreased by 9% and 45%, respectively, which is in line with changes recently presented in the adult population in Iceland [5]. Decreased intake of saturated fat and trans fatty acids might have contributed to improved lipid profile in 2011-2012 study compared with the one conducted 10 years previously [8-10]. Other positive trends towards healthier diet in Icelandic six-year-olds include improved carbohydrate quality.

A significant decrease in total cholesterol and LDL levels was found in the six-year-old population from 2001-2002 to 2011-2012 (-0·2 and -0·3 mmol/L). Other studies have observed similar decrease in blood lipids when the quality of fat intake has changed to the same degree as found in the current study [23-24]. Similar trends in blood lipid levels have been observed in US youth from 1988 to 2010 [25]. Milk and milk products are the main sources of saturated fatty acids in the diets of Icelandic children. Even though milk consumption is still high in the population studied a steady decrease in milk and dairy product consumption has occurred in the past decades [26] which might have contributed to the decrease in cholesterol and LDL levels in the past 10 years.

It is not certain to what extent reduction in cholesterol in children will decrease later risk of CHD. Subjects in the Bogalusa Heart Study with LDL cholesterol concentrations above 3·35 mmol/L in childhood had significantly higher prevalence of adult dyslipidemia, compared with subjects with LDL cholesterol concentration below 2·84 mmol/L in childhood [27]. It has been estimated that, a one mmol/L reduction in cholesterol will reduce CHD mortality rates by approximately 50% in middle aged individuals [28]. According to a study of the Icelandic Heart Association cholesterol concentration decreased from 6·01 mmol/L to 5·14 mmol/L in the adult population from 1981 to2006 [29]. At the same time contribution of saturated fatty acids plus trans fatty acids to total energy intake reduced from 19·0E% to 15·2E% [5, 20]. Coronary heart disease (CHD) mortality rates have declined considerably in Iceland during the past two decades [29], by 80% in men and women. The decline was mostly attributed to risk factors reductions, i.e., cholesterol, smoking and physical inactivity. The 0·3 mmol/L reduction in average LDL cholesterol concentration in 6-year-olds seen in the present study on the population level is suggested to be of clinical relevance, as it will have decreased the number of children considered to be at risk of adult dyslipidemia.

There are several limitations associated with the interpretation of results from two independent cohort studies conducted 10 years apart. However, the present study brings important messages to health authorities implementing different strategies in order to improve health on the population level. The results can therefore be considered of value when developing and supporting concepts in public health nutrition related to improved fat quality. Given the relatively low number of subjects in the present analysis the confidence intervals in our estimation of the association between 1E% increase in the intake of saturated fat and cholesterol intake is broad. However, our results are in line with results presented by large cohort studies and intervention studies [10, 24, 27, 30].

Monitoring trends in dietary intake is of uttermost importance, as knowledge of dietary habits of different age groups is the foundation for policy making in nutrition recommendations [31]. The results of the present study show that actions aiming at improving dietary habits among Icelandic children have been successful. The results are of importance locally in Iceland, but also at the global level, as similar approach has been used in Iceland as in many other countries emphasising healthy eating and overall healthy lifestyle. These include governmental actions [32], dietary intervention trials [33] and actions at the community level [34].

Conclusion

Lower intake of saturated fat and trans fatty acids, replaced by unsaturated fat, might have contributed to improved lipid profile on the population level among six year old children. Carbohydrate quality has also improved in the population studied.

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5. RESULTS

The main results describing change in the intake of energy-providing nutrients and lipid profile among six-year-old children in 2001-2002 and 2011-2012 are presented in the Manuscript (chapter 4). In this section food intake, quality of the total diet as well as intake of vitamin and mineral intake in the 2001-2002 and 2011-2012 studies are presented. Additional results on the association between the contribution of energy-providing nutrients and lipid profile that could not be fitted into the above-mentioned manuscript are also presented in this section.

5.1 Dietary intake

The average consumption of common foods and food categories in grams per day, in 2001-2002 and 2011-2012 among six-year-old children, are presented in Table 1.

Table 1 Average consumption of common food and food categories in grams per day.

	2001-2002 (n=131)		2011-2012 (n=162)			
	mean	SD*	mean	SD*	P value	Difference (%)
Milk and milk products total	440.8	227.9	357.3	193.3	**0.001**	**-19**
Whole milk, drinks	118.0	157.3	78.2	117.5	**0.04**	**-34**
Low fat milk, drinks	135.6	160.9	102.4	144.2	**0.04**	**-24**
Skimmed milk, drinks	2.8	27.5	6.7	36.3	0.1	139
Chocolate milk, drinks	29.4	79.6	32.3	52.7	0.1	10
Cultured products	98.3	78.1	98.7	93.9	0.6	0
Cheeses	12.2	13.2	17.2	13.8	**0.0002**	**41**
Bread total	71.9	40.5	80.0	39.0	0.1	11
Cakes	52.7	39.1	44.9	37.4	**0.05**	**-15**
Breakfast cereals	26.5	20.2	42.7	37.9	**0.0002**	**61**
Oatmeal	10.4	31.0	15.8	35.7	0.1	52
Potatoes	29.7	27.3	21.1	28.4	**0.004**	**-29**
French fries	6.8	14.7	5.6	12.4	0.5	-18
Vegetables and fruit	114.4	86.7	186.5	118.9	**<0.0001**	**63**
Vegetables total	29.5	34.1	51.9	47.0	**<0.0001**	**76**
Fresh vegetables	26.1	33.8	47.0	45.9	**<0.0001**	**80**
Fruit and berries total	84.9	69.0	134.6	102.3	**<0.0001**	**59**
Fresh fruit and berries total	80.6	67.2	128.7	99.8	**<0.0001**	**60**
Fish and fish products	19.8	23.3	21.3	24.0	0.4	7
Meat and meat products	51.5	36.8	65.0	34.8	**0.0002**	**26**
Cod liver oil	1.4	1.9	2.4	3.0	0.05	72
Sweets	13.4	17.8	13.0	19.5	0.2	-3
Soda[1]	74.4	86.2	52.5	85.7	**0.0003**	**-29**
Cool drinks[2]	133.7	124.1	150.1	147.3	**0.0003**	**12**
Fruit juice	59.8	86.7	78.3	100.1	0.08	31

*SD, standard deviation

[1]Soda includes; sugared soda and un-sugared soda.

[2] Cool drinks include; fresh juices, sugared fruit juices and other sugared drinks (other than soda).

The subjects consumed more fruits and vegetables in 2011-2012 than in 2001-2002 (114.4 g vs. 186.5 g, p=<0.0001). There was a greater increase in vegetable consumption than in fruit and berries consumption (76% vs. 59% increase). They also consumed significantly more meat and meat products, cheeses, breakfast cereals and cool drinks. In 2011-2012 six-year-old children consumed less of milk and milk products compared to 2001-2002 (357.3 g vs. 440.8, p= 0.001). Consumption of cakes, potatoes and soda was also lower in 2011-2012 compared to 2001-2002.

Table 2 shows the number and percentages of subjects following each food-based dietary guideline.

Table 2 Number of participants following food-based dietary guidelines, and recommendatons related to fat and carbohydrate quality, in the 2001-2002 and 2011-2012 studies.

	2001-2002 (*n* 131)	2011-2012 (*n* 162)		
	n (%)	n (%)	OR[1]	CI 95%[2]
Fruits, vegetables, pure juices intake (≥400 g/d)	8 (6)	30 (19)	3.4	1.5; 7.6
Fish intake (≥ 34 g/d)	30 (23)	37 (23)	1.0	0.6; 1.7
Fish liver oil intake (≥ 5 g/d)	16 (12)	44 (27)	2.7	1.4; 5.0
Dairy products (≥ two servings/d)	119 (91)	141 (87)	0.7	0.3; 1.4
Salt intake (≤ 3.2 g/d)	5 (4)	7 (4)	1.1	0.4; 3.7
Fibre intake (≥2.5 g/MJ/d)	14 (11)	28 (17)	1.7	0.9; 3.5
Saturated fat incl. trans fatty acids (≤ 10 E%/d)	5 (4)	10 (6)	1.7	0.6; 5.0
Added sugar intake (≤ 10 E%/d)	34 (26)	70 (43)	2.2	1.3; 3.6

[1] OR, Odds ratio, reflecting the odds of meeting each of the dietary recomendations when comparing the dietary intake in the 2001-2002 cohort (referent) with the 2011-2012 cohort

[2] 95% confidence limit

In the 2011-2012 study, subjects were about three times more likely to consume ≥ 400 g/d fruits and vegetables and five ml of fish liver oil (p=0.003 and p=0.002, respectively) than subjects in the 2001-2002 study. Subjects in the 2011-2012 study were two times more likely to consume ≤ 10 E% added sugar than subjects in the 2001-2002 study (p=0.002). The median total HEI score was significantly higher in 2011-2012 compared with 2001-2002 (2 vs. 3 p<0.0001), where more subjects followed three or more FBDG in 2011-2012 compared to 2001-2012, see Figure 2.

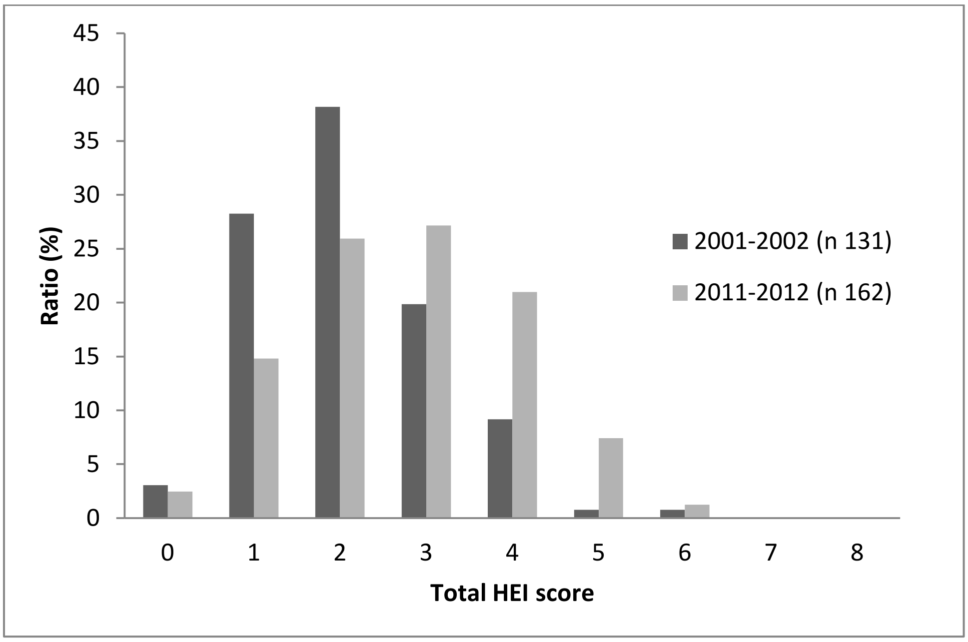

Figure 2 Ratio of subjects following the FBDG used to define the HEI score (a total HEI score of 1 represents one followed FBDG) in 2001-2002 compared to 2011-2012.

5.2 Associations between LDL and saturated fatty acid intake

The association between LDL concentration (mmol/L) and contribution of saturated fatty acids (SFA) to total energy intake (E%) was assessed and is described using a spline function in Figure 3.

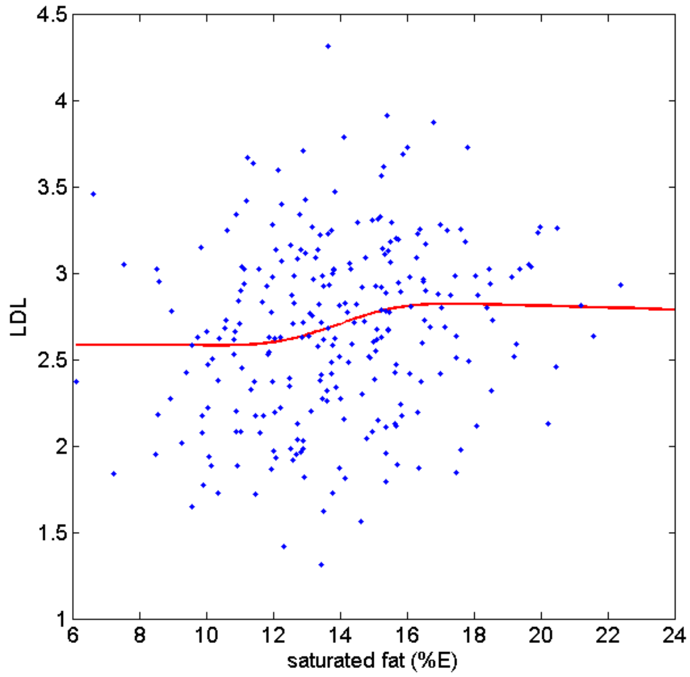

Figure 3 Association between SFA intake (E%) and LDL concentration (mmol/L) (Spline function).

It seems that LDL level in blood serum starts to increase when SFA intake is above 12% of total energy intake.

5.3 Associations between healthy eating index scores and blood lipids

Using a multivariate linear regression model and adjusting for gender, energy intake and BMI, no significant relationship was found between blood lipids and FBDG or HEI score.

5.4 Vitamins and mineral intake

Changes in the consumption of food were reflected in vitamins and mineral intake in six-year-old children. The average daily intake of vitamins, minerals and heavy metals are presented in Table 3.

Table 3. Average daily intake of vitamins, minerals and heavy metals.

		RDI	2001-2002 (n=131)		2011-2012 (n=162)			
			mean	SD†	mean	SD†	P value	Difference %
Vitamin A	RJ	400	1478.2	1318.2	963.4	1051.1	**<0.0001**	**-35**
Vitamin D	µg	10	7.5	8.3	7.5	6.4	0.2	0
Vitamin E	α-TJ	8	4.8	2.2	8.1	4.9	**<0.0001**	**68**
Thiamin (B_1)	mg	0.9	1.1	0.4	1.2	0.5	0.1	11
Riboflavin (B_2)	mg	1.1	1.7	0.5	1.7	0.7	0.7	2
Niacin	NJ	12	22.2	6.3	24.9	8.1	**0.009**	**12**
Vitamin B_6	mg	1.0	1.4	0.5	1.6	0.8	0.5	10
Folate	µg	130	256.1	90.4	309.1	158.0	**0.01**	**21**
Vitamin B_{12}	µg	1.3	5.2	2.8	5.3	3.3	0.8	3
Vitamin C	mg	40	78.3	48.9	98.6	60.9	**0.002**	**26**
Calcium	mg	800	874.1	283.0	811.6	245.9	**0.05**	**-7**
Phosphate	mg	600	1130.1	268.9	1133.8	277.3	0.9	0
Magnesium	mg	200	188.8	43.2	205.9	50.5	**0.002**	**9**
Sodium	mg	-	2061.0	498.0	2032.3	562.8	0.6	-1.4
Potassium	mg	2000	2007.3	523.0	2034.0	517.6	0.7	1
Iron	mg	9	10.3	4.2	11.1	5.1	0.1	8
Zink	mg	7	8.3	2.6	9.5	4.8	0.1	14
Iodine	µg	120	119.8	63.1	122.7	73.3	0.7	2
Selenium	µg	30	48.1	15.4	48.5	20.8	0.9	1

*Recommended daily intake of vitamins and minerals for 6-9 year old children [76].

†SD, standard deviation

In 2001-2002 and 2011-2012, the average intake of most vitamins and minerals was higher than recommended intake [75-76]. Average intake of vitamin A had significantly decreased in 2011-2012 compared to 2001-2002 (1478.2 RJ vs. 963.4 RJ, *p*=<0.0001). Vitamin E intake had increased (4.8 α-TJ vs. 8.1 α-TJ, *p*=<0.0001). Intake of niacin, folate and vitamin C had slightly increased.

6. DISCUSSION

The results for energy and nutrient intake of six-year-old children in 2001-2002 and 2011-2012 and the association with blood lipids are mainly discussed in the Manuscript (see chapter 4).

The results of the present study indicate overall improvements in dietary habits among six-year-old children in Iceland. The diet seems to provide them with sufficient amounts of vitamins and minerals on average, except for vitamin D. According to Gunnarsdottir et al. (2012) only a quarter of six-year-olds in 2011-2012 reached the RDI and above for vitamin D intake [65]. It also indicated that some group of children might not be consuming sufficient amount of iodine, vitamin E and vitamin A. Quality of the children‘s diet is greater in 2011-2012 compared to 2001-2002 and can be measured by more food-based dietary guidelines being followed on average and higher percentage of children reaching the goals. The diet is moving towards increased consumption of vegetables, whole grain products, fish liver oil, less consumption of soda, whole milk and low fat milk and decreased intake of added sugar and saturated fatty acids (SFA) and trans fatty acids. However, more actions are needed since the total diet quality is still relatively low.

The overall diet quality improvements and changes in the diet are in accordance with the trend seen in many other European countries [88-91]. However, some studies show worsening in dietary trends, e.g. in children in Greece [92]. The quality of diet among Danish children (aged 4-14 years) had improved in 2003-2008 compared to in 2000-2002, where consumption of fruits and vegetables had increased and sugar sweetened beverages intake had decreased [89]. The nutrient density of the diet had improved and vitamin and mineral intake was sufficient on average except for vitamin D, which was too low in all age groups. Contribution of total fat and carbohydrates to total energy intake among Danish children had not changed during the period, while intake of SFA had decreased from 15 to 14 E%, added sugar from 14 to 12 E% and dietary fibre increased from 20 to 22 g/10MJ. In Britain similar trends in intake of energy providing nutrients, as seen in present thesis, were observed from 1997 to 2008-2010, where intake of SFA and added sugar in children decreased through the years [91]. However, the intake of SFA and added sugar was higher than recommended (13.4 E% and 14.4 E%, respectively). In 2003 dietary intake in Swedish children was similar as it was in Iceland in 2001-2002, in regards to energy intake and contribution of energy providing nutrients [90], but more recent data is not available and therefore not possible to predict if similar positive changes have occurred in Sweden as seen in Iceland and Denmark the past 10 years.

New Nordic Nutrition Recommendations (NNR5) are in the making and are to be launched in October 2013 [93]. The Nordic Council of Ministers does the revision and a working group, nominated by the council, has been focusing on revising areas where new scientific knowledge has emerged. That includes fat and carbohydrate quality, protein, alcohol, vitamin D, calcium, folate, iodine, iron and eating patterns. Systematic literature reviews have been used to minimise potential reporting bias. According to NNR5, current evidence suggest that to keep future health and well-being of a Nordic population the diet should be rich of vegetables, fruits and berries, nuts and seeds, whole grains, fish and seafood, vegetables oils and low-fat dairy products [93]. It should contain limited amounts of processed meats, red meat, refined and highly processed food products and food rich in added sugar and salt and SFA and trans fatty acids. This is the main focus in current food-based dietary guidelines in Iceland and needs to be more emphasised.

In the past decade, emphasis on public health actions by Directorate of Health in Iceland has been on fruit and vegetable intake, among others, which seems to have resulted in improvements according to the results of the present study. However, there is still a long way to go as very few children in the present study consumed fruit and vegetables in adherence to recommendations (19%). Low intake of fruits and vegetables has been found in other age groups among Icelandic children [88, 94]. Consumption of whole grains and fruits and vegetables has been related to higher intake of vitamins and minerals [31, 75]. Increased fruit and vegetable consumption and whole grain consumption could improve nutritional value of the diet of six-year-old children. It could also influence their health, where improved carbohydrate quality has shown to contribute to a decrease in blood cholesterol levels in children.

Association between diet quality and blood lipids was not found in the present study, contrary to what has been shown in a few other studies [1, 54, 95]. One possible explanation might be related to a difference in the definition of diet quality, but there is no universal way of defining diet quality and different approaches have been used in past studies. Using data-driven methods with cluster analysis to develop dietary patterns might have been more suitable [84]. There is also a possibility that the size of the sample might have been too small or simply using a healthy eating index isn't a good method for evaluating causality between diet and blood lipids.

The updated Nordic Nutrition Recommendations for fat and fatty acids has put more focus on quality of fat and its dietary sources [93]. The recommended range for MUFA and PUFA is 10-20 E% and 5-10 E%, respectively. MUFA and PUFA should be minimum 2/3 of total fat. SFA should be less then 10% of total energy intake and trans fatty acids as low as possible. In the recommendations for adults and children from two years of age the lower intake level of total fat is set to 25 E% because very low-fat diets seem to reduce HDL levels and increase TAG levels [93]. If a diet consist of fat intake lower than 20 E% it can be difficult to ensure sufficient intake of fat-soluble vitamins and essential fatty acids.

Results of the current study indicate that LDL levels in blood serum started to increase when SFA intake was above 12% of total energy intake (Figure 3) and that the LDL levels were significantly higher when intake was between 15-17 E% (Manuscript). These results raise the question where there, is a reason to change the recommendations on SFA intake among children. Should the emphasis in FBDG on SFA intake (<10 E%), for children, be unchanged or is it safe to aim for a higher population average if the CVD risk doesn't increase until the SFA are 15% of total energy intake?

Results from the National dietary survey among six-year-olds revealed the contribution of certain food groups to the total consumption of SFA and added sugar [65]. The food group who contributed the most to the total consumption of SFA in the six-year-old's diet was milk and milk products (37%). Thereafter were fats, meat and meat products and cakes and biscuits. The food groups which contributed the most to added sugar intake were sweets and ice cream, cakes and biscuits, drinks (other than milk) and milk products, approximately 60% in total. Associations have been found between high intake of added sugar among children and lower intake of nutrients and lower consumption of fruit and vegetables [96].

These results can be a good foundation for preventive measures and campaigns towards healthier choices among six-year-old children. It is important to establish healthy dietary habits among children because nutrition and dietary habits in the first years of life track through adulthood and influence development and health [1-6].

7. FUTURE PERSPECTIVES

Presently studies are focusing more on whole diet or food patterns. There is a decreased interest in studying the effects of fatty acid groups on classical variables. It might be beneficial to establish a broader healthy eating index and using cluster analysis, for both adults and children.

It would be interesting to study the difference in children's diet quality in weekdays versus weekends, as was done in Denmark in 2012 [97]. The study indicated a significant difference in dietary patterns during weekdays and weekends. They suggest these results to be used in prevention against obesity among children by targeting the high energy intake on Fridays and weekend days. This might be relevant to Icelandic children because of the "one candy day per week" recommended by dentists. In past years, this has backfired and resulted in massive consumption of sweets and soda on Saturdays, throughout the whole day.

It is important to keep studying dietary trends of the Icelandic population, both adults and children. Present study is useful for further research and hopefully the results will be used for a longitudinal study on the nutrition and health of the Icelandic population. Furthermore, as a comparison for a cross-sectional study on the diet of six-year-old children conducted within the next ten years.

By presenting the status of dietary habits of six-year-old children in Iceland this thesis helps contribute to a healthier lifestyle of Icelandic children. Results from this study can be used as a basis for preventions and campaigns regarding lower added sugar and SFA intake, increased fruits and vegetables, whole grains and fish consumption and so forth. The actions need to be on the children's level, both as individuals and as a part of a society, where peers, teachers, parents and other social factors are influential. The results should also play as a role for Policy making and governmental actions that contribute to the health of Icelandic children.

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