Thesis for the degree of Master of Science in Human Nutrition
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Autumn vitamin D intake and status in 7-year-old school children

- Vitamin D status *vs.* week in autumn, intake of vitamin D containing foods, and cardiorespiratory fitness -

- And comparison of the diet of 7-year-olds in 2006 and the diet of 6-year-olds in 2011 –

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HÁSKÓLI ÍSLANDS

D-vítamínbúskapur og D-vítamínneysla meðal 7 ára íslenskra skólabarna á haustmánuðum

- Tengsl D-vítamínbúskapar við vikunúmer að hausti, inntöku á matvælum sem innihalda D-vítamín og þol -

- Samanburður á mataræði 7 ára barna 2006 og 6 ára barna 2011 -

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Lokaverkefni til MS-gráðu í næringarfræði
Leiðbeinendur: Inga Þórsdóttir prófessor og Dr. Ása Guðrún Kristjánsdóttir

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I. Ágrip

Markmið:

Markmiðið var að rannsaka D-vítamínneyslu í fæði og D-vítamínbúskap 7 ára íslenskra skólabarna á haustmánuðum, september til nóvember 2006. Tengsl við líkamsþyngdarstuðul og þrek voru einnig könnuð. Mataræði 7 ára barna 2006 var einnig borið saman við mataræði 6 ára barna frá 2011 til að slá mati á hugsanlegan D-vítamínbúskap barna í dag.

Aðferðir:

Þriggja daga fæðuskráningum var safnað með jöfnu millibili frá september til nóvember (vika 36-46). Blóðsýni voru tekin eftir næturföstu og þrek var mælt með þrekhjóli. Fæðu- og næringarefnainntaka var reiknuð út og blóðgildi (s)-25(OH)D og kalkkirtilshormóns (PTH) greind. Ófullnægjandi D-vítamínbúskapur barna er skilgreindur sem <50 nmol/L, og D-vítamínskortur við <25 nmol/L. Gögn um 6 ára börn voru meðaltöl úr þriggja daga skráningum, fengin úr töflum úr vísindagrein um mataræði 6 ára barna frá 2012.

Staðsetning:

Rannsókn í grunnskólum í Reykjavík, um 6% allra barna í öðrum bekk á Íslandi 2006. Sex ára börn eru handahófsúrtak frá Hagstofunni tekið á fyrsta aldursári þeirra, frá öllu landinu.

Þátttakendur:

7 ára börn (n=265), 65% gáfu gildar upplýsingar um inntöku (n=165), 60% gáfu blóðsýni (n=158) og 45% gáfu bæði gildar upplýsingar um inntöku og blóðsýni (n=120). Einnig 6 ára börn (n=162) sem gáfu gildar upplýsingar í Landskönnun á mataræði 6 ára barna 2011-2012.

Niðurstöður:

Um 22.4% barnanna náði ráðlagri neyslu af D-vítamíni (10 µg/d), og 65.2% höfðu ófullnægjandi s-25(OH)D stöðu (<50 nmol/L). Miðgildi s-25(OH)D var hærra fyrir börn sem tóku D-vítamínfæðubót, 49.2 nmol/L samanborið við 43.2 nmol/L (P<0.001). Miðgildi s-25(OH)D lækkaði frá viku 36 (september) (59.9 nmol/L) til 46 (nóvember) (36.7 nmol/L) (P<0.001). Aðhvarfsgreiningarmódel sýndi að 19.3% í breytileika á s-25(OH)D mátti rekja til vikunúmers að hausti (P<0.001), 5.5% til D-vítamíninntöku (P=0.003) og 4.7% til þreks (P=0.004). Strákar neyttu meira D-vítamíns (P<0.01), lýsis (P=0.03) og fisks (P=0.04), og höfðu meira þrek (P<0.01) en stelpur. Meðal D-vítamínneysla var svipuð 2006; 6.7 µg/d (SD 6.1), og 2011; 7.5 µg/d (SD 6.4) (P=0.29) og tíðni lýsisneyslu (≥5 ml/d) var 12% árið 2006 en 27% meðal barnanna 2011.

Ályktanir:

Minnihluti barnanna fylgdu ráðleggingum um D-vítamíninntöku og 65% höfðu ófullnægjandi blóðgildi D-vítamíns að hausti. Vikunúmer að hausti hafði sterkari tengsl við D-vítamínbúskap heldur en mataræði eða þrek, sem tengdist D-vítamínbúskap í svipuðum mæli. Mataræði ungra barna hefur ekki breyst mikið þegar kemur að D-vítamínuppsprettum frá 2006 til 2011, en lýsisinntaka virðist algengari. Þessar niðurstöður sýna fram á mikilvægi sólarljóss sem uppsprettu D-vítamíns til að koma í veg fyrir ófullnægjandi D-vítamínbúskap í ungum skólabörnum að hausti á norðlægum slóðum. Aukið átak þarf til að auka fylgni við D-vítamínráðleggingarnar og auka útiveru yfir sumartímann.

II. Abstract

Objective:
The aim was to investigate autumn vitamin D intake and status in 7-year-old Icelandic schoolchildren during autumn (September-November) 2006. Associations to BMI and cardiorespiratory fitness (CRF) were also investigated. The diet of 7-year-old children in 2006 was compared with the diet of 6-year-old children in 2011 to estimate possible vitamin D status of children at present day.
Design:
Three-day-food records were collected evenly from September-November (week 36-46). Fasting blood samples were collected after overnight fast and CRF was measured with an ergometer bike. Food and nutrient intake were calculated and serum (s)-25(OH)D and parathyroid hormone (PTH) analysed. Sub-optimal vitamin D status was defined as <50 nmol/L, and deficient status <25 nmol/L. Data on 6-year-olds were mean values from three day food records that were obtained from tables in an article on the diet of 6-year-old children from 2011.
Setting:
School based study in Reykjavík, representing 6% of all children in the second grade in Iceland 2006 (7-years-old). Data on 6-year-old children was a random sample taken in infancy from Statistics Iceland, from all over the country.
Subjects:
7-year-olds (n=265), 65% gave valid intake information (n=165), 60% gave blood samples (n=158) and 45% gave both valid intake information and blood samples (n=120). Also 6-year-old children (n=162) who gave valid information on dietary intake in the National Dietary Survey in Iceland 2011-2012.
Results:
Recommended vitamin D intake (10 μg/d) was reached by 22.4% of the children and 65.2% had sub-optimal s-25(OH)D status. Median s-25(OH)D were higher for children taking vitamin D supplements, 49.2 nmol/L vs. 43.2 nmol/L, respectively ($P<0.001$). Median s-25(OH)D decreased from week 36 (september) (59.9 nmol/L) to 46 (november) (36.7 nmol/L) ($P<0.001$). Regression model showed that week of autumn accounted for 19.3% of the variance in s-25(OH)D ($P<0.001$), vitamin D intake 5.5% ($P=0.003$) and CRF 4.7% ($P=0.004$). Boys had higher intake of vitamin D ($P<0.01$), fish liver oil ($P=0.03$) and fish ($P=0.04$) than girls, and also had higher CFR ($P<0.01$). Mean vitamin D intake was similar 2006; 6.7 μg/d (SD 6.1), and 2011; 7.5 μg/d (SD 6.4) ($P=0.29$), and fish liver oil intake (≥5 ml/d) was 12% in 2006 and 27% in 2011.
Conclusions:
Minority of the children had vitamin D intake according to the recommendations and 65% had sub-optimal serum vitamin D during the autumn. Week of autumn was more strongly associated with vitamin D status than diet or CRF, which associated with vitamin D status to a similar extent. The diet of young children has not changed much regarding vitamin D sources between 2006 and 2011, but fish liver oil seems to have increased slightly. These results demonstrate the importance of sunlight exposure during summer to prevent sub-optimal vitamin D status in young schoolchildren during autumn in northern countries. Increased effort is needed for enabling adherence to the vitamin D recommendations and promoting outdoor activities.

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The Icelandic Infant nutrition prospective cohort – status at 6 years of age, from 2011 was supported by the Icelandic Centre for Research and The Landspitali Science Fund, as well as RANNÍS which supported the Masters studies of one of the researchers. The study was approved by the National Bioethics Committee (VSNb2011010008) as well as the Icelandic Data Protection Commission (2010111049AMK).

IV. Abbreviations

BMI	-	Body Mass Index (kg/m^2)
EI	-	Energy intake (kkal/d)
E%	-	Percent of energy intake
CRF	-	Cardiorespiratory Fitness (w/kg)
FBDG	-	Food Based Dietary Guidelines
IOM	-	Institute of Medicine
IQR	-	Interquartile range (25, 75%)
NHANES	-	National Health and Nutrition Examination Survey
PTH	-	Parathyroid hormone
RDA	-	Recommended daily amount
RDI	-	Recommended dietary intake
SPF	-	Sun protection factor
s-25(OH)D	-	Serum hydroxyvitamin D

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Introduction

Vitamin D has attracted a lot of interest in recent years regarding its beneficial effects, indicating that sub-optimal levels of vitamin D may have an impact on our health beyond bone homeostasis (Adams & Hewison, 2008; Chun, Adams, & Hewison, 2008; Hewison, 2008). Sub-optimal status of vitamin D has been documented among otherwise healthy children, adolescents and young adults from various countries (Andersen et al., 2005; T. Hill et al., 2008; Kumar, Muntner, Kaskel, Hailpern, & Melamed, 2009; Rockell et al., 2005) but few studies have explored the association between vitamin D intake and serum vitamin D concentrations in children. Populations at high latitudes appear to be at high risk of sub-optimal vitamin D status especially during winter (Dalgård et al., 2010; M.F. Holick, 2003), and sub-optimal levels of vitamin D have been recorded at similar latitudes as Reykjavík (64°N), making it interesting to look at the intake and status of Icelandic children.

The recommended dietary intake of vitamin D for Icelandic children up to 10 years of age is 10 μg/d, equivalent to 1 serving of fatty fish or 5 ml of cod liver oil (The Directorate of Health, 2013; The Public Health Institute of Iceland, 2006), which has been the main dietary source of vitamin D in the country for decades. However food habits have changed at the cost of both fish- and fish liver oil intake in children and adults (Gunnarsdottir, Helgadottir, Thorisdottir, & Thorsdottir, 2012; Thorgeirsdottir et al., 2011), limiting the intake of dietary vitamin D. Research has repeatedly shown that the adherence to the Food Based Dietary Guidelines (FBDG) on fish and fish liver oil as well as fruit and vegetables are rarely met in the Icelandic population (Gunnarsdottir et al., 2012; Kristjansdottir & Thorsdottir, 2009; Thorsdottir & Gunnarsdottir, 2006). The vitamin D recommendation is also badly fulfilled by Icelandic children (Thorsdottir & Gunnarsdottir, 2005) .

Vitamin D consumption only partly explains the variability in vitamin D status, measured as serum vitamin D (s-25(OH)D) (Sigurdsson, Franzson, Thorgeirsdottir, & Steingrimsdottir, 1999). The main sources of vitamin D are exposure of the skin to sunlight and vitamin D containing foods such as oily fish, fish liver oil and eggs (Gunnarsdottir et al., 2012; Matís ehf, 2013; Thorgeirsdottir et al., 2011). Season, ethnicity, fortified foods, body mass index (BMI) and physical activity levels have all been shown to be independent predictors of vitamin D insufficiency in adolescents in the international literature (Gordon, DePeter, Feldman, Grace, & Emans, 2004).

It has been stated that vitamin D levels are inversely correlated with parathyroid hormone (PTH) levels in children (Gordon et al., 2004), but the optimal level of vitamin D for maximal suppression of PTH in actively growing children has not been determined (Greer, 2009). Higher vitamin D concentrations have been reported in boys compared to girls both during childhood and adolescence (1-21 yrs) (T. Hill et al., 2008; Kumar et al., 2009; Rockell et al., 2005). The influence of physical activity or cardiorespiratory fitness (CRF) as predictors of vitamin D status is virtually unknown, but sedentary behavior has been found to be a risk factor for low vitamin D status among children and adolescents (Kumar et al., 2009). It is possible that CRF acts as a surrogate marker for outdoor activities, sunlight exposure or sunscreen use, or through its own mechanisms related to vitamin D status, but exercise for at least one hour per day is advised for children (The Public Health Institute of Iceland, 2006).

The aims of the thesis and manuscript were to:

1. Investigate vitamin D intake and status in a cohort of 7-year old children in the Reykjavík area (64°N) from September until November in 2006.
2. To assess associations of s-25(OH)D with total vitamin D intake, as well as with the locally specific vitamin D sources, physical variables like CRF and BMI, and explore possible gender differences.
3. To compare the diet of 7-year-old children in 2006 to the diet of 6-year-old children in 2011, to look at the adherence to the Food Based Dietary Guidelines (FBDG) and nutrient intake, and to see if the diets are comparable. From that we can predict if our results (aim 1 and 2) are still relevant to children in Iceland.

Review of the literature

An updated literature review from the manuscript (Insufficient autumn vitamin-D intake and low vitamin-D status in 7-year-old Icelandic children) will be discussed in this chapter, repetition does occur.

Vitamin D intake and status

Iceland

The Icelandic Food Based Dietary Guidelines (FBDG) state that fish liver oil or another vitamin D supplement should be used, especially during winter. The recommended dietary intake (RDI) of vitamin D for Icelandic children up to 10 years of age is 10 μg/d, equivalent to 5 ml of cod liver oil (The Directorate of Health, 2013; The Public Health Institute of Iceland, 2006; Thorsdottir & Gunnarsdottir, 2006), which has been the main dietary source of vitamin D in the country for decades. No rich tradition is for vitamin D fortification of foods, but vitamin D fortified skim milk and formula or follow-on milk (6 months-2 years) have been put on the market in the last decade (100 ml giving approximately 1 μg of vitamin D) (Matís ehf, 2013; MS Iceland Dairies, 2013). Vitamin D fortification can also be found in imported foods such as cereals and margarine (Matís ehf, 2013).

There are indications that food habits among children and adults have changed at the cost of both fish- and fish liver oil intake (Gunnarsdottir et al., 2012; Thorgeirsdottir et al., 2011). According to the most recent Icelandic National Dietary Surveys, intake of vitamin D is far below the recommendation for those who do not take fish liver oil or other sources of vitamin D. The dietary survey also showed that even adult individuals who ate fish according to the FBDG but did not take fish liver oil, were not meeting the RDI for vitamin D. This is primarily because the fish consumed is lean (cod and haddock) and generally does not have high amounts of vitamin D (Thorgeirsdottir et al., 2011). The vitamin D status of healthy but slightly overweight Icelandic individuals (20-40 years) has been found to be low, with 11% of tested individuals with levels below 25 nmol/L (deficiency) and 66% below 50 nmol/L (sub-optimal levels) during mid- or late winter (Muldowney et al., 2011). One child serving of fatty fish (salmon) (120 g) (Kristjansdottir & Thorsdottir, 2009) contains about 11.3 μg vitamin D. For comparison, one serving (120 g) of lean haddock contributes only 1.3 μg vitamin D (Matís ehf, 2013), and one serving of fortified reduced-fat milk (200 g) contains 0.8-2.0 μg vitamin D (Matís ehf, 2013; MS Iceland Dairies, 2013).

In the most recent study on 6 year olds from 2011, only 25% of the children reported fish or fish liver oil intake during 3 food record days (Gunnarsdottir et al., 2012). Children that did not take fish liver oil or other vitamin D supplements from an older Icelandic cohort of infants, 2-year-olds and 6-year-olds, reported that they only had a vitamin D intake of 2-3 μg/d, while those who consumed fish liver oil on a daily basis reached the RDI for vitamin D (10 μg/d) (Thorsdottir & Gunnarsdottir, 2005).

Europe and worldwide

In general, vitamin D intake is also relatively low in Europe (≤ 2–3 μg/d) (Ovesen, Andersen, & Jakobsen, 2003) and more than one-third of adolescent girls (12 years) in Northern Europe have vitamin D status below 25 nmol/L and almost all are below 50 nmol/L during winter (Andersen et al., 2005). Vitamin D status in European children has been the focus of several investigations, and high prevalence of vitamin D deficiency has been reported during winter, especially above the 51.9° N latitude (Tylavsky et al., 2006). Sub-optimal levels of vitamin D and low dietary intake has also been recorded in the USA among children and adolescents (9-15 years), where up to 75.5% of the children had sub optimal levels in January-March (Au et al., 2012).

History of fish liver oil intake

Iceland is one of few countries in the world where fish liver oil is considered a part of the FBDG (The Public Health Institute of Iceland, 2006). Both because of the history of fish liver oil intake, and also because of the Nordic latitude of the country and limited sun exposure during large parts of the year, making it the main and local vitamin D source.

Fish liver oil has been a part of the Icelandic nation for centuries and fish liver oil production is an ancient industry in Iceland. The oldest sources on fish liver oil can be found in the ancient laws on tithing from 1096 (Jonsdottir, 2007). During the 14^{th} century fish liver oil was the main export product along with stockfish, but in those days fish liver oil was used for many purposes e.g. for illumination, applied on garments for waterproofing, to calm waves in heavy sea conditions and as a remedy for medical purposes (L. Kristjansson, 1987). Fish liver oil and fusion (ísl: bræðingur) (consisting of fused rendered sheep tallow and fish liver oil) was also the most common spread for those living at the seaside and replaced butter, especially during the spring in the early 1900s (Jonsdottir, 2007). Those living in fishing villages were reported to have the highest vitamin D intake througout the year in 1939-1940, attributed to the fish liver oil and "sheep-fish-fusion" consumption. The bigger towns had lower intake and farms inland had the lowest vitamin D intake due to low fish and seafood intake (Sigurjonsson, 1943).

Local authorities in Iceland decided early in the last century, as a response to bad health of children, to provide the children with fish liver oil at the schools (Jonsdottir, 2007), which was shown to be effective. The fish liver oil was mandatory at school for children in Iceland from ~1930 until ~1970 (Styrkársdóttir, 2008). A description of the execution of the fish liver oil feedings from a teacher in Akureyri from that time says:

> *„The one giving the cod liver oil stands next to a table with the bottle and a small cup, in which he pours in for each child. The child approaches the table, tilts its head far back and opens its mouth while the cod liver oil is poured into its mouth from the cup, without to cup touching the lips, then tilts its head back up and swallows the sip. This works great and so quickly that one man can pour fish liver oil into 10 children per minute" (Styrkársdóttir, 2008).*

Around 1950 the fish liver oil beads replaced the oil, which made life a lot easier for most people. These fish liver oil feedings were not popular among the children as it had an unpleasant taste and texture, and it also depended on how neat the teacher was whether the children got a small or large sip of the fish oil (Styrkársdóttir, 2008). Fish liver oil is still given in kindergartens in Iceland for the children who have breakfast at the school (The Public Health Institute of Iceland, 2009).

Cut-off levels of s-25(OH)D

Much debate has taken place over the definition of vitamin D deficiency for bone health and other outcomes. Many agree that a s-25(OH)D concentration <50 nmol/L is an indication of vitamin D deficiency, 51-74 nmol/L is considered to indicate insufficiency and concentrations >75 nmol/L are considered to be sufficient for children and adults (M. Holick, 2007; M. F. Holick et al., 2011; M. F. Holick & Chen, 2008). These cut-off levels are based on observations of maximized intestinal calcium absorption and PTH decline in adults at approximately 80 nmol/L, assuming that children have the same requirement as adults (M. F. Holick & Chen, 2008), and these values are hard to reach without adequate sun exposure or large supplemental doses. Official Nordic records characterize s-25(OH)D values of >50 nmol/L as acceptable, 25-50 nmol/L as sub-optimal or reflective of vitamin D insufficiency, 12.5-25 nmol/L as indicative of vitamin D deficiency and <12.5 nmol/L as serious vitamin D deficiency (Pedersen, 2008).

Vitamin D deficiency (<25-30 nmol/L) can cause growth retardation, signs and symptoms of rickets and muscle weakness in children, and will exacerbate both osteopenia, osteomalacia and osteoporosis in adults and increase the risk of fracture (M. F. Holick & Chen, 2008; Pedersen, 2008). The development of common cancers, type 1 diabetes, multiple sclerosis, hypertension and secondary hyperparathyroidism, among other, has also been linked with vitamin D deficiency (M. F. Holick & Chen, 2008). However, the evidence is still much weaker than for the link between bone health and vitamin D. Signs of hyperparathyroidism have been seen in the range of 38-80 nmol/L (Pedersen, 2008), however a large proportion of healthy subjects do not develop secondary hyperparathyroidism at low vitamin D levels (Mosekilde, 2008). Low 25(OH)D levels are associated with increased parathyroid hormone levels (PTH) leading to progressive bone loss (Saliba, Barnett, Rennert, Lavi, & Rennert, 2011). A threshold of 46 nmol/L s-25(OH)D was found to plateau parathyroid hormone and minimize bone loss in healthy adult populations with normal renal function (Saliba et al., 2011), and in 2010 the Institute of Medicine (IOM) concluded in their report of "Dietary Reference Intakes for Calcium and Vitamin D" that s-25(OH)D levels of ≥50 nmol/L meet the requirement of ≥97.5% of the US population. The IOM Committee did not find higher s-25(OH)D levels (>75 nmol/L) to be consistently associated with increased benefit for bone health or other outcomes, and found risks to be identified for some outcomes at levels above 125 nmol/L (Ross et al., 2011).

The pragmatic use of >50 nmol/L to indicate sufficiency and <25 nmol/L to indicate deficiency is thus recommended for children (Braegger et al., 2013), and those values are used in the present thesis and paper.

Parathyroid hormone (PTH)

Some studies suggest that the threshold for vitamin D deficiency should be set on the basis of the relation between vitamin D status and PTH according to the theory that suppression of PTH is beneficial for bone health (Vieth, Chan, & MacFarlane, 2001). A physiologic increase in serum PTH concentrations has been observed in adolescents (Lamberg-Allardt & Viljakainen, 2008). Serum PTH concentrations in healthy children have been found to be lower and at a narrower range than in adults (Cioffi et al., 2000) but limited information is available about whether a plateau in PTH concentrations is evident in young children, as in older children and adults (Houghton et al., 2010). In a study on 4-8 year old children who had average vitamin D intakes below the dietary recommendations, supplementations with 25 μg/d vitamin D_3 for 8 weeks increased s-25(OH)D and decreased s-PTH levels significantly, suggesting that young children have responses in PTH concentration to vitamin D intake, similar to those in older children and adults (Abrams, Hawthorne, & Chen, 2013). Normal values for PTH concentration in actively growing children have not been defined, in part because the range of PTH concentrations that correlate with normal bone turnover in children has yet to be determined (Greer, 2009). An attempt to determine the inflection point of the relationship between s-25(OH)D and PTH for maximal suppression of PTH in children and adolescents in a large US cohort failed to find an inflection point, thus speculating if PTH suppression is an appropriate method for determining optimal s-25(OH)D for healthy children and adolescents (K. M. Hill et al., 2010). PTH values in children should be interpreted with caution.

Predictors of vitamin D status

Dietary intake

Vitamin D consumption only partly explains the variability in serum vitamin D, and studies have shown the correlation between consumption of vitamin D and serum vitamin D to be around r = 0.2-0.5 for children and adolescents 12-15 years of age (Sigurdsson et al., 1999). Sub-optimal levels (<50 nmol/L) of s-25(OH)D have recently been recorded during winter in pre-school children in northern Sweden (63°N) despite intake levels up to 7.5 μg/d (Ohlund, Silfverdal, Hernell, & Lind, 2012). However, dietary vitamin D was found to be a predictor of serum vitamin D in 6-12 year-old children during winter in Pittsburgh (40.4°N) in a cohort with mean daily consumption of vitamin D over the RDI (10 μg/d) (Rajakumar et al., 2011). A new Nordic study on healthy adults (19-48 years) in Norway also showed that supplementation with 10 μg/d of vitamin D over four weeks period during winter, either in the form of fish liver oil or multivitamin tablet, increased serum vitamin D (s-25(OH)D) considerably, not differing between supplement types (Holvik, Madar, Meyer, Lofthus, & Stene, 2007). That suggests that if children were following the recommended intake of 10 μg/d of vitamin D, they could probably maintain their serum vitamin D above sub-optimal levels during fall and winter. Less consumption of milk and vitamin D supplements and sedentary behavior (surrogating for less sun exposure) were the risk factors for low vitamin D status in the US NHANES 2001–2004 survey of 1–21 yr old children and adolescents (Kumar et al., 2009).

Season, latitude and sunlight exposure

Sunlight has long been recognized as a major provider of vitamin D for humans. Radiation in the UVB (290-315 nm) portion of the solar spectrum synthesizes previtamin D_3 in the skin, which is then converted by a thermal process to vitamin D_3 (Webb, Kline, & Holick, 1988). The determinant of the available UVB exposure is the solar zenith angle, varying between latitudes, seasons and time of day in the world (Webb, 2006; Webb et al., 1988).

In general, healthy young adults at latitudes north or south of the equator have a marked seasonal fluctuations in serum vitamin D, with lower concentrations during winter than summer (Gozdzik et al., 2010). Season was found to be one of the strongest determinants of vitamin D status in New Zealand children aged 5-14 years (Rockell et al., 2005), and more than 90% of the vitamin D requirement for most people has been shown to come from casual exposure to sunlight (M.F. Holick, 2003; M. F. Holick, 2004). The fear of skin cancer and sunburns has promoted sunscreen and sunblock use, especially on children. Data has shown that the correct usage of sunscreen reduces vitamin D levels by blocking the cutaneous absorption of UVB radiation (Fuller & Casparian, 2001; Matsuoka, Ide, Wortsman, MacLaughlin, & Holick, 1987). However, other studies have shown that this is not the case in practice, demonstrating that sunscreens are rarely applied correctly, in the right dosages and with appropriate frequencies, and therefore could result in production of vitamin D among those wearing sunscreen under normal usage terms (Burnett & Wang, 2011;

Norval & Wulf, 2009). A proper use of sunscreen of SPF 15 can reduce vitamin D synthesis by more then 98% (Quatrano & Dinulos, 2013), but frequent sunscreen use was not linked with vitamin D deficiency in the US NHANES 2003-2006. Sunscreen use is advised in Iceland by the Directorate of Health, with protection against both A and B rays and with SPF of at least 15, every two hours spent in the sun (S. Kristjansson, 2006).

Populations at high latitudes appear to be at high risk of low vitamin D status during winter (Dalgård et al., 2010; M.F. Holick, 2003), particularly in countries with minimal fortification practices (Kimlin, 2008). At Nordic latitudes the highest vitamin D level is reached in the late summer or early autumn, related to sunlight exposure during summer. During the late spring, summer and early autumn cutaneous vitamin D can be synthesized from sunlight exposure, and enough vitamin D is produced in the skin to be stored in the body fat (M. F. Holick, 2004). These vitamin D stores can then be mobilized during winter months when little or no vitamin D is synthesised and vitamin D status is dependent on reserve vitamin D stores and sufficient intake (M. F. Holick, 2004). Above latitudes 35-42°N exposure to sunlight during the winter months is not sufficient to initiate cutaneous production of vitamin D (Tsiaras & Weinstock, 2011; Webb et al., 1988). Researchers in Tromsø (69°N) concluded that cutaneous skin synthesis of vitamin D was not present from October-March and was peaking in June-July (Brustad et al., 2007). Similar time frame can be expected in Iceland given the similar latitudes (Reykjavík is at 64°N). Up to 30% of variation in serum vitamin D has been shown to be explained by sun exposure and vitamin D supplements in women (25-40 years) in Sweden (57-58°N), concluding that cutaneous vitamin D synthesis seems to be a major contributor to vitamin D status at nordic latitudes (Hedlund, Brembeck, & Olausson, 2013).

Cardiorespiratory fitness, gender and obesity

Several mechanisms for a correlation between cardiorespiratory fitness (CRF) (VO_2 – max) and s-25(OH)D have been proposed, but greater CRF has been associated with higher levels of s-25(OH)D in adolescents and adults (Ardestani et al., 2011; Mowry, Costello, & Heelan, 2009; Valtuena et al., 2013). Fitness might be a confounder in the association between body fat tissue and vitamin D (Wortsman, Matsuoka, Chen, Lu, & Holick, 2000), or might possibly act as a surrogate marker for increased outdoor activites and exposure to sunlight (Nilson et al., 2009; Rockell et al., 2005). It has also been proposed in 12-14 year old girls that vitamin D affects muscle contractility where those with low s-25(OH)D concentrations have been shown to generate less power than those with higher concentrations (Ward et al., 2009), suggesting that a lower CRF as a consequence of lower 25(OH)D could be one potential explanation for the positive association between CRF and 25(OH)D.

Several studies worldwide have reported higher vitamin D status in boys compared with girls (T. Hill et al., 2008; Kumar et al., 2009; Rockell et al., 2005), but these studies were only examining vitamin D status, irrelevant of intake. Data from the Northern Ireland Young Hearts 2000 study (54-55°N) found that the strongest predictors of vitamin D inadequacy during winter for children aged 12-15 years were low vitamin D intake and gender, where 46% of the children had sub-optimal levels of vitamin D during winter (38% boys, 55% girls) and vitamin D intake was generally very low (T. Hill et al., 2008). Gender and obesity have been shown to have an independent effect on serum vitamin D concentration, although of lesser magnitude than season or ethnicity. Physical inactivity has also been shown to be higher in girls than in boys, and higher in children who are obese rather than normal weight. It is possible that gender and obesity act as surrogate markers for sunlight exposure through their association with physical activity (Rockell et al., 2005).

Heritability of s-25(OH)D levels

Heritability of s-25(OH)D levels suggest that genetic determinants also play a role in regulating ciruculating s-25(OH)D. Studies have shown that the presence of certain deleterious genes at defined locations more than double the odds of vitamin D insufficiency in European descendents. Hence, only about a quarter of the inter-individual variability in serum vitamin D concentration can be considered to be attributable to season, latitude, or reported vitamin D intake (Wang et al., 2010). However, despite undisputable genetic influences on vitamin D status, the amount of variation explained by genetic determinants is considered small compared with environmental exposures and is only partially understood (Berry & Hypponen, 2011).

Autoimmune diseases and vitamin D

Vitamin D´s proposed role as a regulator of adaptive immunity has been closely studied in relation to autoimmune diseases such as multiple sclerosis and type 1 diabetes, where vitamin D supplementation has been shown to reduce the disease risk

(Harris, 2005; Hyppönen, Läärä, Reunanen, Järvelin, & Virtanen, 2001; Munger, Levin, Hollis, Howard, & Ascherio, 2006; Papandreou, Karabouta, & Rousso, 2010) up to 80% (Hyppönen et al., 2001), which is more prevalent at higher latitudes (Ponsonby, Lucas, & van der Mei, 2005; Ponsonby, McMichael, & van der Mei, 2002). Epidemiological studies also hint a possible protective effect of vitamin D (Mohr, Garland, Gorham, & Garland, 2008; Zittermann, 2003). Studies have associated fish liver oil intake during the first year of life to lower risk of type 1 diabetes (Stene & Joner, 2003) and studies of diabetic children have shown that the majority of them have sub-optimal vitamin D levels (Svoren, Volkening, Wood, & Laffel, 2009), hinting a causal connection between the two. However, according to the draft proposal of the 2012 Nordic Nutrition Recommendations the evidence is still found to be lacking in randomized clinical trials, and is thus limited and inconclusive in demonstrating a causal relationship or an association between vitamin D and diabetes (Lamberg-Allardt, Brustad, Meyer, & Steingrimsdottir, 2013).

Main present study: 7-year-old children 2006

The present data was obtained from an intervention study on the lifestyle of 7-9 year old children performed by advanced students from Educational and Health sciences at the University of Iceland in 2006-2008. The project aimed to increase the health of children, to increase physical activity, influence their diet and educate children, parents and teachers on healthy diets and the FBDG provided by the Directorate of Health. The intervention was performed in six schools in the Reykjavík area, three intervention schools and three reference schools. This group of children has been studied in a variety of papers regarding physical activity (K. T. Magnusson, Hrafnkelsson, Sigurgeirsson, Johannsson, & Sveinsson, 2012; K. T. Magnusson, Sigurgeirsson, Sveinsson, & Johannsson, 2011), bone mineral density (Hrafnkelsson, Sigrudsson, Magnusson, Johannsson, & Sigurdsson, 2010; Hrafnkelsson, Sigurdsson, Magnusson, Sigurdsson, & Johannsson, 2013), cardiovascular disease risk (Hrafnkelsson, Magnusson, Sigurdsson, & Johannsson, 2009) and dietary intake (Kristjansdottir, Johannsson, & Thorsdottir, 2010; Kristjansdottir & Thorsdottir, 2009). The project was performed on the need for an intervention in children, based on the results of an observational study on 9-15 year old Icelandic children performed in 2003-2004 on lifestyle related factors, e.g. diet, physical activity, weight and fitness (K. T. Magnusson, Arngrimsson, Sveinsson, & Johannsson, 2011; Thorsdottir &

Gunnarsdottir, 2006). In the present thesis, including the manuscript, only the baseline values from all six schools were used. They should give a good example of the vitamin D status and dietary intake of vitamin D in 7-year-old Icelandic children.

Icelandic Infant Nutrition Prospective Cohort: Intake at 6 years of age 2011. Comparison to present cohort

The participants were 6-year-old children who had earlier participated in a longitudinal study on the diet and health of Icelandic infants. Infants born in 2005 were randomly chosen from the National Registry by Statistics Iceland, four times that year. At each randomization the children were within 4 months old. The participation requirements were met by 250 children, but the requirements were Icelandic parents, single birth, gestational age 37-41 weeks, birth weight between the 10^{th} and 90^{th} percentage, no birth defects or congenital diseases and that the mother had antenatal care. In total 219 children finished at least one part of the investigation at 12 months of age and they were invited to participate in a follow up at 6 years of age. 162 children returned adequate food records at 6 years of age (Gunnarsdottir et al., 2012). This group of children has been studied in a variety of papers regarding early nutrition (Thorisdottir, Gunnarsdottir, & Thorsdottir, 2013), iron status in infancy (Thorisdottir, Ramel, Palsson, Tomassson, & Thorsdottir, 2013; Thorisdottir, Thorsdottir, & Palsson, 2011), iron status and developmental scores at 6 years of age (Thorisdottir, Gunnarsdottir, Palsson, Gretarsson, & Thorsdottir, 2013) and growth development and associations to diet and intake at 6 years of age (B. Thorisdottir et al., 2013).

Summary

The vitamin D status of Icelandic children is of great relevance, given the low intake of vitamin D and the lack of adherence to the FBDG regarding fish and fish liver oil. Fish liver oil has been the main provider of vitamin D in the Icelandic population for decades, but the majority of children in Iceland do not take in fish liver oil (>50%). Children worldwide have been reported to have sub-optimal levels of vitamin D during autumn and winter, especially at nordic latitudes. The effects of vitamin D deficiency on bone health is established, and the evidence also hint an association of poor vitamin D status during childhood to chronic diseases like cancer, type 1 diabetes and multiple sclerosis. Dietary intake, season, latitude, sun exposure, cardiorespiratory fitness (CRF) and gender have previously all been shown to be predictors of serum vitamin D status. Our aim of investigating the vitamin D intake and status of 7-year-old Icelandic children, to explore gender differences and associations of vitamin D status with total vitamin D intake, fish and fish liver oil intake and CRF is therefore relevant to the literature. Furthermore it is important to compare the diet of 7-year-old children from 2006 to the diet of 6-year-old children in 2011 to estimate if our results are still relevant to children in Iceland.

Methods

The methods concerning the first two aims are described in detail in the manuscript.

Statistical analysis of the additional results on intake of 7-year-olds and comparison between cohorts

As the intake variables were not normally distributed, the values presented in Table 1 and 2 are median and interquartile range (IQR). Statistical difference between supplemented and not supplemented groups was tested with the Mann-Whitney U-test. Statistical testing was done with Chi^2-test for grouping by blood values (deficiency, sub-optimal and sufficiency), gender and adherence to the FBDG. The independent T-test was used for comparing height, weight and BMI between gender.

Statistical testing between grouping by blood values of the 7-year-olds (deficiency, sub-optimal and sufficiency) and the macronutrient- and fiber distribution and vitamin D intake was done with one-way ANOVA. Gender differences were determined by Mann-Whitney U-test for macronutrient, fiber and added sugar.

The data on the diet of 6-year-old children were obtained from a published article; „Diet of six-year-old Icelandic children – National dietary survey 2011-2012", from the Icelandic Medical Journal (Gunnarsdottir et al., 2012). The adjusted FBDG were obtained from the same article.

The dietary intake and adherence to the FBDG were compared between our 7-year-old children who both returned valid 3-day records and had blood tested (n=120) in 2006, and a cohort of 6-year-old Icelandic children who returned valid food records in a National Dietary Survey in 2011 (n=162), to see if the results on the 7-year old children were still relevant.

Comparison between 2006 and 2011 for mean vitamin D-, total energy- and added sugar intake, were calculated with an independent t-test between means.

Levels of ≤50 nmol/L are used to indicate sub-optimal levels and <25 nmol/L to indicate deficiency levels in the present thesis and manuscript.

Author contribution

From February 2012 to June 2012 I reviewed the relevant literature, reviewed the data, did the statistical analysis and drafted the first version of the manuscript. I had maternity leave for six months.

From January 2013 to May 2013 I continued the work and writing of the manuscript and analysing the data and then submitted the manuscript for publication.

From May 2013 to August 2013 I wrote the thesis.

From August 2013 to November 2013 I revised the manuscript and thesis, and on December 6th, the manuscript was accepted for publication in the Public Health Nutrition journal.

I wrote the the final draft of the manuscript before a thorough review and addition by co-authors, as well as the thesis, under supervision from Ása Guðrún Kristjánsdóttir and Inga Þórsdóttir, which supervised my masters program.

Manuscript

Insufficient autumn vitamin-D intake and low vitamin-D status in 7-year-old Icelandic children.

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Insufficient autumn vitamin-D intake and low vitamin-D status in 7-year-old Icelandic children

Short title: Low vitamin-D intake and status in children.

Key words: Vitamin-D, 25(OH)D, children, sub-optimal levels, deficiency

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Ethics of Human Subject Participation: This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the National Bioethics Committee (VSN b2006050002/03) as well as the Icelandic Data Protection Commission. Informed consent was obtained from all subjects.

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Conflict of Interest: None.

Authorship: Bjarnadottir is a nutrition student who explored the data for associations between intake, fitness and status and wrote the first draft of the paper. Kristjansdottir, Hrafnkelsson and Magnusson were all PhD students when the original data was collected and have participated in the analysis and interpretation of the data. Johannsson is principal investigator and administered the collection of data and Thorsdottir is co-ordinating the nutritional studies in the project. All authors have participated in writing the manuscript.

Abstract

Objective:

The aim was to investigate autumn vitamin-D intake and status in 7-year-old Icelanders, fitting BMI and cardiorespiratory fitness (CRF) as predictors.

Design:

Three-day-food records and fasting blood samples were collected evenly from September-November, and CRF measured with an ergometer bike. Food and nutrient intake were calculated and serum (s)-25(OH)D and parathyroid hormone (PTH) analyzed. Sub-optimal vitamin-D status was defined <50 nmol/L, and deficient status <25 nmol/L.

Setting:

School based study in Reykjavík, Iceland in 2006.

Subjects:

7-year-olds (n=265), 165 returned valid intake information (62%), 158 gave blood samples (60%), 120 gave both (45%).

Results:

Recommended vitamin-D intake (10 μg/d) was reached by 22.4% of the children and 65.2% had s-25(OH)D status <50 nmol/L. Median s-25(OH)D were higher for children taking vitamin-D supplements, 49.2 nmol/L vs. 43.2 nmol/L respectively (P<0.001). Median S-25(OH)D was lower in November (36.7 nmol/L) than in September (59.9 nmol/L) (P<0.001). Regression model showed that week of autumn accounted for 18.7% of the variance in s-25(OH)D (P<0.001), vitamin-D intake 5.2% (P<0.004) and CRF 4.6% (P<0.005).

Conclusions:

Minority of children followed vitamin-D recommendations and 65% had sub-optimal serum vitamin-D during the autumn. Week of autumn was more strongly associated with vitamin-D status than diet or CRF, which associated with vitamin-D status to a similar extent. These results demonstrate the importance of sunlight exposure during summer and to prevent sub-optimal vitamin-D status in young schoolchildren during autumn in northern countries. An increased effort is needed for enabling adherence to the vitamin-D recommendations and increasing outdoor activities for sunlight exposure.

Introduction

Vitamin-D has attracted a lot of interest as new knowledge regarding its non-hormonal, intracrine, paracrine and immunological actions have been revealed. It indicates that sub-optimal levels of vitamin-D may have an impact on our health beyond bone homeostasis [1; 2; 3] and may possibly play a role in the onset of diseases such as diabetes type 1 [4; 5]. Current studies from various countries have documented a high prevalence of sub-optimal status of vitamin-D among children, adolescents and young adults [6; 7; 8]. The main sources of vitamin-D come from exposure of the skin to sunlight [9] as well as vitamin-D containing foods [10] such as fish liver oil [11; 12].

Few studies have explored the correlation between vitamin-D intake and serum vitamin-D concentrations in children. The majority of studies are carried out in adolescents and adults. However a recent study on pre-school children in northern Sweden (63°N) showed that 40% of children had sub-optimal serum vitamin-D (s-25(OH)D) (<50 nmol/L) during winter, despite intake of vitamin-D in accordance with the Nordic and Swedish nutrition recommendations (7.5 μg/d) [13]. Season, ethnicity, fortified foods, body mass index (BMI) and physical activity levels have been shown to be independent predictors of vitamin-D insufficiency in adolescents [14].

Populations at high latitudes appear to be at high risk of low vitamin-D status especially during winter [15; 16]. The recommended dietary intake of vitamin-D for Icelandic children and adults up to 60 years of age is 10 μg/d, equivalent to 5 ml of cod liver oil [17], which has been the main dietary source of vitamin-D in the country for decades, but no tradition is for vitamin-D fortification of foods. Recently food habits among adults have changed at the cost of both fish- and fish liver oil intake [11]. Fish liver oil intake used to be mandatory for children in Iceland and was given in schools until around 1970 [18]. Fish liver oil is still given in kindergartens with breakfast, but given the recent changes in dietary intake among adults it could be questioned if the vitamin-D status of Icelandic schoolchildren is sufficient.

The variability in the density of vitamin-D in the blood is only partly explained by vitamin-D consumption [19]. It has been stated that s-25(OH)D levels are up to 24% lower during winter compared to summer and they are inversely correlated with parathyroid hormone (PTH) levels in 14 year old children, but the association between s-25(OH)D and PTH seem to be dependent of age, increasing with increasing age [14]. Higher s-25(OH)D concentrations have been reported in boys compared to girls both during childhood and adolescence (1-21 yrs) although the reason is unclear [6; 7; 8]. The influence of physical activity or fitness as predictors of vitamin-D status in children is virtually unknown. In children sedentary behavior seems to be a risk factor for low vitamin-D status, due to lower outdoor activities and sunlight exposure [8]. Cardiorespiratory fitness might act as a marker for outdoor activities and sunlight exposure. Body Mass Index (BMI) has been shown to associate adversely with vitamin-D status, likely because of decreased bioavailability of vitamin-D and its deposition in body fat [20]. We hypothesize that vitamin-D intake and CRF are positively associated with s-25(OH)D and BMI negatively, and that there is no gender difference in s-25(OH)D.

The aim was to investigate vitamin-D intake and status in 7-year-olds in Reykjavík (64°N) in September until November. A secondary aim was to assess associations of s-25(OH)D with total vitamin-D intake, as well as with the locally specific vitamin-D sources, physical variables like cardiorespiratory fitness (CRF) and BMI, and to explore possible gender differences.

Methods

Sample

The study sample was obtained from an intervention study on the lifestyle of 7-9 year old children aiming to increase physical activity and promote healthy diet in 2006-2008. The present study uses the children's baseline values. The children invited came from six randomly selected schools in the Reykjavik area in Iceland 2006 [21]. All children in the second grade (born 1999) in the schools were invited to participate (n=265) and had height and weight measured. Written consent of both parent and child had to be obtained for participation in the study. Of the 265 children invited to participate 187 (70.6%) returned complete three-day food records (FR). After excluding underreports (n=22), 165 (62.3%) valid FRs were included in the data analysis [21] (Figure 1). Due to a lower participation rate and a few unsuccessful attempts at collecting blood specimens, a total of 158 (59.6%) children had usable blood samples taken. There were no significant differences in BMI or ethnicity between the children participating and those not participating in the study. A total of 120 (45.3%) participants both returned valid FRs and had blood samples taken, representing about 2.7% of all children in the second grade in Iceland in 2006. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the National Bioethics Committee (VSN b2006050002/03) as well as the Icelandic Data Protection Commission. Informed consent was obtained from all subjects.

Dietary assessment methods

The dietary assessment method used was a 3-day food record (FR). The FRs were done over two weekdays and one weekend day. Data was collected evenly from September until the end of November [21; 22]. Parental meetings were held at the time of measurements where instructions on how to record the diet were given. The parents weighed all food items for each child, except for the school meal, which was recorded for each child by a trained nutritionist [22]. Under-reports were excluded and not used in the calculations (energy intake < 1.2 (estimated BMR)). Nutrient calculations were performed with ICEFOOD, using the Icelandic Nutrient Database as well as the Icelandic Nutrition Council Recipe database 2002. All data on food and drink as well as fish liver oil and supplement consumption was included in the data analysis [22]. Total intake of vitamin-D was calculated from all food and supplement intake [21]. Among the children with s-25(OH)D concentrations higher than 50 nmol/L there were two children who had consumed a non-specified multivitamin, which may indicate that the multivitamin included vitamin-D.

Biochemical measurements

Blood samples were collected evenly at each school from the beginning of September until the end of November. Fasting blood samples were obtained using standard procedures after overnight fasting and serum samples were frozen immediately and kept at -80°C until analysis. The serum vitamin-D (25(OH)D) was measured with DiaSorin 25OHD RIA assay (DiaSorin, Stillwater, MN, USA) and PTH was measured with ECLIA (ElectroChemiLuminescence Immuno Assay Elecsys 2010, Roche Diagnostics, USA) [23]. Sub-optimal levels of vitamin-D were defined as <50 nmol/L, and deficiency < 25 nmol/L [24].

Fitness measurement

Cardiorespiratory fitness (CRF) was measured with a Monark ergometer bike with the main outcome as maximum watts/kg/person (w/kg). The study protocol from the European Youth Heart Study was followed when this test was administered [25]. This maximal fitness test was run such that every three minutes the weight on the front wheel was increased by 20-25 watts, depending on the participant's weight. Each participant had to keep a steady pace during the test of about 60 rpm and bike either until exhaustion or until no longer being able keep up the desired pace. A trained individual performed all CRF assessments. The same verbal encouragement protocol was used on all participants to motivate them during the test.

Statistical analyses

The statistical analyses were performed using Statistical Package for Social Sciences (SPSS) statistical software package version 20 for Windows (SPSS Inc., Chicaco, II, USA). Distribution assessment was performed on the outcome variables by means of histograms and QQ-plots. The distribution of intake variables were skewed, hence the Mann Whitney U-test was used when comparing gender differences for intake variables and week of autumn. S-25(OH)D was normally distributed, and the independent t-test was used when comparing continuous parametric variables between gender (s-25(OH)D height, weight, BMI, PTH and CRF). The Chi^2-test was used to assess the relationship between sub-optimacy and deficiency of s-25(OH)D, and supplement intake. The variables were presented as median and interquartile range (IQR) in Table 1. Spearman's correlation analysis was used to assess correlations between s-25(OH)D and week number in the autumn, intake variables and physical variables. The level of significance used in all analyses was $P \leq 0.05$. Simple linear regression and multiple linear regression analyses were performed to model the association between the primary dependent variable (s-25(OH)D) and independent variables (week of autumn, CRF and vitamin-D intake), which had the strongest correlations with s-25(OH)D of the variables tested.

Results

Diet and evaluation of serum Vitamin-D

The recommended intake of vitamin-D (10 μg/d) was reached by 37 children (22.4%) and the recommended intake of fish liver oil (5 ml/d) was reached by 21 children (12.7%). Sub-optimal vitamin-D status was present in 103 children (65.2%), thereof 5 children (3%) had deficient status (<25 nmol/L). In total 56 of the children giving blood samples were taking fish liver oil or other vitamin-D supplements. Of the group of 56 children 28 children reached optimal vitamin-D status (≥50 nmol/L) with a median fish liver oil intake of 4 ml/d, and median total vitamin-D intake of 9.6 μg/d. Of the same group 28 children had sub-optimal vitamin-D status (<50 nmol/L) with a median fish liver oil intake of 2.7 ml/d, and 8.1 μg/d of vitamin-D, and thereof was one child with deficient vitamin-D status (<25 nmol/L) which had an intake of 1.7 ml/d of fish liver oil and 4.2 μg/d of vitamin-D.

Table 1 shows a significant gender difference for vitamin-D intake and fish liver oil intake, where boys had higher intakes in both cases (P=0.003 and P=0.03 respectively). The median s-25(OH)D for the children was 45.1 nmol/L, with no significant gender differences.

No gender difference was found in the frequency of sub-optimal status of vitamin-D (<50 nmol/L) or vitamin-D deficiency (<25 nmol/L) (P=0.68) (Table 2), despite the different intake levels of vitamin-D. However there was a significant difference between the frequency of sub-optimal status of vitamin-D and vitamin-D deficiency between the groups taking and not taking fish liver oil or other vitamin-D supplements (P=0.02), irrespective of gender.

Seasonal and dietary influences on s-25(OH)D

Figure 2 shows the effect of week in autumn on s-25(OH)D during the sampling period from early September to late November (week 36-46). The s-25(OH)D ranged from 17.4 – 94.8 nmol/L. The s-25(OH)D levels were significantly lower in November (36.7 nmol/L) than in September (59.9 nmol/L) (P=0.001). Median values were below 50 nmol/L for both supplemented children and non-supplemented children in November (figure not shown). The median s-25(OH)D for the vitamin-D supplemented children was higher than for those not supplemented 49.2 nmol/L and 43.4 nmol/L respectively (P=0.009) (figure 3). The median intake of vitamin-D for the supplemented group was 8.8 μg/d, vs. 2.0 μg/d for the non-supplemented group (figure not shown). The correlation between s-25(OH)D (nmol/L) and s-PTH (nmol/L) was $R^2 = 0.047$ (Spearman's ρ=-0.159, P=0.05) (figure not shown).

Selected predictors associated with serum Vitamin-D

Table 3 shows that s-25(OH)D was negatively correlated with week of autumn, BMI and PTH, but positively correlated with intake of fish liver oil, intake of vitamin-D and CRF. The correlations were dependent on gender for the following; BMI and s-PTH were only significant in boys.

The stepwise linear regression model shows that week of autumn accounted for 18.7% of the variation in s-25(OH)D (P<0.001) (Table 4). Vitamin-D intake accounted for extra 5.2% (P<0.004) and CRF 4.6% (P<0.005), in total explaining 28.7% of the variance in s-25(OH)D. Gender and BMI did not have a significant effect on the model (P=0.266 and P=0.981, respectively).

Discussion

The present study shows that a minority of 7 year-old children were following the dietary guidelines for fish liver oil or vitamin-D supplement intake in autumn, despite a rich tradition in Iceland for fish liver oil intake. Only 42.5% of the children took fish liver oil or other vitamin-D supplements some of the recording days and for those children, only 50% reached optimal serum vitamin-D status (>50 nmol/L). Among the children that did not take vitamin-D supplements, only 30% reached optimal serum vitamin-D status in autumn. These results indicate that those autumn weeks have a great impact on serum vitamin-D status in children, as the median values had decreased to below 50 nmol/L in both the supplemented and the non-supplemented groups at the end of the measurement period. Sunlight exposure and outdoor activities during summer and early autumn are important to increase vitamin-D synthesis and status, and affect serum vitamin-D concentrations during autumn and winter.

The strongest correlations with vitamin-D status in the present study were intake of vitamin-D, week of autumn and CRF, but BMI was not significantly correlated with vitamin-D status. Studies have shown the correlation between consumption of vitamin-D and vitamin-D status to be about r = 0.20-0.54 for children over 12 years of age, which suggests that the variability in vitamin-D status in the blood is only partly explained by vitamin-D consumption [19]. Furthermore, only about a quarter of the inter-individual variability in serum vitamin-D concentration is considered to be attributable to season, latitude, or reported vitamin-D intake [26]. Data from the Northern Ireland Young Hearts 2000 study found that the strongest predictors of vitamin-D inadequacy during winter for children aged 12-15 years were low vitamin-D intake and gender [7], which is not in line with our results as vitamin-D status did not differ between gender in present study during the autumn season, despite different intake levels. Vitamin-D intake might play a larger role in increasing serum vitamin-D during the winter season than in the autumn. Season was found to be one of the strongest determinants of vitamin-D status in New Zealand children aged 5-14 years [6]. The interesting gender difference in diet observed in the present study is similar to former results and attributed to different food choices [27; 28; 29; 30]. In the present cohort boys had higher protein E% and the gender difference in vitamin-D intake is attributed to more vitamin-D density in protein rich foods like fish liver oil, fish and eggs. Since the blood samples from boys and girls, respectively, were equally distributed over the sampling weeks and there was no confounding by BMI between gender these factors do not explain why there was no gender difference in s-25(OH)D despite different intake levels. This suggests that girls get more sun exposure, perhaps attributed to different clothing during summer (tank tops vs. t-shirts, and skirts vs. pants). However, exclusion of different vitamin-D metabolism in the genders or a stronger negative influence of BMI in boys, as indicated in Table 3, is not possible.

The Icelandic Food Based Dietary Guidelines (FBDG) state that fish liver oil or another vitamin-D supplement should be used, especially during the winter. The recommended dietary intake of vitamin-D for children and adults up to 60 years of age is 10 µg/d, equivalent to 5 ml of cod liver oil [17; 29]. Only a minority of the children reached the recommendations for fish liver oil intake (12.7 %) or vitamin-D intake (22.4 %) and about 50% of children who took fish liver oil or other vitamin-D supplements in present study did neither reach the optimal level of vitamin-D in their blood (≥50 nmol/L), nor the recommended intake of vitamin-D (10 μg/d). These findings relate to the vitamin-D intake and status in the adult population in Iceland, where only approximately 17% and 30% of women and men, respectively, reach the RDI for vitamin-D, and 21% take fish liver oil daily according to the most recent National Dietary Survey conducted in 2010 [11]. This as well as results from studies on vitamin D intake among Icelandic children [12; 30] support that the present study sample taken from randomly chosen schools in Reykjavik is representative for the population of 7-year-olds in Iceland. Since the median vitamin-D value is not far from the reference value (50 nmol/L), emphasizing nutrition education campaigns, increasing adherence to the vitamin-D recommendations and promoting outdoor activities for sunlight exposure could elevate values to more optimal levels. The vitamin-D status decreased rapidly in the autumn weeks, and it can be assumed from Figure 2 that they could decrease even further before spring (April/May). A new Nordic study on healthy adults (19-48 years) in Norway showed that supplementation with 10 μg/d of vitamin-D over a four weeks period during winter, either in the form of fish liver oil or multivitamin tablet, increased serum vitamin-D (s-25(OH)D) by a mean of 34.1 nmol/L (SD 13.1) [31]. This suggests that if the children were following the recommended intake of 10 μg/d of vitamin-D, they would probably have better maintained their serum vitamin-D status.

In an Icelandic cohort investigating vitamin-D status of infants, 2 year olds and 6 year olds, the children who did not take fish liver oil or other sources of vitamin-D were within half of the RDI (2-3 μg/d) while those who consumed fish liver oil on a daily basis reached the RDI for vitamin-D (9.5 μg/d for the 2 year olds and 12.3 μg/d for the 6 year olds) [32]. In general, vitamin-D intake is low in Europe (≤ 2–3 μg/d) [33] and more than one-third of adolescent girls (12 years) in Northern Europe had vitamin-D status below 25 nmol/L and almost all were below 50 nmol/L during winter [34]. Vitamin-D status in European children has been the focus of several investigations, and high prevalence of vitamin-D deficiency has been reported during winter, especially above the 51.9° N latitude [35]. Latitude has shown to be a well-established risk factor for sub-optimal levels of vitamin-D, particularly in countries with minimal fortification practices [36]. At Nordic latitudes the highest vitamin-D concentration is reached in the late summer or early autumn, related to sunlight exposure during summer. Children with more sunlight exposure during summer should have higher serum vitamin-D, and better maintain their serum vitamin-D during autumn and winter where little or no vitamin-D is synthesized and vitamin-D status is dependent on reserve vitamin-D stores and sufficient vitamin-D intake. The ideal daily dose of vitamin-D has been studied in detail in the past years, but a consensus has not been reached [37].

Several mechanisms for a correlation between fitness and vitamin-D status have been proposed. One being that fitness may be a confounder in the association between body fat tissue and vitamin-D [20]. Fitness may also be associated with vitamin-D concentrations because greater fitness is likely linked to increased outdoors activities [38] and thus exposure to sunlight [6], as predicted in the present study. It should however also be mentioned that girls (12-14 years) with low s-25(OH)D have been shown to generate less power, jump less height and have lower velocity than girls with higher s-25(OH)D concentrations, measured as "Esslinger Fitness Index" which represents efficiency and asymmetry of movement, and maximum voluntary force of each leg [39], suggesting that a lower CRF as a consequence of lower 25(OH)D could be one potential explanation for the positive association between CRF and 25(OH)D. Several studies worldwide have reported higher vitamin-D status in boys compared with girls, although the reason is unclear [6; 7; 8], but these studies were only examining vitamin-D status, irrelevant of intake. In the present study boys had higher fitness and higher intakes of vitamin-D than girls, but there was no gender difference in serum vitamin-D status. That might suggest that girls have similar outdoors activities or sun exposure as boys in Iceland, irrespective of fitness.

The correlation between vitamin-D status and PTH was significant, but weak, in the present study. Some studies suggest that the threshold for vitamin-D deficiency should be set on the basis of the relation between vitamin-D status and PTH according to the theory that suppression of PTH is beneficial for bone health [40]. However, limited information is available about whether a plateau in PTH concentrations is evident in young children, as in older children and adults [41]. A physiologic increase in serum PTH concentrations has been observed in adolescents [42] and Cioffi et al found that serum PTH concentrations in healthy children were lower and had a narrower range than in adults [43]. Normal values for PTH concentration in actively growing children have not been defined, in part because the range of PTH concentrations that correlate with normal bone turnover in children has yet to be determined [44].

The limitations of the study may be related to the method used to measure serum vitamin-D or the method used to assess vitamin-D intake. The method used to measure s-25(OH)D was DiaSorin RIA, which has decreased in popularity since 2001 [45], mainly because it may underestimate 25(OH)D compared to the HPLC method [46; 47]. It has also been shown to give lower values than LC-MS/MS [48]. Some experiments suggest that the method does not equally recognize 25(OH)D_2 and 25(OH)D_3, but the method only gives one 25(OH)D value [45]. The values might therefore have been different if other methods had been used, and the proportions for sub-optimacy and deficiency could have been different. The method used to measure vitamin-D intake was FRs. FR´s have widely been used as a dietary assessment tool and as a reference method to validate other dietary assessment methods. However, due to day-to-day and seasonal variations they can be misleading, perhaps omitting some food groups that are rarely consumed. Intake levels of fish liver oil or vitamin-D intake is based on evaluated amounts from spoons, beads, tablets and sips which is not precise enough to draw conclusions about exact intake. It may therefore be questioned if this method is valid to evaluate the diet of children. However a recent systematic review concluded that 3 day food records that includes weekdays and weekend days, with parents as co-reporters, is the most accurate method to estimate total energy intake in children aged 4-11 years old [(49)]. Another limitation is that there is no information on sunlight exposure or outdoor activities during summer and early autumn. Week of autumn and CRF were positively correlated with serum vitamin-D as discussed, which may be partly related to outdoor activities and sunlight exposure, but other mechanisms may also be involved.

Conclusions

This study concludes that only a minority of 7 year-old children (22.4%) was following the vitamin-D intake recommendations during autumn in Iceland. About 65% of the children had blood values of vitamin-D below 50 nmol/L (sub-optimal levels). The predictors for vitamin-D status were week of autumn, total vitamin-D intake and cardiorespiratory fitness (CRF). Week of autumn seemed to have more effect on vitamin-D status than diet or CRF, which demonstrate the importance of sunlight exposure during summer. There was a poor correlation between serum vitamin-D and PTH. It is suggested that adherence to the advice on one hour physical activity daily could improve the vitamin-D status through increased outdoor activities and sunlight exposure. To prevent sub-optimal vitamin-D status in young schoolchildren during autumn in northern countries an increased effort is needed for enabling adherence to the vitamin-D recommendations and promoting the importance of outdoor activities for sunlight exposure.

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Table 1. Median and interquartile range (IQR) serum 25(OH)D concentrations (nmol/L), intake of Vitamin-D (µg/d) and fish liver oil (ml/d) in 7-year-old Icelandic girls and boys.

			n	Median	IQR	P-value[1]
Serum Vitamin-D (nmol/L)	All participants	total	158	45.1	17.8	0.52
		girls	85	44.2	17.9	
		boys	73	46.9	17.8	
Vitamin-D intake (µg/d)	All participants	total	165	3.9	7.5	0.003
		girls	89	2.9	6.0	
		boys	77	6.2	9.9	
	Those taking fish liver oil or other vitamin-D supplementation	total	73	9.0	7.8	0.02
		girls	33	8.0	6.3	
		boys	40	11.0	9.0	
Fish liver oil intake (ml/d)	All participants	total	165	0	2.7	0.03
		girls	89	0	1.3	
		boys	76	0	3.3	
	Those taking fish liver oil	total	67	3.3	3.3	0.03
		girls	31	2.7	2.7	
		boys	36	3.7	3.4	

[1]Variables were tested for gender differences by using the Mann Whitney U-test except serum vitamin-D which was tested using the independent t-test.

Table 2. Frequency of sub-optimal levels of vitamin-D (<50 nmol/L) and vitamin-D deficiency (<25 nmol/L) for the children in total (all blood values, n=158), and the groups taking and not taking fish liver oil or other vitamin-D supplements. The children presented in the table returned valid 3-day food records and had blood sampled (n=120).

		All blood samples (n=158)	Food records (n=120)[1]	
			Take fish liver oil or other vitamin-D supplement (n=51)	Do not take fish liver oil or other vitamin-D supplement (n=69)
		n (%)	n (%)	n (%)
Sub-optimal levels of vitamin-D (< 50 nmol/L)		103 (65)	25 (49)	49 (71)
	Girls	58 (68)	12 (55)	30 (73)
	Boys	45 (62)	13 (45)	19 (68)
Thereof: Vitamin-D deficiency (< 25 nmol/L)		5 (3)	1 (2)	3 (4)
	Girls	3 (3)	0	2 (5)
	Boys	2 (3)	1 (3)	1 (4)

Significance values were computed with Chi^2-tests. There was no gender difference in sub-optimal levels or vitamin-D deficiency for all samples (P=0,68). There was a significant difference in sub-optimal levels and vitamin-D deficiency between taking and not taking fish liver oil or other vitamin-D supplements (P=0.02) irrelevant of gender.

[1]A total of 63 girls and 57 boys both returned 3 day food records and had blood tested (n=120).

Table 3. Correlation coefficients between serum s-25(OH)D (nmol/L) and seasonal variable (n=158), physical variables (n=158) and intake variables (n=120). P-values are shown for gender differences.

		Spearman's Correlation (r)	P-value (2-tailed)[1]
Seasonal variable			
Week of autumn (36-46)	Total	-0.50**	
	Girls	-0.57**	
	Boys	-0.40**	0.41
Physical variables			
Height (cm)	Total	-0.06	
	Girls	-0.12	
	Boys	-0.02	0.25
Weight (kg)	Total	-0.12	
	Girls	-0.11	
	Boys	-0.18	0.59
BMI (kg/m^2)	Total	-0.20*	
	Girls	-0.11	
	Boys	-0.27*	0.72
Cardio respiratory fitness (w/kg)	Total	0.34**	
	Girls	0.36**	
	Boys	0.29**	<0.001
Serum PTH (nmol/L)	Total	-0.16*	
	Girls	-0.06	
	Boys	-0.25*	<0.01
Intake variables			
Intake of fish liver oil (ml/d)	Total	0.28**	
	Girls	0.32**	
	Boys	0.27*	0.03
Intake of vitamin-D (µg/d)	Total	0.30**	
	Girls	0.27*	
	Boys	0.32**	<0.01

[1]Gender differences were computed with Mann Whitney U-test for week of autumn, BMI, vitamin-D intake and fish liver oil intake, and with the independent t-test for height, weight and cardiorespiratory fitness.

*Correlations are significant at the P=0.05 level

**Correlations are significant at the P=0.01 level

Table 4. Stepwise linear regression model explaining the variance in s-25(OH)D (nmol/L) dependent on background, seasonal, intake and physical variables based on the food records (n=120).

	Step 1		Step 2		Step 3		Step 4	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Background variable								
Gender	0.103	0.266	0.06	0.473	-0.008	0.926	-0.074	0.386
Seasonal variable								
Week of autumn*			-0.441	<0.001	-0.415	<0.001	-0.389	<0.001
Intake variable								
Vitamin-D intake (mg/d)					0.251	0.004	0.231	0.006
Physical variables								
BMI (kg/m^2)							-0.002	0.981
Cardiorespiratory fitness (w/kg)**							0.252	0.005
Adjusted R'2	0.002		0.189		0.241		0.287	

*Week 36-46

**Marker for outdoor activities or sunlight exposure

Figure 1. Flowchart showing the participation rate of the study.

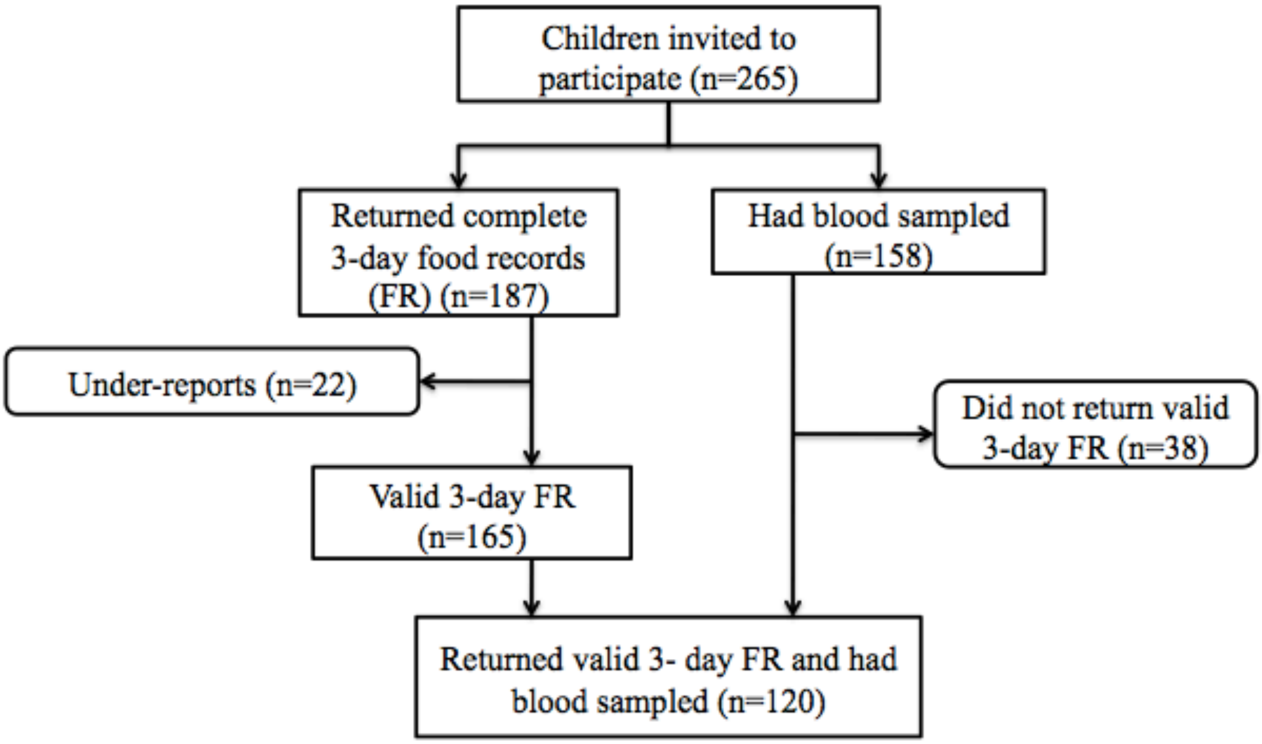
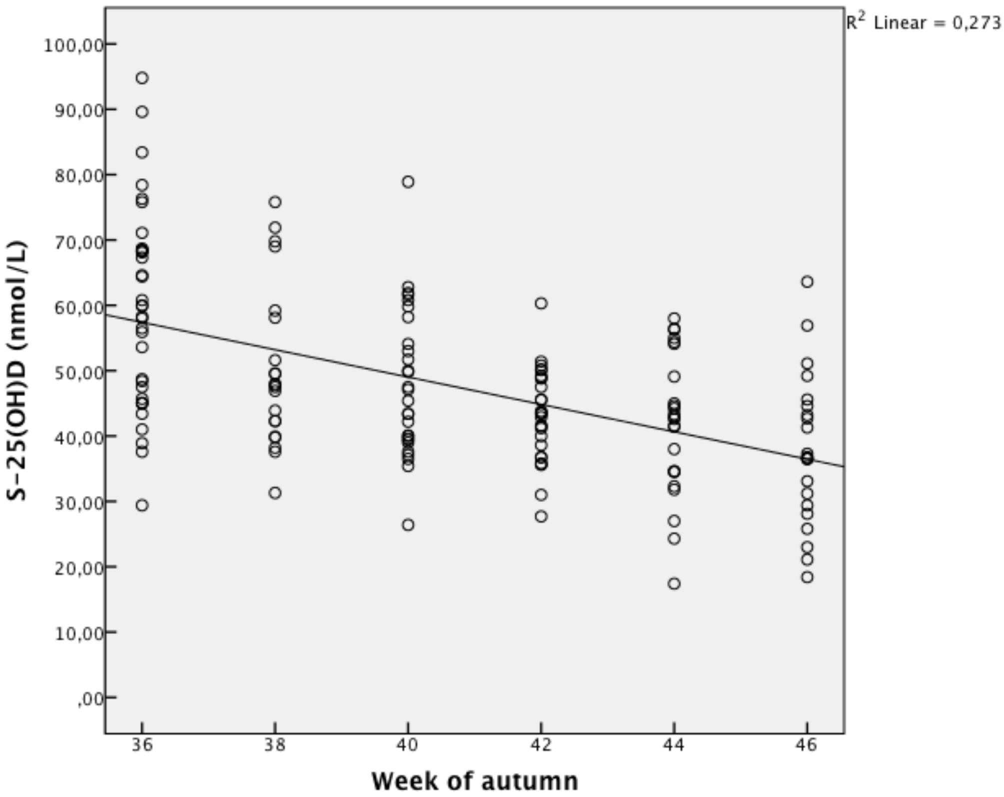
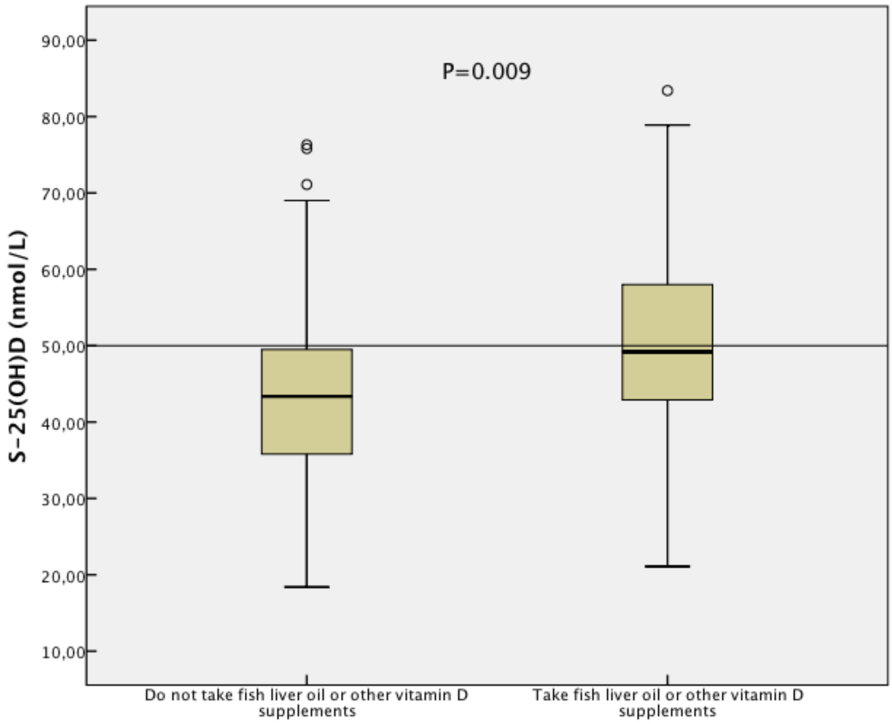


Figure 2: Correlation between blood sample timing in autumn weeks (evenly distributed from week 36-46) and s-25(OH)D (nmol/L) (n=158). Spearman's ρ = -0.496, P=0.01.



The difference in s-25(OH)D decreased significantly between week 36 (59.9 nmol/L) and 46 (36.7 nmol/L) (P<0.001) according to the independent t-test.

Figure 3: The difference in s-25(OH)D (nmol/L) for those taking vitamin-D supplements (fish liver oil or other vitamin-D supplements) and those not taking (n=120). P-value is shown for difference in s-25(OH)D between groups.



[1]P-value for difference in s-25(OH)D was calculated using the independent t-test.

[2]Median vitamin-D intakes for the group taking vitamin-D supplements were 8.8 µg/d (equivalent to about 4,5 ml of fish liver oil) and 2.0 µg/d for the group that did not take vitamin-D supplements.

[3]There was a statistical difference in vitamin-D intake between the group taking vitamin-D supplements and the one that did not (P<0.001), based on the Mann-Whitney U test.

Additional results

The results on the distribution of s-25(OH)D, dietary vitamin D intake and fish liver oil intake are described in the manuscript, as well as the proportion of children that fall within the definition of sub-optimal vitamin D levels and deficiency. The manuscript also covers the correlations of independent factors to vitamin D status, regression models for s-25(OH)D and correlations of s-25(OH)D to weeks in autumn. For further knowledge additional results are presented below. Vitamin D intake, serum vitamin D and fitness levels are described further based on fish liver oil intake and gender (Table 1 and 2). Adherence to the Food Based Dietary Guidelines (FBDG) is compared between 7-year-olds in 2006 and 6-year-olds in 2011 (Table 3), as well as comparing macronutrient and vitamin D intake (Table 4) for estimating if our results are still relevant to children at the present day in Iceland.

The vitamin D intake and status of 7-year-olds - gender and fish liver oil intake

Table 1. Table describing intake variables, serum vitamin D and fitness levels dependent on fish liver oil intake (n=120[1]). P-value is shown for difference between groups taking and not taking fish liver oil.

	Take fish liver oil			Do not take fish liver oil			
	N	Median	IQR	N	Median	IQR	P-value*
Total vitamin D intake (µg/d)	56	8.9	5.9, 14.0	64	2.0	1.3, 3.9	<0.001
Fish intake (g/d)	56	22.5	0, 33.7	64	5.0	0, 31.3	0.32
S-25(OH)D (nmol/L)	56	49.2	42.8, 58.0	64	43.4	35.8, 50.7	<0.01
Fitness (w/kg)	56	2.5	2.1, 2.9	64	2.6	2.2, 2.9	0.50

[1] Number of children that both returned 3-day food records and had blood sampled.
*P-value for difference between groups according to Mann-Whitney U-test.

Total vitamin D intake is considerably higher for the group taking fish liver oil or other supplements than for the group who does not (8.9 µg/d vs. 2.0 µg/d respectively, P<0.001), and they also have significantly better serum vitamin D (P<0.01). Fish intake and fitness do not vary significantly between groups (P=0.32 and P=0.50, respectively).

Table 2. Table describing intake variables, serum vitamin D and fitness levels for girls (n=63) and boys (n=57) separately. P-value is shown for gender differences.

		Median	IQR	P-value*
Total vitamin D intake (µg/d)	girls	2.9	1.6, 8.0	<0.01
	boys	6.7	2.6, 13.3	
Fish intake (g/d)	girls	0	0, 26.7	0.04
	boys	23.7	0, 42.0	
Fish liver oil intake (ml/d)	girls	0	0, 1.3	0.03
	boys	0.3	0, 3.3	
S-25(OH)D (nmol/L)	girls	43.5	37.6, 55.0	0.22
	boys	47.1	41.4, 57.3	
Cardiorespiratory fitness (w/kg)	girls	2.4	2.0, 2.7	<0.01
	boys	2.7	2.2, 3.1	

*P-value for gender difference according to Mann-Whitney U-test.

Boys have significantly higher total vitamin D intake (P<0.01), fish intake (P=0.04), fish liver oil intake (P=0.03) and fitness (P=0.01) than girls. However, the difference in s-25(OH)D measure was insignificant (P=0.22) between gender.

Fish consumption was not found to be a major contributor of vitamin D after adjusting for week of autumn and fish liver oil intake (regression model not shown). It measured insignificant (P=0.19) in explaining around 0.5% of the variability in s-25(OH)D, irrespective of gender.

No statistical difference between gender was found for height (P=0.25), weight (P=0.59) or BMI (P=0.96) (model not shown).

Comparison of intake between cohorts of 7-year-olds in 2006 and 6-year-olds in 2011

Table 3. Number and proportions of children following the Food Based Dietary Guidelines in 2006 (7-year olds) and 2011 (6-year-olds).

		2006 (n=120)		**2011**[3] (n=162)	
Dietary Recommendations[1]	**Guidelines adjusted for 6-7 year old children**[2]	N	%	N	%
Fish liver oil or other vitamin D source	≥5 ml per day, equivalent to the RDI of vitamin D	14	12	44	27
Fish at least two times per week	≥34 g/day, corresponding to a fish meal twice per week	26	22	37	23
Fruit and vegetables	≥400 g of fruit and vegetables per day	6	5	30	19
Whole grain bread and other fibre rich grain foods	≥2.5 g dietary fiber/MJ/day	20	17	28	17
Oil or unsaturated fats instead of saturated fats	<10% of the energy intake from saturated fats	7	6	10	6
Salt in moderation	<3.2 grams salt per day	0	0*	7	4
Water is the best refreshing drink	<10% of the energy intake from added sugars	38	32	70	43

[1] Food based Dietary Guidelines from the Directorate of Health (The Public Health Institute of Iceland, 2006)
[2] Adjusted guidelines used in the National Dietary Survey of 6-year-olds in 2011
[3] Values obtained from an article on the diet of 6-year-old children in 2011 (Gunnarsdottir et al., 2012)
*(Sodium (mg) • 2.5)/1000 (The Public Health Institute of Iceland, 2006)

The adherence to the National Dietary Recommendations has changed mainly in few ways regarding vitamin D sources between 2006 and 2011. The intake of total vitamin D has increased slightly along with the intake of fish liver oil, but no change was visible for fish intake. The total diet of children is under positive development, with more children in 2011 consuming diets lower in salt and added sugars and richer in fruit and vegetables than in 2006. However, when calculating nutrient intake none or little change was seen in intake of dietary fiber or saturated fat. The comparison of milk products is not shown due to the lack of available data on cheese, skyr and yogurt intake in the 2006 dataset.

When addressing the issue of whether the children with higher vitamin D status had better overall diets, we looked separately at the groups of children (7-year-olds) with sufficient vitamin D status (>50 nmol/L), sub-optimal (<50 nmol/L) and deficiency (< 25 nmol/L), but did not find significant difference in adherence to the FBDG except for the fish liver oil guideline (P<0.001), where those with the highest serum values had the highest adherence demonstrating that the children had similar diets except regarding intake of fish liver oil.

Table 4. Daily consumption (mean ± SD) and relative distribution of macronutrients, added sugar, fiber content (g/MJ) and vitamin D intake (μg/d) in 7-year-old children in 2006 (n=120) and 6-year-old children in 2011 (n=162)[1].

	Guidelines[2]	7-year-olds 2006	6-year-olds 2011
Orka (kcal)	1600-2000[3]	1765 ± 338	1543 ± 324
Protein (% EI)	10-20	15.8 ± 2.5	15.4 ± 2.9
Fat, total (% EI)	25-35	31.3 ± 4.7	32.2 ± 4.9
Carbohydrates, total (% EI)	50-60	52.2 ± 5.3	50.3 ± 5.5
Added sugars (% EI)	<10	12.2 ± 4.6	11.2 ± 4.5
Trefjar (g/MJ)	>2.5	2.1 ± 0.5	2.1 ± 0.5
D-vítamín (μg)	10	6.7 ± 6.1	7.5 ± 6.4

[1] Article on the diet of 6-year-olds in 2011 (Gunnarsdottir et al., 2012)
[2] Recommendations on diet and nutrients for adults and children from 2 years of age (The Public Health Institute of Iceland, 2006) and Nordic reference values (Nordic Council of Ministers, 2004).
[3] Common energy requirement for 6-8 year old children based on mean weight and moderate exercise. Energy requirement of children varies.

The vitamin D intake was similar in the 7-year-olds in 2006 and 6-year-olds in 2011 (P=0.29), but energy intake was higher in 2006 than in 2011 (P<0.001), as well as the intake of added sugar of borderline significance (P=0.07). The diets were very similar regarding macronutrient distribution and fiber content. No difference in macronutrient distribution was found among the 7-year-olds based on grouping the children by blood values (<25, ≤50, >50 nmol/L) rejecting the hypothesis that those with higher serum vitamin D status have better overall diet (model not shown). However significant difference was found in intake of vitamin D between blood value groups (P<0.001), as expected (model not shown).

No gender difference was found for food choices in the 7-year-olds when looking at intake of fruit, vegetables, meat, candy, chips and fries, sugared drinks, biscuits, cakes or milk. The only significant gender difference in food choice was for fish and fish liver oil intake. Furthermore, the only significant gender difference among energy intake, macronutrient distribution and fiber was for protein intake (P=0.015), where boys had higher protein proportion of total energy intake, but girls had a tendency of higher carbohydrate intake than boys (P=0.08) (model not shown).

Discussions and conclusion

Discussions, limitations and conclusions regarding the subject of first two aims of the thesis can be found in the manuscript on page 16. Reflections on fish liver oil, gender differences and comparison of diet between 2006 and 2011 will be discussed in the following chapter.

It is known that fish liver oil intake strongly influences the total vitamin D intake and status in children and has significant effects on serum levels of vitamin D, and our present study verifies those results. Fish consumption has been shown to have limited effects on vitamin D status in Iceland, due to the fish being lean and containing limited amounts of vitamin D. However, if the fish consumed is oily, then it significantly affects the vitamin D intake and status. The children in the present study, 7 years old in 2006, had very low fish consumption and it did not differ between those taking and not taking fish liver oil or supplements. Fish was not found to be a major contributor of vitamin D, which in addition to the fish consumption being low, fits that the fish mostly consumed is lean haddock or codfish which contain very little vitamin D.

Significant gender differences were found for total vitamin D intake, fish liver oil intake and fish intake in the present cohort of 7-year-olds, where boys had higher intake. Given the young age of the children, it is interesting that the intake of this type of basic foods differs between the genders. Gender differences have been recorded before in children in England, attributed to different food choices where boys tended to eat more breakfast cereals and girls to eat more fruit and vegetables (Glynn, Emmett, & Rogers, 2005), and in the USA where boys demonstrated higher preference for meat, fish and poultry over girls, but girls preferred fruits and vegetables over boys (Caine-Bish & Scheule, 2009). Studies on Icelandic children have reported higher energy intake among boys than girls (3-, 5-, 9- and 15-year-olds), and higher protein-, carbohydrate- and fat intake due to the increased EI for boys, but difference in food choice other than different sources of added sugar and bread consumption has not been stated (Gunnarsdottir, Eysteinsdottir, & Thorsdottir, 2008; Thorsdottir & Gunnarsdottir, 2006). The difference seems to be more apparent with increasing age (15 years and older) (Steingrimsdottir, Thorgeirsdottir, & Olafsdottir, 2002; Thorgeirsdottir et al., 2011). No gender difference was found in the present cohort for food choice except for fish and fish liver oil intake, and no difference was found for total energy intake, fiber, added sugar or macronutrient distribution except boys having higher E% for protein, but girls tended to eat more carbohydrates than boys. This gender difference in vitamin D intake can possibly be attributed to more nutrient density, fish liver oil intake and vitamin D content in protein rich foods like fish, fish liver oil and eggs compared to carbohydrate rich foods. No difference in baseline characteristics (height, weight, BMI), energy intake, added sugar or fat content indicates that the difference is attributed to food choice, other than those tested in present thesis.

The question has been proposed if the vitamin D "awakening" in the society has resulted in higher vitamin D intake and values since the data collection in 2006. Importance of sufficient dietary or supplemental vitamin D has been emphasized by the Directorate of Health and by the media, and supplements containing large doses of vitamin D are readily available in most stores (up to 50 μg per tablet). Some nutrition enthusiasts have also questionably recommended up to 5-10 times the RDI of vitamin D for children and adults (K. Gunnarsson, 2011; K. Gunnarsson, 2011; H. Magnusson, 2011). The current study and other Nordic studies show that the vitamin D intake is not according to recommendations. First concern would be to get the public to follow the recommendations. Most countries recommend more than they did 10 years ago, but the current literature does not support larger doses of vitamin D, especially among children.

There have been positive changes in children´s adherence towards the FBDG in 2011 in comparison to 2006. Slightly increased fish liver oil intake as well as increased fruit and vegetable consumption and diets lower in salt and added sugars were found in 6 year old children in 2011. The vitamin D intake did not differ between 2011 and 2006 (P=0.29) . No change was visible for intake of dietary fiber, saturated fat or fish. The results shown in Table 3 demonstrate that the diet of the majority of young children is still not in accordance with the FBDG in 2011, although it has improved since 2006. The macronutrient distribution is very similar in 2006 and 2011. The mean total energy intake was slightly higher in 2006 than in 2011. That could be a result of the children in 2006 being one year older and in active growth, but the energy intake is in line with dietary records of other 7-year-old children (Glynn et al., 2005) and within the norms of common energy needs of 6-8 year-olds given by the

Directorate of Health (~1600-2000 kcal) (The Public Health Institute of Iceland, 2006).

The main emphasis of recent dietary interventions in Icelandic children has been aiming to increase overall health, diet and exercise, but focusing on increasing fruit and vegetable intake (ProChildren, ProGreens), lowering intake of added sugars, increasing the intake of fish liver oil or other vitamin D supplements (Lifestyle of 7-9 year-old children) as well as on implementing a healthier lifestyle and diet in the school environment (e.g. "Everything affects us, especially ourselves"). Although the diet of young children has not been meeting the FBDG it has been shown to provide adequate amounts of vitamins and minerals, with the exception of vitamin D (Gunnarsdottir et al., 2012). Based on this comparison between 2006 and 2011 we can draw the conclusion that our results are still relevant to children in the present day, especially concerning the vitamin D sources. The fish consumption had not changed, and although the fish liver oil intake (≥5 ml/d) had increased from 12% to 27% in young children from 2006 to 2011, only about a quarter of the children met the recommendation. The other three quarters of 6-year-old children are not reaching the recommended vitamin D intake from their diet in 2011. Based on this, it is probable that the vitamin D status of young children is at a similar point as those in 2006, with the majority of children having sub-optimal serum levels during autumn and winter. Autumn, winter and springtime vitamin D status is thus still a great concern in Iceland among young children.

Outdoor activities among children have decreased in the past years, where indoor activities such as computer use and hours spent watching TV have increased. As many outdoor activities are coupled with physical activity and sun exposure, it is possible that the marker for cardiorespiratory fitness (CRF) is a surrogate marker for cutaneous synthesis of vitamin D, and thus masking the true association between CRF and vitamin D. Other mechanisms related to fitness may also be involved, e.g. muscle strength and stamina, but it is probable that the children who spend more time doing outdoor activities have better fitness as well. The use of sunscreen probably diminishes the cutaneous synthesis of vitamin D at some level, but the use of sun protection has been increasing in tandem with recommendations and public awareness. However, CRF was highly correlated with s-25(OH)D in a regression model, even after adjusting for week of autumn, indicating that fitness also has its own relation to serum vitamin D status, irrespective of outdoor activities or sun exposure.

Speculations on the adequate intake (AI) needed to maintain optimal serum values are common, but the AI is hard to evaluate and varies between many factors like age, race, latitude, time of year, sun exposure, clothing etc. The Insitute of Medicine (IOM) claims in their report on dietary reference intakes for calcium and vitamin D that a RDA of 15 μg/d gives a corresponding serum vitamin D level of 50 nmol/L for children and adolescents (1-18 years old). They conclude that this level of serum vitamin D would meet the needs of approximately 97.5% of the US population under conditions of minimal sun exposure, not finding higher levels to confer greater benefit (Ross et al., 2011). The Nordic Nutrition Recommendations (NNR 2004) estimated that the daily intake of 7.5 μg/d in all Nordic countries except Iceland (RDI 10 μg/d) is sufficient to diminish the seasonal drop in s-25(OH)D during the winter months and maintain vitamin D levels above 50 nmol/L for people up to 74 years of age (Pedersen, 2008), but an estimated intake of up to 12.5 μg/d may be needed to completely alleviate the drop (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003). However in the most recent NNR which are to be published, a daily intake of 10 μg/d in all Nordic countries is recommended (NNR 2012 working group, 2013), except in Iceland where 10 μg/d is recommended for children up to 10 years of age, and 15 μg/d for adults (up to 60 years of age) (The Directorate of Health, 2013).

Vitamin D results must be interpreted with caution concerning children, as optimal levels have not been established either for serum vitamin D or optimal intake, since they are affected by many factors like fat mass, calcium intake, latitude and sunlight exposure. The median value in children in the present cohort was just below the reference value of 50 nmol/L, hinting, based on the conclusion of the IOM, that at least 50% of them are meeting their vitamin D requirement despite being classified with sub-optimal serum levels. These results are no proof that these children will develop diseases, but it is a hint that the matter requires attention and preventive measures, to counter further decline in vitamin D status and intake in children.

Limitations of the additional results are that the two cohorts may not be completely comparable, as the older one only covers the Reykjavík area but the 2011 cohort covers the whole country. Diets have been shown to vary between parts of the country, from e.g. rural fishing villages to villages inland, or bigger cities (Gudjonsdottir, 2012). Further analysis and comparisons could be made in the future as the data from the Reykjavík area exists in the 2011 dataset.

As a fishing nation with good access to top quality fish and being one of very few countries in the world where fish liver oil is considered a part of the FBDG, it is unfortunate that the consumption of fish, fish products and fish liver oil is as little as research has shown. Similar findings on fish intake have nevertheless been reported in the other nordic countries among children (Fagt et al., 2012). New ways to improve the dietary habits of children and their families have to be implemented, and hopefully the diet of young Icelanders will continue to evolve towards the FBDG in coming years, along with increasing outdoor- and physical activities, vitamin D intake and sunlight exposure.

Future perspectives

More research is needed on the optimal intake of vitamin D and deficiency cut off levels of serum 25(OH)D in children. More accurate listings are needed to further examine the relation between dietary vitamin D to vitamin D status of the body, and determine the effects of physical activity and sunlight exposure at nordic latitudes.

New strategies need to be implemented to ensure compliance to the Food Based Dietary Guidelines in Icelandic children, as nutrition during infancy and childhood makes the foundation for future physical health and wellbeing.

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