

VITAMIN D
AND CARDIOMETABOLIC RISK FACTORS
IN CHILDREN

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VITAMIN D AND CARDIOMETABOLIC RISK FACTORS IN CHILDREN

Vitamin D deficiency has been traditionally linked with rickets and bone health. However, the discovery of the vitamin D receptor in almost all cells of the body led to a potentially wider role of the vitamin D. Vitamin D deficiency has been associated with metabolic syndrome (MetS) and cardiovascular diseases in observational studies. As cardiovascular diseases originate in childhood, it would be of major public health importance to prevent vitamin D deficiency in children.

The aim of the thesis was to study the association between serum 25-hydroxyvitamin D (25(OH)D) concentration and the components of the cardiometabolic risk (CMR) factors in children. A cross-sectional analysis was performed from the baseline data of the children participating in the Physical Activity and Nutrition in Children (PANIC) study. A total of 736 children aged 6-8 years who started the first grade in 2007-2009 were invited to participate in the study to have a representative population sample of the children from Kuopio. Of the invited children, 512 participated in the baseline examinations and serum 25(OH)D concentration was available for 447 subjects (214 girls, 233 boys).

Association of serum 25(OH)D with the CMR score, systolic blood pressure (SBP) and diastolic blood pressure (DBP), glucose metabolism, insulin resistance, serum lipids, liver enzymes, body fat percentage (%), waist circumference, body mass index (BMI) and high sensitivity C-reactive protein (hs-CRP) were tested by performing multiple linear regression analyses. Three different models were made. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, physical activity and Dietary Approach to Stop Hypertension (DASH) score. Model 3 used a stepwise regression model and a backward elimination method, which included serum 25(OH)D and confounding factors: age, sex, physical activity, body fat %, DASH score, ratio of unsaturated to saturated fat in diet, vitamin D intake, length of day, skin types, travel to sunny locations, parental education, pubertal status, race and use of sunscreen. Models 1 and 2 showed that a higher serum 25(OH)D concentration was associated with lower SBP, lower plasma concentration of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG), and a higher plasma concentration of alanine aminotransferase (ALT). The same results remained in model 3 after adjustment for all confounders, at the exception of TG, which association became no more statistically significant. Also, having an hs-CRP value between 1.0 and 5.0 mg/l was associated with an increased serum 25(OH)D concentration, compared with having a hs-CRP value <1.0 mg/l.

Serum 25(OH)D concentration could have a protective role in some components of CMR, such as SBP, TC and LDL-C. However, it may also be associated with a higher level of hs-CRP and with a lower HDL-C. Randomized controlled trials should be performed to find a possible causal association for those findings.

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The journey has been longer than expected, and not without obstacles, but I have learned a lot on myself and on the research world. I have now a deeper understanding and respect toward researchers who devote their career in order to make progresses in science and transfer their knowledge in order to achieve a better understanding of this world. In this thesis, I got a better understanding of the complexity of the physiology of vitamin D and its effect on health.

I would like to thank the children of the schools of Rovaniemi, which gave me the motivation to study vitamin D. I was working as a school doctor and was worried about the low level of vitamin D in some of the children. It driven me the need to know more about the health consequences of vitamin D deficiency and study the health implication of vitamin D deficiency among children living in Nordic locations, as the subject for my master thesis in Public Health. I wish that the information of this thesis would be useful in order to pursue further research about vitamin D deficiency and promote a better health among children.

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ABBREVIATIONS

1,25(OH) ₂ D	1,25-dihydroxyvitamin D ₂ and/or D ₃ (or) 1,25-dihydroxycholecalciferol D ₂ and/or D ₃ (or) calcitriol
25(OH)D	25-hydroxyvitamin D ₂ and/or D ₃ or 25-hydroxycholecalciferol D ₂ and/or D ₃ (or) calcifediol (or) calcidiol
ALT	alanine aminotransferase
AR	average requirements
BMI	body mass index
CMR	cardiometabolic risk
DASH	Dietary Approaches to Stop Hypertension
DBP	diastolic blood pressure
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FPG	fasting plasma glucose
GGT	gamma-glutamyl transpeptidase
HDL-C	high-density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment of insulin resistance index
hs-CRP	high-sensitivity C-reactive protein
IQR	Interquartile Range
LDL-C	low-density lipoprotein cholesterol
MetS	metabolic syndrome
MUFA	monounsaturated fatty acids
n	number of subjects
PANIC	Physical Activity and Nutrition in Children
PUFA	polyunsaturated fatty acids
RI	recommended intake
SBP	systolic blood pressure
SD	standard deviation
SFA	saturated fatty acids
TC	total plasma cholesterol
TG	plasma triglyceride
Vitamin D ₂	ergocalciferol
Vitamin D ₃	cholecalciferol

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1. INTRODUCTION

Vitamin D is a hormone precursor synthesized in the skin under the action of the sun. The main action of the active form of vitamin D, calcitriol or 1,25-dihydroxyvitamin D (1,25(OH)₂D), is to maintain a normal serum level of calcium, in order to keep normal functioning of cells (Holick 2002). Vitamin D is essential for bone health and growth. Vitamin D deficiency leads to rickets in children, osteomalacia in teenagers and young adults, and to osteopenia and osteoporosis in the elderly (Welch 2000). The discovery of the vitamin D receptor in almost all tissues of the human body lead to the hypothesis that vitamin D has a wider effect than traditional bone health and calcium homeostasis. Vitamin D receptor has been found in immune, vascular and myocardial cells, pancreatic beta cells, neurons and osteoblasts, suggesting that vitamin D may have a role in the functions of those tissues, including functions related to cardiometabolic health (Prasad & Kochhar 2016).

Populations living at Northern latitudes are at higher risk of having vitamin D deficiency (Gupta et al. 2012) and hypertension (Rostang et al. 1997). Interestingly, vitamin D deficiency has been associated with chronic diseases in adults, including diabetes and metabolic syndrome (MetS) (Hoffmann et al. 2015). However, reviews on vitamin D and cardiovascular health in generally healthy adults and in children could not draw clear conclusions, due to the lack of data from randomized controlled trials (Pittas et al. 2010).

Cardiovascular diseases (CVD) originate already from childhood (Berenson et al. 1998, Raitakari et al. 2003), and the presence of MetS in childhood increases the likelihood of developing CVD in adulthood (Morisson et al.2007). In children, a review of cross-sectional trials found inconsistent findings regarding vitamin D deficiency and cardiometabolic risk (CMR). However, few randomized controlled trial and fairly few cross-sectional studies have been made in children in order to assess the association between serum 25-hydroxyvitamin D (25(OH)D) or vitamin D deficiency and CMR. (Dolinsky et al. 2012) Therefore, there is need for more investigations including cross-sectional studies and randomized controlled trials in order to assess that issue. This would be of a major public health interest, since vitamin D deficiency is highly prevalent worldwide, in all age groups (Palacios et al. 2014).

2. LITERATURE REVIEW

2.1 Vitamin D: a demystification of its actions

2.1.1 Evolutionary perspective

Vitamin D has been produced by phytoplankton and zooplankton for more than 500 million years, as a basic protection mechanism against excessive solar ultraviolet B radiation. Fishes and other sea organisms depend on phytoplankton and zooplankton for their survival. Therefore, the ingested vitamin D accumulates in the fat of oily fishes. When evolution allowed species to survive in a non-sea environment, vitamin D developed a predominant role in bone and calcium homeostasis. Animals, most plants and mushrooms also have the capacity to produce vitamin D from sunlight (Holick 2003).

Humans has the capacity to synthesize vitamin D in skin. The 7-dehydrocholesterol also called provitamin D₃ is stored in the skin and competes with the pigmentation of melanin for ultraviolet B light. Our ancestors, living in Africa, had naturally darker skin with high melanin content, acting as a natural sunscreen. When civilizations started to migrate toward the poles, the skin became gradually lighter, because of the evolutionary process. One possible explanation is that light-skinned women were less likely to develop nutritional rickets, therefore less likely to have pelvic malformations due to rickets, and more likely to give birth without complications (Holick 2007a, Holick 2011a).

2.1.2 From rickets recovery to vitamin D discovery

A disease of bone malformation affecting children was already described in 100-200 AD, but it is only by the middle of the 17th century that rickets was identified as a proper disease, affecting children of the cities of England (Rajakumar et al. 2003). During the industrial revolution (1760-1840), populations migrated toward cities. As a consequence, rickets became more prevalent among children, due to a shift to a more sedentary life and to limited access to sunlight. In 1898, 80% of infants under the age of 2 years had rickets. In 1923, Eliot showed that cod liver oil and regular sun bathing during the first year of age could prevent rickets. The “X-factor” healing rickets was called vitamin D. This finding led to the common utilization of cod liver oil, and, as a result, rickets was almost eradicated (Welch 2000). In 1924, Steenbock & Black, as well as Hess & Winstock, found that the irradiation of food had an antirachitic activity on rats, and this led to the common use of irradiated milk

and cereals (Rajakumar et al. 2003). It was only in 1932 that vitamin D₂ (ergocalciferol) was isolated from an irradiation mixture of ergosterol. In 1935, Windaus isolated 7-DHC, and, in 1937, Windaus & Bauck identified Vitamin D₃ (cholecalciferol) (DeLuca 2014). The vitamin D receptor was discovered in 1974 in the intestinal cells of vitamin D-deficient chickens (Brumbaugh & Haussler 1974). Since then, vitamin D receptor was discovered in almost all tissues of the human body, including osteoblasts, pancreatic beta-islets cells, nerve cells, brain, colon tissue, small intestine, heart, skin including immature keratinocytes, hair follicles, gonads, breast, prostate, adrenal medulla, immune cells including activated T and B lymphocytes, mononuclear cells and lymphoid granulomata (Zehnder et al. 2001, Holick 2003, Holick 2007a, Misra et al. 2008, Bikle et al. 2015).

Also, 25-hydroxyvitamin D-1 alfa hydroxylase, the enzyme converting 25(OH)D into its active form, was found in other tissues of the body, including prostate (Swartz et al. 1998), colon (Cross et al. 2001), breast, immune system, alveolar and activated macrophages, lymph nodes, placenta, osteoblasts and keratinocytes (Misra et al. 2008). Therefore, 1,25 (OH)₂D has not only endocrine, but also autocrine and paracrine properties (McCullough et al. 2009, Morris & Anderson 2010, Carlberg & Molnár 2015). Interestingly, cells also have the machinery to produce the enzyme 25-hydroxyvitamin D-24-hydroxylase, which degrades 1,25 (OH)₂D locally (McCullough ML et al. 2009). In late 1970, there was a reemergence of rickets in children in the industrialized world, which could be due to the promotion of long-term breastfeeding (Welch 2000).

2.1.3 Vitamin D: from photosynthesis to bone synthesis

Vitamin D₃ is synthesized in the skin, under the action of sunlight. Ultraviolet B light of wavelength 290-315 nm breaks down the solid cholesterol ring of 7-DHC, becoming a secosteroid called previtamin D₃. Previtamin D₃ is then rapidly converted into vitamin D₃, through a heat-dependant process (Holick 2007b). The conversion of provitamin D₃ into previtamin D₃ is more efficient during initial sun exposure. It takes about 8 hours to convert 7-DHC into vitamin D₃ (Wacker & Holick 2013). Continued exposure to sunlight causes the isomerization of previtamin D₃ into its inactive metabolite lumisterol. For that reason, sun exposure cannot cause vitamin D intoxication (Alshahrani & Aljohani 2013).

The skin-synthesized vitamin D₃ is transported through the blood stream into the liver, where it undergoes a process of hydroxylation at its 25th position by the vitamin D 25-hydroxylase. The end-product is 25(OH)D₃, which is biologically inactive.

Vitamin D₂ and vitamin D₃ can also be ingested through diet. Dietary intake of vitamin D₂ and D₃ are incorporated into chylomicrons, and transported by lymph, which drains into the venous system. After reaching the liver, vitamin D₂ and D₃ undergo the same hydroxylation process as the vitamin D₃ produced in the skin, therefore becoming 25(OH)D₂ and 25(OH)D₃.

The molecule 25(OH)D, also called calcidiol or calcifediol, is then transported in the blood stream until it reaches the kidney, its main target organ. In the kidney, 25(OH)D undergoes a second hydroxylation at its first position by the enzyme 25-hydroxyvitamin D- 1- α -hydroxylase. The end-product is 1,25(OH)₂D, also called calcitriol, which is the active form of vitamin D. 1,25(OH)₂D is finally catabolized in the liver by the 25-hydroxyvitamin D 24-hydroxylase into its water-soluble inactive form called calcitronic acid, which is then excreted in the bile (Holick 2007a). 24-OHase is part of the cytochrome CYP24A1 in P450 (Jones et al. 2012). 25(OH)D is a highly liposoluble compound, which is stored in fat tissue (Rosenstreich et al. 1971), and then released from fat tissues (Ziaie et al. 2016).

The 1,25 (OH)₂D has an important role in maintaining the serum level of calcium constant (Holick et al. 2007b). Indeed, a normal serum level of phosphate and calcium are essential to promote normal calcification of bones (Holick et al. 2007b, Matikainen 2014). In case of hypocalcemia, parathyroid hormone is secreted by the parathyroid gland, which activates 25-hydroxyvitamin D-1 α hydroxylase, which then enhances the production of 1,25(OH)₂D in the kidney. 1,25(OH)₂D increases the absorption of calcium from the small intestine, reestablishing a normal serum concentration of calcium. The 1,25(OH)₂D connects with the vitamin D receptor in the osteoblasts, inducing the maturation of preosteoclasts into osteoclasts, and causing the release of calcium and phosphate in the blood stream through bone resorption. The 1,25(OH)₂D also enhances the calcium reabsorption from the renal tubule. The parathyroid hormone also has a direct stimulating effect on bone resorption (Holick et al. 2007b). The parathyroid hormone is down-regulated by the serum concentration of calcium and phosphate (Holick et al. 2007b, Kumar et al. 2011). 1,25(OH)₂D is regulated by the serum level of calcium and phosphate, the fibroblast growth factor 23 and the parathyroid hormone (Holick et al. 2007b).

2.1.4 Dietary and supplemental sources of vitamin D

Vitamin D₃ is found in fatty fish, like sardines, salmon, mackerel, tuna and in caviar, egg yolks, vitamin D fortified dairy products like milk and margarines. Vitamin D₂ is found in certain kinds of mushroom. Vitamin D₂ and D₃ are also available in the form of supplements (Lamberg-Allardt et al. 2013, Nordic Nutritional recommendations 2012). In Finland, most vitamin D fortified foods contains vitamin D₃, and most of the vitamin D supplements contains vitamin D₃. Vitamin D₂ and D₃ are both efficiently absorbed by the intestinal wall, but vitamin D₃ raises the serum 25(OH)D concentration to a higher level and for a longer time (28 d versus 3 d) than vitamin D₂ (Armas et al. 2004). Excessive intake of vitamin D from supplements may cause vitamin D intoxication and hypercalcemia (Netter 1987, Taylor & Davies 2018).

2.2. Epidemiology of vitamin D deficiency

2.2.1 Definition

Vitamin D deficiency refers to a low serum level of 25(OH)D, which may be accompanied by impaired bone mineralization. Until 2016, there was no international consensus about the cut-off point of vitamin D deficiency and sufficiency for children. The recent Nordic Nutrition recommendations 2012 (2014) recommended a cut-off point of 30 nmol/l for vitamin D deficiency and 50 nmol/l for vitamin D sufficiency and the American Academy of Pediatrics defined vitamin D sufficiency as a serum 25(OH)D concentration > 50 nmol/l (Wagner et al. 2008) (Table 1). Braegger et al. (2013) suggested using a cut-off point of 25 nmol/l for severe vitamin D deficiency among European children (not in table).

Most recently, international experts in pediatric endocrinology, nutrition and public health from 10 pediatric societies published Global Consensus Recommendations on Prevention and Management of Nutritional Rickets (Munns et al. 2016), setting vitamin D deficiency as < 30 nmol/l, insufficiency as 30-50 nmol/l, and sufficiency as > 50 nmol/l for infants, children and adolescents. The Institute of Medicine (2011) suggested that serum level of 25(OH)D above 125 nmol/l may have adverse effects on health. More recently the Global Consensus Recommendation 2016 assessed vitamin D toxicity as a level of 25(OH)D > 250 nmol/l (Munns et al. 2016).

Table 1 Vitamin D status in relation to serum 25(OH)D concentration in nmol/l in children and adolescents

Vitamin D status	25(OH)D ¹ concentration for children			25(OH)D ¹ concentration for children and adults	
	American Academy of Pediatrics 2008 ²	Nordic Nutrition Recommendations 2012	Global Consensus Recommendations on Prevention and Management of Nutritional Rickets 2016 ³	Institute of Medicine 2011	Endocrine society 2011 ⁴
Deficiency	ns	<30	<30	<30.0	<50
Insufficiency	ns	30-50	30-50	30-50	52.5-72.5
Sufficiency	>50	>50	>50	>50	>75.0
Excess	ns	Ns	Ns	>125	Ns
Toxicity	ns	Ns	>250	ns	Ns

ns, not specified by the authors ¹ 25-hydroxyvitamin D in nmol/l, ²Wagner et al. 2008, ³Munns et al. 2016, ⁴Holick et al. 2011b.

2.2.2 Prevalence of vitamin D deficiency

Vitamin D deficiency is highly prevalent worldwide (Palacios et al. 2014). A possible reason could be a shift toward a sedentary lifestyle, for instance due to a lack of time spent outside (Musson & Collin 2015).

The cut-off point for vitamin D deficiency and insufficiency differed between studies. A total of 25 % of Brazilian adolescents had a serum 25(OH)D concentration below 25 nmol/l (Oliveira et al. 2013), and 43% of Canadian Cree had serum 25(OH)D concentration below 30 nmol/L (Riverin et al. 2014). Serum 25(OH)D concentration below 50 nmol/l was found in 29% of English children (Tolppanen et al. 2012), 43% of children in Boston (Gordon et al. 2004), 75 % of Korean adolescents (Nam et al. 2012), 80% of South Arabian boys and 90% of South Arabian girls (Al-Daghri et al. 2015), 98% of Turkish children (Andiran et al. 2012) and 83.7% of obese Dutch children (Radhakishun et al. 2015). In one study, 43 % of Finnish girls had a serum 25(OH)D below 40 nmol/l, and as much of 32 % of Finnish girls had a serum 25(OH)D concentration below 25 nmol/l (Cheng et al. 2003). Published data from the same study population as this thesis of children 6-8 years of age participating in the Physical

Activity and Nutrition in Children (PANIC) study, showed that the prevalence of serum 25(OH)D concentration below 50 nmol/l was 19.5% (Soininen et al. 2016).

2.2.3 Determinants of vitamin D status

Factors associated with a higher prevalence of vitamin D deficiency are a lack of sun exposure, northern latitude, winter season or shorter length of day (Holick 2003, Holick & Chen 2008, Rajakumar et al. 2011, Holick et al. 2011b, Tolppanen et al. 2012). In Helsinki (60° N), there is no skin production of vitamin D between mid-October and mid-March (Lamberg-Allardt et al. 2013).

Other factors that impair vitamin D synthesis in the skin are a sedentary life and a lack of time spent outside (Tolppanen et al. 2012), the use of a veil (Holick & Chen 2008), the use of sunscreen (Holick et al. 2011b, Tolppanen et al. 2012), and a higher amount of melanin in the skin, which acts as a natural sunscreen (Gordon et al. 2004, Holick and Chen 2008, Holick et al. 2011b, Rajakumar et al. 2011, Tolppanen et al. 2012, Whiting et al. 2011). For instance, dark skinned individuals require 5-10 times more exposure to the sun in order to produce the same amount of vitamin D as light skinned individual (Holick et al. 2007b).

Lower dietary intake of vitamin D has been associated with lower serum concentration of 25(OH)D (Tolppanen et al. 2012, Soininen et al. 2016). Medical conditions that cause malabsorption of vitamins and nutrients, and other drugs affecting the absorption and liver metabolism of vitamin D (rifampicin, glucocorticoids and anticonvulsants), as well as liver diseases, also cause a decreased serum level of 25(OH)D (Holick & Chen 2008). 25(OH)D is stored in the fat. Obesity, higher body mass index (BMI) and higher visceral fat % are linked with a poorer vitamin D status (Rajakumar et al. 2011, Gupta et al. 2012, Tolppanen et al. 2012).

Socio-economic factors such as lower maternal education and household income also predict a poorer vitamin D status (Tolppanen et al. 2012). Older age, a more advanced pubertal stage and female sex are also associated with a lower level of serum (25(OH)D concentration (Holick & Chen 2008, Holick et al. 2011b, Tolppanen et al. 2012, Rajakumar et al. 2011). A previously published paper from the same study population as this thesis, showed that older age was associated with lower 25(OH)D in girls, but not in boys (Soininen et al. 2016). Also,

in that same paper, a higher parental education was associated with higher 25(OH)D in boys, but it was explained by other factors.

2.2.4 Vitamin D requirements

There have been discrepancies in opinions from experts regarding the adequate intake of vitamin D in order to prevent vitamin D deficiency (Holick 2002, Heaney et al. 2003, Viljakainen et al. 2006, Harel et al. 2011, Garland et al. 2014). According to the recent Nordic Nutrition Recommendation panel 2012 (2014), a total daily intake of vitamin D of 10 µg would provide vitamin D sufficiency (>50 nmol/l to 95% of the population of children and adults) (Table 2). The Nordic recommendation for total daily intake of vitamin D has increased from 7.5 µg/d to 10 µg/d in 2014 (Nordic Nutrition Recommendations 2012 (2014)).

Table 2 Vitamin D recommendations from diet and supplement in Finland. From the Nordic Nutrition Recommendations 2012 (2014) and the National Nutrition Council 2014 (Valtion ravitsemusneuvottelukunta 2014).

	Vitamin D intake in µg/d					
	infants (0-12 months)	younger children (2-10 y)	older children (11-17 y)	younger adults (18-60 y)	older adults (61-74 y)	Elderly (≥75 y)
AR, ¹	Ns	7.5	7.5	7.5	7.5	7.5
RI from diet and supplements ¹	10	10	10	10	(10) ³ (20) ⁴	20
RI from supplements (Valtion ravitsemusneuvottelukunta 2014)	10	7.5	7.5	(10) ⁵	(10) ⁵	20
Lower Intake ¹	Ns	ns	Ns	ns	2.5	2.5
Upper Intake ¹	25	(50) ²	100	100	100	100

AR, average requirements (vitamin D intake in order to achieve vitamin D sufficiency among 50% of the population); RI, recommended intake of vitamin D, in order to achieve vitamin D sufficiency among 95% of the population; y, year(s) ¹ daily intake from diet and supplements, ²1-10 y ³ if sufficient sun exposure, ⁴if little or no sun exposure. ⁵if no daily consumption of dairy products or margarines supplemented with vitamin D between October and March, or/and if not sufficient consumption of fish between October and March.

A sufficient supplementation of vitamin D during childhood and adolescence promotes bone mineralization and prevents growth retardation (Holick 2011a). In adults and elderly, vitamin D sufficiency promotes a better absorption of calcium (Heaney et al. 2003). Vitamin D sufficiency has also been associated with a better bone and muscle health, and with prevention of falls and fractures (Bischoff-Ferrari et al. 2006).

Carlberg (2016) suggested that some individuals need a higher serum concentration of 25(OH)D in order to achieve an optimal health effect. The personal range for optimal vitamin D status may vary between individuals and depends on genetics and epigenetics factors (Carlberg et al. 2016).

2.3 Vitamin D and health

2.3.1 Vitamin D: a role beyond traditional bone health

Vitamin D deficiency has a well-established role to play in bone diseases: rickets and osteomalacia in children (Misra et al. 2008), and osteoporosis in the elderly (Holick 2007a). Serum concentration of 25(OH)D also has been associated with other health conditions. For instance, vitamin D sufficiency has been linked with a better pregnancy outcome and improved health of the developing infant (Holick 2011a). A pioneering Finnish study on health effects of vitamin D supplementation by Hyppönen et al. (2001) found that giving 50 µg/d of vitamin D to 10366 children during their first year of life reduced their risk of developing type 1 diabetes by 80% in the following 30 years. Vitamin D supplementation in children has been found to reduce the risk of developing asthma and wheezing disorders, as well as contracting upper respiratory tract infections caused by influenza and other infectious agents (Holick 2011a).

In adults, studies found that a higher serum level of 25(OH)D has been associated with a decreased all-cause mortality (Bjelakovic 2014, Garland et al. 2014, Lamberg-Allardt et al. 2013, Thomas et al. 2012, Virtanen et al. 2011), as well as a decreased risk of developing autoimmune diseases (Hyppönen 2001, Holick et al. 2011b), type II diabetes (Holick et al. 2011b), CVD (Wang et al. 2008, 2013, Parker et al. 2010, Holick et al. 2011b, Birken et al. 2015, Rai & Agrawal 2017), hypertension (Pils et al. 2009, Pittas et al. 2010, Jeong et al. 2017), dyslipidemia (Jorde & Grimnes 2011), MetS (Wang et al. 2013), colorectal cancer (Bischoff-Ferrari et al. 2006), cognitive dysfunctions and infectious diseases (Holick et al.

2011b). Vitamin D supplementation may improve fasting plasma glucose (FPG) level, insulin sensitivity and heart rate among pre-diabetic adults (Saksa et al. 2015).

Vitamin D is a regulator of the immune system (Cantorna & Mahon 2005), inhibits the inflammatory response and induces antimicrobial mechanisms (Gupta et al. 2012). It also acts as a natural antimycobacterial agent (Gombard 2009). The 1,25(OH)D regulates cellular growth, and it has been suggested that the intracrine production of 1,25(OH) in the cells may have an activity against cancer (Holick 2003).

2.3.2 Behind the scene: Vitamin D receptor

The potential health effects of vitamin D might be explained by their effects on the genome. The vitamin D receptor is pleiotropic, meaning that it influences many different genes in many different cells of the body (Carlberg & Molnár 2015). The vitamin D receptor has been found to directly or indirectly regulate 100-1250 different genes and 0.5-5% of the total human genome (McCullough et al. 2009, Ramagopalan et al. 2010, Hossein-Nezhad et al. 2013, Carlberg 2014a, Carlberg & Molnár 2015). Vitamin D receptor has a role in the regulation of cell proliferation, differentiation, apoptosis, modulation of growth and inflammatory factor, immunomodulation, and may have a role in DNA repair, angiogenesis, cell adhesion, bile acid and xenobiotic metabolism as well as protecting from oxidative stress (McCullough et al. 2009). This opens interesting avenues for possible therapeutic effects of vitamin D in the treatment of certain diseases (Carlberg 2014b, Bikle et al. 2015), like cancer (Misra et al. 2008).

2.4 Vitamin D, cardiovascular health and metabolic syndrome in children and adolescents

In 2000, it was estimated that more than 2 million US adolescents have the MetS (Duncan et al. 2004). Since 1970, the prevalence of childhood obesity and MetS has increased (Rokholm et al. 2010). Cardiovascular diseases originate in childhood (Berenson et al. 1998, Raitakari et al. 2003). Autopsies made on 2876 young adults aged 15-34 years who died from external causes between 1987 and 1994, showed that intimal lesions already appeared in aortas and right coronary arteries of the youngest age group (15-19 years old) (Strong et al. 1999). Raitakari et al. 2003, found a positive association between systolic blood pressure (SBP), low-density lipoprotein cholesterol (LDL-C), BMI and smoking in childhood and common carotid artery intima-media thickness 21 years later. Having the MetS in childhood increases

the risk of developing cardiovascular diseases by 14 times (CI 95% 4.8-45.3) in adulthood, compared with subjects, who did not have the MetS as a child (Morisson et al. 2007).

Vitamin D sufficiency during childhood has been suggested to promote cardiovascular health later in life (Birken et al. 2015). The most recent review on children stated that a poorer vitamin D status was possibly associated with hypertension and cardiovascular diseases (Xu et al. 2017).

2.4.1 Definition of metabolic syndrome

The MetS is defined, in children over 10 years and in adults, as an increased waist circumference, and at least 2 of the following criteria: elevated plasma triglyceride level (TG), low high-density lipoprotein cholesterol (HDL-C), elevated blood pressure, and increased FPG. Reference values for children aged 10-15.9 years are age-specific. The diagnosis of MetS cannot be made for children younger than 10 years, but special attention should be given to young children having an increased waist circumference and with familial history of CVD, diabetes, dyslipidemia and MetS (Alberti et al. 2006a, 2006b).

Table 3 International Diabetes Federation consensus worldwide definition of the MetS in children, adolescents and adults (Alberti et al. 2006a, 2006b).

Criteria	For children <10 y ¹	For children aged ≥10 and < 16 y ²	For children ≥16 y and adults ²
Increased waist circumference	≥ 90 th of age-specific waist circumference	≥90 th percentile for age or adult cut-off point ³	Increased waist circumference (population specific) or BMI ≥ 30kg/m ²
High TG	_____	≥1.7 mmol/l (≥150 mg/dl)	TGs ≥150mg/dl (1.7 mmol/l) or use of TG-lowering medication.
Low HDL-C	_____	<1.03 mmol/l (<40 mg/dl)	HDL-C <40mg/dl (1.03 mmol/l) in men or <50mg/dl (1.29 mmol/l) in women or use of HDL-C increasing medication.
High blood pressure	_____	Systolic ≥130/ diastolic ≥85 mm Hg	SBP ≥130mmHg or DBP ≥85mmHg or use of medication for high blood pressure.
Impaired fasting glucose	_____	≥5.6 mmol/l (100 mg/dl)	Impaired fasting glucose ≥ 5.6 mmol/l (100mg/dl) or diabetes.

DBP, diastolic blood pressure; MetS metabolic syndrome, TG, triglycerides, HDL-C, high-density lipoprotein cholesterol, BMI, body mass index, SBP systolic blood pressure; y, year(s) ¹ MetS cannot be diagnosed. ² For diagnostic of MetS in individuals ≥10 y, abdominal obesity and the presence of two or more other clinical

features are required. ³If the measurement of waist circumference is above the adult cut-off point, it can be used as diagnostic criteria for abdominal obesity in children aged 10-16 years.

2.4.2 Vitamin D and the metabolic syndrome

Some cross-sectional studies among children and adolescents showed that vitamin D deficiency was positively associated with MetS among adolescents (Reis et al. 2009) and was associated with an increased SBP and BMI and decreased HDL-C among obese children and adolescents of 7-18 years old living in New York (Smotkin-Tangorra et al. 2007). Vitamin D deficiency was also associated with an increased FPG in children of 9-16 years (Delvin et al. 2010) and increased insulin resistance in white adolescents (Ashraf et al. 2011). However, an extensive systematic review made on children and adolescents, including mostly cross-sectional studies, concluded that evidence was insufficient to conclude that vitamin D supplementation would bring any cardiometabolic benefits (Dolinsky et al. 2012). There is, indeed, a need for large randomized-controlled trial to determine the health effect of vitamin D among children and adults (Wang et al. 2013).

Many studies found, that for a lower serum level of vitamin D, there was a higher prevalence of having MetS, in children (Pacifico et al. 2011, Lee et al. 2012) and adolescents (Reis et al. 2009, Gangi et al. 2011, Nam et al. 2012). Lee et al. (2012) showed that 9 year-old vitamin D deficient children (<38.5 nmol/l) had a 4.5 times increased risk (OR 4.25, 95% CI 1.84-9.85) of having MetS than vitamin D sufficient children (> 54 nmol/l). In children, lower vitamin D level was associated with a higher BMI, waist circumference and TG, a lower level of HDL-C (Reis et al 2009, Lee et al 2012), as well as a higher SBP and FPG (Reis et al 2009). Other studies did not find an association between vitamin D and MetS (Delvin et al. 2010, Cabral et al. 2016).

2.4.3. Vitamin D and the cardiometabolic risk

In many studies on children, a CMR score is used in the analyses, instead of the MetS. Cross-sectional studies among children and adolescents have displayed divergent results concerning the association of serum 25(OH)D concentration and the CMR score. A cross-sectional analysis of a large prospective birth cohort study of lean and physically active rural children living in South India found no statistically significant association between serum 25(OH)D concentration and CMR after adjusting for age, lifestyle, body fat % and season (Baker et al. 2015). However, high physical activity level (in average 186 min/d) and low BMI were suggested to be protective factors in that study. A cross-sectional study on 114 obese and

non-obese Turkish children and adolescents, found that adiponectin and low serum 25(OH)D concentration were strongly associated with the CMR factors: decreased HDL-C and increased LDL-C, total plasma cholesterol (TC), TG, FPG, homeostatic model assessment of insulin resistance index (HOMA-IR), SBP, diastolic blood pressure (DBP) and BMI (Kardas et al 2013). However, another cross-sectional study among Turkish adolescents did not find an association between the serum 25(OH)D concentration and CMR score (Aypak et al 2014). A cross-sectional study of Portuguese living in Azorean Islands (36.5°-43°N) indicated that teenagers having a higher dietary intake of vitamin D had a lower population-specific CMR score. No study subject was taking vitamin D supplements, and total sun exposure was not measured (Moreira et al. 2014).

In a triple-masked controlled trial among 50 vitamin D deficient Iranian adolescents aged 10-16 years, the intervention group, which received 300 000 IU (7500 µg) of vitamin D per week for 12 weeks, raised its serum 25(OH)D concentration from 45.6 +/- 5.1 nmol/l to 79.9 +/- 5.3 nmol/l and showed a significant improvement of fasting insulin, HOMA-IR, TG and also an improvement of the continuous metabolic score, compared with the baseline and the placebo group (Kelishadi et al. 2014b).

Results for the pediatric population have been inconsistent in finding an association between serum 25(OH)D concentration and the MetS or/and CMR score. However, some studies did not adjust for important confounders including physical activity, season and puberty, which make the results difficult to compare.

2.4.4 Vitamin D and adiposity

Numerous studies have found a negative association between serum 25(OH)D concentration and BMI, obesity, waist circumference or total fat mass and visceral adipose tissue in both children and adolescents in different countries (Reis et al. 2009, Ganji et al. 2011, Pacifico et al. 2011, Rajakumar et al. 2011, Andiran et al. 2012, Kouda et al. 2013, Lee et al. 2012, Nam et al. 2012, Kardas et al. 2013, Kao et al. 2015, Cediél et al. 2016). A plausible reason could be that vitamin D is stored in, and slowly released from fat tissues (Worstman et al. 2000).

Vitamin D deficiency and a lower vitamin D intake have been associated with obesity (Cabral et al. 2016), but cross-sectional studies cannot prove causality, nor the direction of the plausible association. These conditions may coexist together, and the observed association

may be caused by one or more unmeasured factor(s). However, there is some evidence that supplementation of vitamin D early in life may have beneficial effects on the body composition later in life. For instance, in a trial on vitamin D supplementation of 10 µg/d versus 30 µg/d during the first year of life, toddlers who achieved a serum 25(OH)D concentration above 75 nmol/l had a leaner body composition at 3-year-old than toddlers whose serum 25(OH)D concentration was below 75 nmol/l (Hazell et al. 2017).

2.4.5 Vitamin D and glucose metabolism

Children with suboptimal serum 25(OH)D concentration (<30 ng/ml, corresponding to < 75 nmol/l) had a 2-3 times higher risk of being obese, and a 3-4 times higher risk of insulin resistance (Cediel et al 2016). Aypak et al. (2014) found that the association between serum 25(OH)D concentration and insulin resistance was only found in obese pubertal subjects. The association between serum 25(OH)D concentration and insulin resistance might be mediated by body fat mass, as suggested by Alemzadeh et al. (2008).

Some cross-sectional and retrospective studies among children and adolescents have found that an inverse association between serum 25(OH)D concentration and glucose profile, also occurs after adjustment for adiposity (Alemzadeh et al. 2008, Johnson et al. 2010, Ashraf et al. 2011, Ford et al. 2011, Jang et al. 2013, Oliveira et al. 2013, Cediel et al. 2016). A cross-sectional study in adolescents found that for a higher serum 25(OH)D concentration, there was lower fasting insulin, after adjustment for BMI and physical activity (Ford et al.2011). Ashraf et al. (2011) found an inverse association between serum 25(OH)D concentration and FPG and insulin resistance in obese adolescents, after adjustment for BMI and race. Alemzadeh et al. (2008) found an inverse association between serum 25(OH)D and plasmatic glycohemoglobin in adolescents after adjustment for body fat mass. A Brazilian study on teenagers found an inverse correlation between serum 25(OH)D concentration, insulin and HOMA-IR, but did not adjust for puberty (Oliveira et al. 2013). On the other hand, some cross-sectional analysis did not find association between serum 25(OH)D concentration and measures of insulin resistance in 7-9-year-old children (Kwon et al. 2015), adolescents (Erdönmez et al. 2011), and physically active obese children (Poomthavorn et al. 2012).

Puberty is associated with a physiological reduction in insulin sensitivity, independent of adiposity (Hannon et al. 2006). Therefore, previously mentioned results on adolescents should be taken with caution, if no adjustment was made for puberty. Kelly et al. (2011)

found that for a higher serum 25(OH)D, there was a lower FPG, glycohemoglobin and fasting insulin among 4-18-year-old children, after adjusting for BMI and puberty.

Only a few intervention studies on vitamin D supplementation have been made among children and adolescents and most of them included a limited number of subjects. For instance, an intervention study on obese adolescent girls showed that the vitamin D deficient sub-group, who received vitamin D supplementation, improved their FPG (Ashraf et al. 2011). Belenchia et al. (2013) conducted a randomized double-blind controlled trial, where 35 obese American teenagers with vitamin D deficiency received 1000 µg/d for 6 months. The comparison group received a placebo. The treatment group improved their insulin sensitivity and their leptin/adiponectin ratio, which has been suggested as a possible mediator for insulin resistance.

2.4.6. Vitamin D and lipids

A recent systematic review and meta-analysis of serum 25(OH)D concentration and lipids in children and adolescents, mostly composed of cross-sectional studies, showed that for a higher serum 25(OH)D concentration, there was a significantly improved lipid profile (Kelishadi et al 2014a). Congruent with those results, cross-sectional studies made on children and adolescents showed that, for a lower serum 25(OH)D concentration, there was a higher TC and a higher LDL-C (Hassan et al. 2014, Cabral et al. 2016), a higher TG (Rodriguez-Rodriguez et al. 2011), or both (Kelishadi et al. 2014b, Petersen et al. 2015).

Other cross-sectional studies found a positive association between serum 25(OH)D concentration and HDL-C (Rajakumar et al. 2011, Williams et al. 2012). Johnson et al. (2010) found a positive association between 25(OH)D and HDL-C among 2-18 years old children, independent of age, sex, BMI and season. Supportively, Birken et al. (2015) found that for a higher level of vitamin D, there was a better lipid profile (non-HDL-C, TC and TG) among 1 to 5 years old Canadian children, independent of BMI, milk intake and physical activity. To my knowledge, only one randomized control trial showed that vitamin D supplementation in 10-14-year-old children succeeded to increase their HDL-C level (Tavakoli et al. 2016). A cross-sectional analysis of a prospective cohort study, made on 205 Korean 7-9-year-old children, found that for a higher serum 25(OH)D concentration, there was a decreased serum level of TG, after adjustment for age, sex and BMI (Kwon et al. 2015). However, there were no associations between serum 25(OH)D concentration and

HDL-C, LDL-C, or LDL-C/HDL-C ratio (Kwon et al. 2015). Nwosu et al. (2013) found that pre-pubertal subjects with serum 25(OH)D \geq 50 nmol/l had a better lipid profile including lower levels of non-HDL-C, TC/HDL-C ratio and TG than subjects with serum 25(OH)D concentration $<$ 50 nmol/l. However, no adjustment for physical activity was performed.

Some studies found contradictory results. For example, Delvin et al. (2010) found a slightly positive association between serum 25(OH)D and TC, TG and apolipoprotein A-1, but only in girls. It was proposed that those results could be due to sex difference and hormonal sensitivity regarding lipid metabolism (Delvin et al. 2010). Delvin did not control for puberty, which is known to play a role in lipid metabolism and insulin sensitivity. Also, Ashraf et al. (2011) found a positive association between vitamin D and LDL-C in African and Caucasian American obese female adolescents, but this association may have been due to dietary factors.

In a cross-sectional study by Jang et al. (2013), the positive association between serum 25(OH)D and HDL-C became non-significant after adjustment for physical activity and BMI, suggesting that physical activity and visceral fat are important confounders. In conclusion, a higher serum 25(OH)D concentration seems to be associated with a better lipid profile. However, randomized control trials are needed in order to confirm the relationship and prove the possible causality.

2.4.7 Vitamin D and blood pressure

It has been previously showed that blood pressure varies seasonally in children, with a higher SBP and DBP during the winter months in Leipzig, Germany (Miersch et al. 2013). However, the author concluded that it was less likely to be explained by the variation in serum 25(OH)D concentration between seasons. A cross-sectional analysis made on 205 Korean children aged 7-9 years found that for a higher serum 25(OH)D concentration, there was a lower blood pressure (Kwon et al. 2015). A review in children showed that vitamin D deficiency or insufficiency may be associated with hypertension and other pediatric cardiovascular diseases, like orthostatic intolerance and Kawasaki disease (Xu et al. 2017). In a cross-sectional study of obese children and adolescents, vitamin D deficiency was independently associated with a higher SBP and DBP, after adjustment for BMI or total fat mass (Kao et al. 2015). Serum 25(OH)D concentration was inversely correlated with blood pressure among Brazilian teenagers (Oliveira et al. 2013).

2.4.8 Vitamin D and liver enzymes

Ashraf et al. 2011 found that, among African American obese adolescent women, a higher vitamin D level was associated with a higher alanine aminotransferase (ALT). This was not found among Caucasian Americans, suggesting that, in that particular study, ethnicity may have had a potential role in the association, but the plausible explanation remains unclear (Ashraf et al. 2011). Contrary to the results of Ashraf et al. (2011), Nobili et al. (2014) found that vitamin D concentration was lower among 8-18 years old children with non-alcoholic fatty liver disease, suggesting that vitamin D may have a protective role regarding non-alcoholic fatty liver disease, which is usually related to obesity and MetS. Therefore, it is unclear if serum 25(OH)D concentration has a positive or negative association with liver enzymes. To the best of my knowledge, there are no studies on this issue in children.

2.4.9 Vitamin D and inflammation

A pro-inflammatory state has been previously linked with increased cardiovascular risk among subjects with MetS (Devaraj et al. 2009). Vitamin D deficiency has been associated with inflammatory diseases, but a causal role has not yet been established (Yin & Agrawal 2014). In mice, serum 25(OH)D concentration mediated the inhibition of tumor necrosis factor alpha (Cantorna & Mahon 2005) and may have a role in the pathophysiology of inflammatory diseases. Tumor necrosis factor alpha is a pro-inflammatory factor, which is secreted in the adipocytes, and linked to insulin resistance and inflammation (Ruan 2003).

Vitamin D deficiency has been associated with a pro-inflammatory state of the vascular wall, and to vascular endothelial dysfunction (Jablonski et al. 2011). More specifically, Jablonsky et al. (2011) showed that vitamin D deficiency was associated with a decreased flow-mediated dilation of the brachial artery in adults. However, it was not associated with tumor necrosis factor- α . The same study showed that vitamin D deficient subjects had a decreased expression of vitamin D receptor and 1- α -hydroxylase in the vascular endothelium, and an increased expression of the pro-inflammatory nuclear factor NF- κ B in the endothelial cell, and increased expression of the inflammatory factor interleukin 6.

3.AIMS

The aim of the thesis was to analyze the association between serum 25(OH)D and CMR in Finnish children, using the data of the PANIC study at the baseline (2007-2009).

4. METHODS

4.1 Study design

This study is part of the PANIC study, which is a long term controlled intervention study on physical activity and nutrition among pre-pubertal children living in Kuopio, Finland. A total of 736 children aged 6-8 years who started the first grade in 2007-2009 were invited to participate in the study. A total of 512 (70%) children participated in the baseline examinations, held between October 2007 and November 2009. Six children were excluded from the intervention study because of severe physical disability or withdrawal from the study during baseline examinations. Serum 25(OH)D concentration and fasting blood samples were available for 447 children (214 girls, 233 boys) . The study protocol of the original study was approved by the Research Ethics Committee of the Hospital District of Northern Savo. A written informed consent was collected from children and their parents. The data of this thesis are cross-sectional from the baseline of the PANIC study.

4.2 Measurements

4.2.1 Biochemical assessments

Venous blood samples were taken after a 12-hour overnight fasting. Blood was immediately centrifuged and stored at a temperature of -75°C until further analyses except for glucose and lipids that were measured from non-frozen plasma samples.

Serum 25(OH)D concentration

Serum 25(OH)D concentration was analyzed by a chemiluminescence immunoassay called the LIAISON® 25 OH Vitamin D TOTAL Assay (DiaSorin Inc., Stillwater, MN, USA) using an automatic immunoanalyzer (DiaSorin S.p.A., Saluggia, Italy). Total variation, including intra-assay and interassay variation, for the assay is 8.2-11.0% in the concentration range of 21-123 nmol/l.

Cardiometabolic risk factors

Biochemical analyses were done using Cobas 6000 analyzers (Hitachi High Technology Co, Tokyo, Japan). FPG was analyzed using a hexokinase method (Roche Diagnostics Co, Mannheim, Germany). Serum insulin was analyzed by using an electrochemiluminescence

immunoassay with the sandwich principle (Roche Diagnostics Co). Total plasma cholesterol and TG were analyzed by using a colorimetric enzymatic assay (Roche Diagnostics Co). HDL-C and LDL-C were analyzed by using a homogeneous enzymatic colorimetric assay (Roche Diagnostics Co). Plasma high sensitivity C-reactive protein (hs-CRP) was measured using enhanced immunoturbidimetric assay with hs-CRP (Latex) High Sensitive Assay reagent (Roche Diagnostics Co). The limit of quantitation for hs-CRP was 0.29 mg/l. ALT and gamma-glutamyl transpeptidase (GGT) were analyzed by a kinetic method according to the International Federation of Clinical Chemistry (Roche Diagnostics Co). HOMA-IR was calculated as fasting Insulin x FPG/405.

4.2.2 Assessments of body composition

Body composition was measured after overnight fasting, standing in light underwear, with empty bladder, and after removing all metal objects. Body fat mass, total body fat %, and lean body mass were measured using a Lunar dual energy X-ray absorptiometry device (Lunar Prodigy Advance; GE Medical Systems, Madison, WI). In this study, body fat % refers to the measured total body fat %. Body weight was measured twice by a calibrated InBody 720 bioelectrical impedance device (Biospace, Korea) to an accuracy of 0.1 kg. The mean value was used as a weight variable for analyses. Body height was measured three times in the Frankfurt plane without shoes by a wall-mounted stadiometer to an accuracy of 0.1 cm. Waist circumference was measured three times after expiration at mid distance between the lower rib and the iliac crest with a measuring tape to an accuracy of 0.1 cm. The mean of the nearest two values of body height and waist circumference were used for the analyses. BMI was calculated as body weight (kg) divided by body height (m) squared.

4.2.3 Assessment of blood pressure

Blood pressure was measured manually by a calibrated aneroid sphygmomanometer (HEINE GAMMA G7, Germany). After an initial rest of 5 minutes, three measurements of blood pressure at 2-minute intervals were taken in a sitting position. The mean of the three values were used as SBP and DBP.

4.2.4 Cardiometabolic risk score

The CMR score was calculated as the sum of standardized variable (Z-scores) of waist circumference, fasting serum concentration of insulin, FPG, TG and HDL-C and the mean of DBP and SBP. The Z-score of HDL-C was multiplied by -1, because it is inversely associated

with the CMR. A lower CMR score indicates a better CMR profile. The CMR score has been validated in children as an appropriate tool to measure the index of MetS for pediatric epidemiological studies (Shafiee et al. 2013).

4.2.5 Other factors affecting serum 25(OH)D concentration

An exercise specialist or a nutritionist instructed the parents to fill out the PANIC questionnaires that included items on several topics including physical activity, race, parent's education, income (Haapala et al. 2014) and skin types (Soininen et al. 2016).

Food questionnaire

The parents received instructions to record all food and drink consumption of their children at home and outside of home. Schools and afterschool clubs were asked for detailed information about the type and preparation of served food. A food record of 4 consecutive days was collected. A clinical nutritionist made a verification of the records and assisted the parents in filling out any missing information. Records consisting of either 3 weekdays + 1 weekend days or 2 weekdays + 2 weekend days were included in the analyses. Among the 428 returned food questionnaires, 423 (98.8%) were filled out sufficiently and included in the analysis.

The Micro Nutrica dietary analysis software (version 2.5, The Social Insurance Institution of Finland, Turku, Finland) was used to analyze the food records (Eloranta et al. 2011, 2016). Vitamin D intake from diet was calculated from the food records using Micro Nutrica dietary analysis software. The vitamin D from diet was calculated from ingested foods fortified with vitamin D including milk, dairy products and margarines, and from other ingested food containing vitamin D including fish, meat and grain products (Soininen et al. 2016). The use of vitamins and supplements was also assessed by the PANIC questionnaire including brand and dosage of supplements, and frequency of supplement use. Because many children used supplements in series only during winter, an average daily dose of supplements of vitamin D for the 1-month period prior to the blood sampling was calculated.

The Dietary Approach to Stop Hypertension (DASH) diet has proved beneficial at preventing CVD among adults with an increased CMR (Siervo et al. 2015). It is an indicator of a healthy diet, which is defined by a higher consumption of vegetables, fruit, nuts, whole grain and legumes, low-fat dairy products, fish, lean meats and by a lower consumption of red

meat, sugar, salt, saturated fat and cholesterol (National Institute of Health 2006). A score for the DASH was calculated from the food records.

The Dietary fat intake ratio was determined as the ratio of unsaturated to saturated fat. It was calculated as a summation of the proportion of daily energy intake from monounsaturated fatty acids (%E MUFA) and polyunsaturated fatty acids (%E PUFA) divided by the proportion of daily energy intake from saturated fat (%E SFA).

Physical activity

Physical activity and sedentary behavior were assessed by the PANIC Physical Activity Questionnaire administered by the parents at home (Haapala et al. 2014, Väistö et al. 2014, Lampinen et al. 2017). The questionnaire included information on organized sports, supervised exercise organized by sports clubs, unsupervised leisure time- and commuting physical activity, and physical activity during recess at school. The amount of each type of physical activity was calculated by a frequency of the physical activity and time spent for each session of physical activity and was expressed in minutes per day. The questionnaire measured the sum of different types of physical activity performed during an average week. In addition, since 90 minutes per week of physical education during school is compulsory for all first-grade children in Kuopio, it was added to the total physical activity for all children participating in the study. Physical activity performed indoors or outdoors was not calculated separately (Haapala et al. 2014). The PANIC Physical Activity Questionnaire was validated using the Actiheart monitor (Actiheart CamNtech, Cambridge, UK) combining heart rate and accelerometer measurements in a subsample of 38 children examined at the baseline of the PANIC study (Väistö et al. 2014). Total physical activity measured by the questionnaire correlated positively with total physical activity measured by the Actiheart monitor ($r= 0.37$, $p= 0.033$).

Sun exposure

The average daylight time during the 3 months before the blood sampling was calculated using the data provided by the Almanac Office, University of Helsinki (Soininen et al. 2016). Kuopio, Finland, is at a latitude 62.89°N . Blood samples were collected between August and June. The average daylight time was expressed as a continuous variable, giving an approximation of the sun exposure prior to the blood test. Information regarding travels to

sunny locations within 3 months before the blood sampling (no, yes), and sunscreen use (no, occasionally, frequently) were recorded from the questionnaires filled by the parents.

Skin type and race

The skin types and information on race were collected by the PANIC study self-administrated questionnaire, filled by the parents. The Fitzpatrick skin types were defined as type I: always burns, never tans; type II: often burns, sometimes tans; type III: sometimes burns, often tans; type IV: never burns, always tans (Fitzpatrick 1988). A dichotomic variable was created using type 1 and 2, and type 3 and 4 as separate groups. The race was dichotomized as Caucasian or non-Caucasian. Non-caucasian was classified as having at least one non-caucasian parent. Missing data were assumed to be Caucasians, due to the high prevalence (98.7%) of Caucasian in the studied population.

Socio-economic factors

The parental education and household income was assessed by the PANIC study self-administrated questionnaire, filled by the parents. The level of education was set as the highest completed or ongoing degree (vocational school or less, university of applied science, university). A dichotomous variable was formed as 1) vocational school or less and vocational high school and 2) university. The reason behind that choice was because children from households with university education had a statistically significantly higher serum 25(OH)D than children from households with a university of applied science degree or from households with a vocational school degree or less. Parental education was chosen as the predominant factor reflecting the socio-economic status of the subjects. Household income was also collected from the questionnaire filled by the parents, but was excluded from the statistical analysis, to avoid multicollinearity.

Pubertal status

A research physician performed a physical examination of all children and assessed the pubertal status. The children were classified as having entered puberty if their Tanner stage was > 1 . Central puberty was defined as breast development at Tanner stage ≥ 2 for girls and testicular volume ≥ 4 ml assessed using an orchidometer for boys (Marshall & Tanner 1969, 1970). A dichotomic variable classified as having entered puberty 1) no 2) yes was created and used in the model 3 of the statistical analysis.

4.3 Statistical analysis

A statistical analysis was performed using SPSS software for Windows, version 25 (Chicago, IL). In all performed analyses, a p-values of ≤ 0.05 were considered statistically significant.

Differences between girls and boys were tested with independent samples t-test for normally distributed variables, Mann-Whitney's U test for skewed variables and Pearson's χ^2 test for categorical variables. Logarithmic transformation was performed for body weight, BMI, waist circumference, body fat %, total vitamin D intake from diet and supplements one month prior to the blood sampling, dietary fat intake ratio, serum 25(OH)D, ALT, GGT, HDL and plasma TG before analyses. Square-root transformation was performed for SBP, total physical activity, TC, LDL-C, FPG and HOMA-IR.

Statistical analysis for serum 25(OH)D and the components of CMR

To find a possible association between serum 25(OH)D concentration and the different components of the CMR, I used three different linear logistic regression models, in which serum 25(OH)D concentration was included as an independent variable. In each model, analysis was made separately for each component of the CMR, set as the dependent variable. Those included lipids, liver enzymes, insulin resistance, blood pressure, inflammation markers and the CMR score.

The first two regression models, model 1 and 2, were built according to predetermined confounders based on literature. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, physical activity, DASH-score, and body fat %.

The third model, or model 3, was created using a backward elimination method in a stepwise multiple linear regression model. The independent variables included in the stepwise backward elimination method were the serum 25(OH)D concentration, age, sex, race, physical activity, body fat %, dietary fat intake ratio, average vitamin D intake from diet and supplement one month prior to the blood sampling, average length of the day three months before the blood sampling, skin types, travel to sunny location three months before blood sampling, use of sunscreen, race, education of the parents and puberty. The least significant independent variable was removed from the model after each regression analysis, until only

statistically significant independent variables were left in the model. Serum 25(OH)D concentration was a variable of interest and was included in the final model. The standardized beta of 25(OH)D was reported. This backward elimination method was repeated separately for each component of the CMR and the standardized beta of 25(OH)D for each component of the CMR was reported for each backward model. Each final regression model was checked for multicollinearity.

Statistical analysis for serum 25(OH)D and hs-CRP groups

In adults, hs-CRP <1.0 mg/l correspond to a low risk of developing CVD diseases (<10%), a hs-CRP 1.0-3.0 mg/l correspond to a moderate risk (10-20%), and an hs-CRP >3.0 mg/l correspond to a high risk (>20%) of developing CVD (Pearson et al. 2003). In the literature, there were no hs-CRP reference values defined for children. Therefore, I based cut-off values for hs-CRP categories according to the threshold values in adults.

The variable hs-CRP had a positively skewed distribution due to the fact that most children had an hs-CRP below the detectable value (0.29 mg/l). Therefore, hs-CRP could not be used as a continuous variable. A dichotomized hs-CRP variable was defined as a low risk group for subjects of hs-CRP <1.0 mg/l versus moderate- and high risk groups for subjects of hs-CRP >1.0 mg/l. Children with hs-CRP level of more than 5.0 mg/l were excluded due to possible inflammatory conditions or infections.

The possible association between serum 25(OH)D concentration and the hs-CRP values between low risk (hs-CRP <1.0mg/l) and moderate and high risk for CVD (hs-CRP 1.0-5.0 mg/l) was tested through a multiple linear regression analysis. Three multiple linear regression models were built, using the logarithmic value of serum 25(OH)D concentration as the continuous dependent variable. Independent variables were the dichotomous variable of hs-CRP and variables of different confounders based on literature: age and sex for model 1, age, sex, physical activity, body fat % and DASH-score for model 2. Model 3 was built using a backward elimination method in the stepwise multiple linear regression model, where age, sex, race, physical activity, body fat %, dietary fat intake ratio, average vitamin D intake from diet and supplement one month previous to the blood sampling, average length of the day three months before the blood sampling, skin types, travel to sunny location three months before blood sampling, use of sunscreen, race, education of the parents and puberty were

included in the initial model. Only statistically significant variables were kept in the final model. Results of the associations were expressed as standardized regression coefficient Beta.

5. RESULTS

5.1 Characteristics of the study population.

The study population included 214 girls (47.9%) and 233 boys (52.1%) (Table 3). The mean age was 7.6 years (range 6.6 - 9.0 years). The majority of the children were Caucasian 98.7%. Parents of children were in general well educated, with 20.4% having a vocational education or less, 45.4% having university of applied science degree and 33.7% having a university degree. Most children (97.5%) had not entered puberty at the time of the study. Children had a mean BMI of 16.1 ranging from 12.8 to 24.6, and a median of 15.6 (IQR 14.7, 17.0). There were no differences in race, parental education, household income, pubertal status and BMI between girls and boys. Boys were taller, slightly heavier, and had a smaller waist circumference and a body fat % than the girls. The median vitamin D intake was 6.4 µg/d (Table 4). There was no statistical difference in vitamin D intake between girls and boys. The boys had a lower DASH-score than the girls. There was no difference in the amount of SFA, MUFA and PUFA consumption between girls and boys.

A total of 19.5% of children had a serum 25(OH)D concentration <50 nmol/l and 69% had a serum 25(OH)D concentration <75 nmol/l (not shown). Serum 25(OH) D concentration varied from 19.4 nmol/l to 199.0 nmol/l, with a mean of 68.0 nmol/l (median 66 nmol/l) (Table 5). Boys were physically more active than the girls. Boys used less frequently sunscreen than girls. Less than 10% of children had travelled to sunny countries 3 months prior to blood sampling. Girls and boys equally travelled to sunny countries. There were no differences in skin pigmentation between girls and boys. There was an equal average daylight time 3 month prior to blood test in both sexes.

Boys had a lower TG, fasting insulin, a slightly lower HOMA-IR and a higher FPG than the girls (Table 6). There was no difference in TC, HDL-C, LDL-C, ALT, GGT, CMR score, SBP and DBP between girls and boys. The median hs-CRP was low (0.2900). The median value of hs-CRP was the same for girls and boys (0.29 mg/l), however, there was a statistically significant difference in the distribution of absolute hs-CRP between girls and

boys, favoring boys for lower hs-CRP values (table 7). There were an equal distribution of girls and boys among hs-CRP categories (<1.0 mg/l vs 1.0-5.0 mg/l).

Table 3. Socio-economic and anthropometric characteristics of children and difference between girls and boys.

	All (n=447) ¹	Girls (n=214)	Boys (n=233)	p ³
Age (y)	7.6 (0.4) ²	7.6 (0.4)	7.7 (0.4)	0.111
Race				
Caucasians	376 (98.7%)	182 (98.4%)	194 (99.0%)	0.677
Non-Caucasians	5 (1.3%)	3 (1.6%)	2 (1.0%)	
Parental education				
Vocational high school or less	293 (66.3%)	153 (72.2%)	140 (60.9%)	0.016
University	149 (33.7%)	59 (27.8%)	38.6 (39.1%)	
Household income				
≤60 000 €/y	283 (65.1%)	146 (69.5%)	137 (60.9%)	0.070
>60 000 €/y	152 (34.9%)	64 (30.5%)	88 (39.1%)	
Pubertal status				
Pre-pubertal	430 (97.5%)	204 (96.2%)	226 (98.7%)	0.129
Pubertal	11 (2.5%)	8 (3.8%)	3 (1.3%)	
Height (cm)	129.0 (125.5-132.0) ²	128.1 (123.6 - 131.0)	130.2 (126.9 -132.7)	<0.001
Weight (kg)	26.0 (23.5 – 29.3)	25.4 (23.2 – 29.0)	25.6 (23.9 – 29.6)	0.047
BMI (kg/m ²)	15.6 (14.7 – 17.0)	15.7 (14.6 – 17.0)	15.6 (14.7 – 17.0)	0.894
Waist circumference (cm)	55.6 (53.0 - 59.0)	55.6 (52.3 - 58.2)	54.8 (53.6 - 59.5)	0.004
Body fat %	18.7 (13.3 - 24.1)	20.7 (17.4 - 27.2)	15.1 (11.4 - 21.5)	<0.001

IQR, interquartile range; SD, standard deviation; BMI, body mass index; body fat %; n=number of children; ¹n= varies between 435 and 447 in different variables: n=447 (214 girls, 233 boys) for age, height and waist circumference; n= 446 (214 girls, 232 boys) for BMI; n=435 (209 girls, 226 boys) for body fat %. ²The values are means (SDs) for normally distributed variables: age; medians (IQR_s) for skewed variables: height, weight, BMI, waist circumference and body fat %; or number of subjects (%) for categorical variables. ³Differences between girls and boys were tested with independent samples t-test for normally distributed variables, Mann-Whitney's U test for skewed variables and Pearson's χ^2 test for categorical variables. Logarithmic transformation was performed for body weight, BMI, waist circumference and body fat %.

Table 4. Dietary intake in boys and girls

	All (n=432) ¹		Girls (n=209)		Boys (n=223)		p ²
Total Vitamin D intake ³	6.4	(4.7 – 9.1) ⁴	6.1	(4.7 – 8.4)	6.9	(4.8 – 9.6)	0.128
DASH-Score	21.0	(18.0 – 24.0)	22.0	(19.0 – 25.0)	21.0	(18.0 – 24.0)	0.004
Dietary fat intake ratio in diet	1.3 (0.3) ⁴		1.3 (0.3)		1.3 (0.3)		0.749
SFA, % of total energy	12.1 (2.7)		12.0 (2.7)		12.2 (2.7)		0.686
MUFA, % of total energy	9.9 (1.8)		9.8 (1.8)		10.0 (1.8)		0.401
PUFA, % of total energy	4.9 (1.3)		4.9 (1.3)		5.0 (1.3)		0.610

IQR, interquartile range; DASH-score, Dietary Approach to Stop Hypertension Score; Dietary fat intake ratio in diet calculated as (MUFA+PUFA)/SFA; SD, standard deviation; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ; SFA, saturated fatty acids; %E, % of total energy; n, number of children. ¹ n varies between 372 and 432 between variables. n=372 (183 girls, 189 boys) for DASH score, dietary fat intake ratio, SFA %E, MUFA %E and PUFA %E; n=432(209 girls, 223 boys) for total vitamin D intake. ² Differences between girls and boys were tested with independent samples t-test for normally distributed variables, Mann-Whitney's U test for skewed variables and Pearson's χ^2 test for categorical variables. Logarithmic transformation was performed for total vitamin D intake, dietary fat intake ratio in diet before analyses; ³ average intake of vitamin D ($\mu\text{g}/\text{d}$) from diet and supplements, 1 month before the blood sampling; ⁴The values are presented as means (SDs) for normally distributed variables: dietary fat intake ratio in diet, SFA E%, MUFA E%, PUFA E% and medians (IQR_s) for skewed variables: total vitamin D intake and DASH score.

Table 5. Serum 25(OH)D concentration, sun exposure and other confounding factors of serum 25(OH)D.

	All (n=447) ¹		Girls (n=214)		Boys (n=233)		p ²
Serum 25(OH)D concentration (nmol/l)	65.8	(52.0 – 79.0) ³	64.4	(53.1 – 74.9)	67.3	(50.4 – 83.5)	0.124
Average daylight time during 3 months before blood sampling (h/d)	11.0	(7.2 – 13.8)	11.0	(7.5 – 14.4)	10.9	(7.1 – 13.6)	0.257
Travel to sunny countries							
yes	28 (8.7%) ³		13 (8.2%)		15 (9.3%)		
no	293 (91.3%)		146 (91.8%)		147 (90.7%)		0.844
Sunscreen use							
Occasionally or frequently	257 (79.1%)		140 (87.5%)		117 (70.9%)		
No	68 (20.9%)		20 (12.5%)		48 (29.1%)		<0.001
Fitzpatrick skin type ⁴							
I or II	93 (28.8%)		49 (30.8%)		44 (26.8%)		
III or IV	230 (71.2%)		110 (69.2%)		120 (73.2%)		0.462
Physical activity (min/d)	107.1	(77.1 – 139.4)	100.0	(72.6 – 130.5)	115.7	(85.0 – 153.0)	<0.001

IQR, interquartile range; 25(OH)D, 25-hydroxyvitamin D; n=number of children. n=447 children, 214 girls and 233 boys unless otherwise specified

¹ n varies between 442 and 447 between variables. n=447 (214 girls, 233 boys) for serum 25(OH)D concentration and average daylight time during 3 months before blood sampling; n=442 (210 girls, 232 boys) for physical activity. ² Differences between girls and boys were tested with independent samples t test for normally distributed variables, Mann-Whitney's U test for skewed variables and Pearson's χ^2 test for categorical variables. Logarithmic transformation was performed for serum 25(OH)D concentration. Square-root transformation was done for total physical activity; ³The values are presented as medians (IQR_s) for skewed variables: serum 25(OH)D concentration, daylight time and physical activity; and as mean (%) for categorical variables: travel to sunny locations, sunscreen use, skin types. ⁴ defined as type I: always burns, never tans; type II: often burns, sometimes tans; type III: sometimes burns, often tans; type IV: never burns, always tans.

Table 6 Metabolic factors and difference between boys and girls

	Median ²	All (n=447) ¹ IQR ² (25; 75 percentile)	Median	Girls (n=214) IQR (25, 75 percentile)	Median	Boys (n=233) IQR (25, 75 percentile)	p ³
TC (mmol/l)	4.2	(3.9, 4.7)	4.3	(3.9, 4.7)	4.2	(3.8, 4.7)	0.154
HDL-C (mmol/l)	1.6	(1.4, 1.8)	1.6	(1.4, 1.7)	1.6	(1.4, 1.8)	0.084
LDL-C (mmol/l)	2.3	(2.0, 2.6)	2.4	(2.0, 2.7)	2.3	(2.0, 2.6)	0.093
TG (mmol/l)	0.6	(0.4, 0.7)	0.6	(0.5, 0.7)	0.5	(0.4, 0.7)	0.040
ALT (U/l)	18.0	(15.0, 21.0)	18.0	(15.0, 21.0)	18.0	(15.0, 22.0)	0.424
GGT (U/l)	11.0	(10.0, 13.0)	11.0	(10.0, 13.0)	12.0	(10.0, 13.0)	0.486
FPG (mmol/l)	4.8	(4.6, 5.0)	4.8	(4.6, 4.9)	4.9	(4.7, 5.1)	<0.001
Fasting insulin (mU/l)	4.1	(2.8, 5.7)	4.6	(3.4, 6.2)	3.9	(2.5, 5.4)	0.003
HOMA-IR	0.89	(0.59, 1.28)	0.98	(0.68, 1.32)	0.85	(0.53, 1.19)	0.022
CMR score mean (SD) ²	0.60	(3.5244)	0.386	(3.5237)	-0.0236	(3.5324)	0.853
SBP (mmHg)	100.0	(95.3, 105.3)	100.0	(94.7, 104.7)	100.4	(96.0, 105.3)	0.515
DBP (mmHg)	61.3	(57.3, 66.7)	61.3	(57.3, 66.0)	61.3	(57.3, 66.7)	0.535

IQR, interquartile range; TC, total plasma cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; FPG, fasting plasma glucose; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance Index; QUICKI, quantitative insulin sensitivity check index; CMR, cardiometabolic risk; SD standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; n=number of children. ¹varies between 442 and 447 between variables: n= 447(214 girls, 233 boys) for TC, HDL-C, LDL-C; TG, ALT, GGT and FPG; n=446 (213 girls, 233 boys) for SBP and DBP; n=443 (211 girls, 232 boys) for HOMA-IR and QUICKI; n=442 (210 girls, 232 boys) for CMR score. ²The values are means (SDs) for normally distributed variables, medians (IQR_s) for skewed variables, or number of subjects (%) for categorical variables. ³Differences between girls and boys were tested with independent samples t-test for normally distributed variables, Mann-Whitney's U test for skewed variables. Logarithmic transformation was performed for ALT, GGT, HDL-C, TG, and QUICKI before statistical analysis. Square-root transformation was performed for TC, LDL-C, FPG, HOMA-IR and SBP.

Table 7 Measure of inflammation and difference between boys and girls

	All (n=435) ¹	Girls (n=207)	Boys (n=228)	p ²
Hs-CRP	0.2900 (0.2900, 0.5500) ³	0.2900 (0.2900, 0.6200)	0.2900 (0.2900, 0.4675)	0.005
hs-CRP low risk group ⁴	376 (86.2%)	175 (84.1%)	201 (88.2%)	
hs-CRP moderate and high risk group ⁵	60 (13.8%)	33 (15.9%)	27 (11.8%)	0.266

IQR, interquartile range; hs-CRP, high-sensitivity C-reactive protein; NC, non-calculable due to small number of subjects; n= number of subjects. ¹n varies between 9 and 376 between groups: n= 376 subjects (175 girls, 201 boys) for hs-CRP low risk category; n=51 (27 girls, 24 boys) for hs-CRP moderate risk category; n= 9 (6 girls, 3 boys) for hs-CRP high risk category. n=435 (207 girls, 228 boys) for all hs-CRP categories. ² statistical difference between girls and boys was tested with Mann-Whitney's U t-test for hs-CRP and pearson chi square test for difference in the amount of girls and boys between different categories of hs-CRP. ³The values are presented as medians (IQR_{s 25;75 percentile}) for skewed continuous variables: hs-CRP and as mean (%) for categorical variables: hs-CRP low, moderate and high risk group. ⁴hs-CRP low risk:<1.0 mg/L, ⁵hs-CRP moderate and high risk group: 1.0-5.0 mg/L

5.2. Associations between serum 25(OH)D concentration and cardiometabolic risk

A higher serum concentration of 25(OH)D was associated with a lower SBP, TC, LDL-C, HDL-C, TG, and with a higher ALT, after adjustment for age and sex (Table 8, Model 1).

A higher serum concentration of 25(OH)D was associated with a lower SBP, TC, LDL-C, HDL-C, and with a higher ALT, after adjustment for age, sex, physical activity, DASH-score and body fat % and TG became not statistically significant (Table 8, Model 2). Serum 25(OH)D concentration was not statistically significantly associated with the CMR score, height, BMI, waist circumference, body fat %, DBP, FPG, fasting insulin and measures of insulin resistance when adjusted for age and sex (Table 8, Model 1), and when adjusted for age, sex, physical activity, DASH-score and total body fat % (Table 8, Model 2).

A higher serum concentration of 25(OH)D was associated with a lower SBP, TC, LDL-C, HDL-C, TG, and with a higher ALT after proceeding to a stepwise backward elimination method including, in the final model, the most relevant confounders specific for each component of the CMR (Table 8, Model 3). In Model 3, the significant confounders were sex, body fat %, dietary fat intake ratio and puberty for SBP; body fat %, puberty and length of the day for TC; body fat % and puberty for LDL-C and HDL-C; body fat %, length of the day and physical activity for TG; and body fat %, sex and physical activity for ALT.

A multiple linear regression analysis, testing the association of categories of hs-CRP to serum 25(OH)D showed that having a hs-CRP between 1.0-5.0 mg/l was associated with a higher serum 25(OH)D concentration, than having a hs-CRP < 1.0 mg/l, after adjustment for age and sex ($p=0.005$) (Table 9). Further adjustments for physical activity, DASH-score and body fat % did not affect the association ($p=0.008$). A multiple linear regression using the stepwise backward method confirmed similar results, after adjustments for most relevant confounders, which were in model 3 age and vitamin D intake.

Table 8. The associations between the serum concentration of 25(OH)D and the different components of the metabolic risk.

Components of the metabolic risk	Model 1 ¹	Model 2 ²	Model 3 ³
Weight (kg)	0.062	0.052	
Height (cm)	0.058	0.046	
BMI (kg/m ²)	0.051	0.050 ⁴	
Waist circumference	0.061	0.056 ⁴	
Body fat %	0.002	0.004 ⁴	
SBP (mmHg)	-0.103*	-0.144**	-0.151**
DBP (mmHg)	0.037	0.043	
TC (mmol/l)	-0.194 ***	-0.212***	-0.191***
HDL-C (mmol/l)	-0.165 ***	-0.142**	-0.184***
LDL-C (mmol/l)	-0.160 ***	-0.196***	-0.151***
TG (mmol/l)	-0.123 **	-0.099	-0.114*
ALT (mmol/l)	0.145 **	0.121*	0.133**
GGT (mmol/l)	0.000	0.017	
FPG (mmol/l)	0.034	0.033	
fasting insulin (mU/l)	0.009	0.013	
HOMA-IR	0.001	0.005	
CMR score	0.012	0.005	

*P≤0.05 **P≤0.01 ***P≤0.001; 25(OH)D, 25-hydroxyvitamin; DASH-score, Dietary Approach to Stop Hypertension Score; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total plasma cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; FPG, fasting plasma glucose; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance Index; CMR, cardiometabolic risk; Multiple linear regression models were performed. Results were expressed as standardized coefficient beta for serum 25(OH)D concentration. ¹ Model 1 was adjusted for age and sex; ² Model 2 was adjusted for age, sex, physical activity, DASH-score and total body fat %. ³ Model 3 was built using a stepwise backward elimination method: SBP was adjusted for total body fat %, sex, Dietary fat intake ratio and puberty; TC was adjusted for total body fat %, puberty and length of the day; HDL-C was adjusted for total body fat % and physical activity; LDL-C were adjusted for total body fat % and puberty; TG were adjusted for length of the day and physical activity; ALT was adjusted for total body fat %, sex and physical activity. ⁴ adjusted for age, sex, DASH-score and physical activity. n= number of subjects. In model 1, n=447 for height, TC, LDL-C, HDL-C, TG, ALT, GGT, FPG, n= 446 for weight, BMI, SBP, DBP, n= 443 for fasting insulin, n=442 for CMR score, n=435 for body fat %. In model 2, n= 365 for height, waist circumference, total body fat %, FPG, TC, LDL-C, HDL-C, TG, ALT, FPG; n=364 for weight, BMI, SBP, DBP; n= 362 for fasting insulin; n=361 for CMR score. In model 3, n=429 for TC, LDL-C; n=433 for HDL-C, TG, ALT; n=358 for SBP.

Table 9. Multiple linear regression for the association of serum 25(OH)D concentration and the hs-CRP in categories, expressed as standardized linear coefficient Beta

	Model 1 ¹		Model 2 ²		Model 3 ³	
	Beta	p	Beta	p	Beta	p
Low hs-CRP group compared with the Moderate- and High hs-CRP group ⁴	0.135	0.005	0.142	0.008	0.152	0.001

¹adjusted for age and sex, ²adjusted for age, sex, physical activity, DASH-score and body fat % ³stepwise backward regression where age, vitamin D intake and CRP categories were including in the final model.

⁴Low hs-CRP : 0.29-0.99 mg/l (corresponding to a low risk of developing CVD in adults)

Moderate hs-CRP: 1.00-3.00 mg/l (corresponding to a moderate risk of developing CVD in adults)

High hs-CRP : 3.01-5.00 mg/l (corresponding to a high risk of developing CVD in adults)

⁵n= 436 for model 1, n= 358 for model 2, n= 244 for model 3.

6. DISCUSSION

6.1 Main findings

For a higher serum 25(OH)D concentration, there was a lower level of SBP, TC, LDL-C, HDL-C, TG, and a higher level of ALT. Only the association of 25(OH)D with TG was partly explained by confounding factors, which included age, sex, BMI, physical activity and DASH-score. Children with higher hs-CRP (1.0-5.0 mg/l) had higher serum 25(OH)D concentration than children with lower hs-CRP (<1.0 mg/l).

The present study did not find an association between serum 25(OH)D concentration and BMI, waist circumference, height, body fat % or DBP. Serum 25(OH)D was neither associated with FPG, fasting insulin or the parameters of insulin resistance.

6.2 Serum 25(OH)D and the cardiometabolic risk

A similar study on serum 25(OH)D concentration and CMR was performed among 8-11-year-old Danish children by Petersen et al. (2015). Their results were adjusted for age, sex, height, ethnicity, puberty, parental education, fat mass, physical activity and serum concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). They found a positive association between serum 25(OH)D concentration and TC, LDL-C and TG, which is in line with the results of this thesis. Additionally, they found a positive association of serum 25(OH)D concentration with DBP and CMR, while in this thesis no statistically significant association was found. A systematic review found an inverse association between serum 25(OH)D concentration and SBP but found inconsistent association between serum 25(OH)D concentration and lipid profile and measures of insulin and glucose metabolism (Dolinsky et al. 2012). Dong et al. (2010) found that vitamin D supplementation of 100 µg/d among black American adolescents succeeded in decreasing arterial stiffness, compared to the placebo group, who received 50 µg/d of vitamin D. There is need for more randomized control trials in order to assess the roles of serum 25(OH)D concentration and vitamin D intake on the CMR.

Interestingly the results of this thesis bring contradictory findings regarding the association of serum 25(OH)D and CMR. On one hand, this thesis found that serum 25(OH)D is linked with a cardioprotective state with lower TC, LDL, TG and SBP, which is in line with previous

studies (Rodriguez-Rodriguez et al. 2011, Oliveira et al. 2013 Hassan et al. 2014, Kelishadi et al. 2014b, Birken et al. 2015, Kao et al. 2015, Petersen et al. 2015, Cabral et al. 2016). On the other hand, this thesis found that a higher serum 25(OH)D concentration is linked with a pro-inflammatory and atherogenic state with higher hs-CRP and a lower HDL. This is in contradiction with some of the previous studies (Johnson et al. 2010, Jablonsky et al. 2011, Tavakoli et al. 2016).

The results of this thesis also show that for a higher 25(OH)D concentration, there was a higher liver enzyme ALT. This is in line with the results of Ashraf et al. (2011). However, Nobili et al. (2014) found the opposite. There is some evidence that vitamin D deficiency is associated with nonalcoholic fatty liver disease among adults (Barchetta et al. 2017). A randomized control trial on vitamin D supplementation in children found that the combined treatment of DHA and vitamin D successfully reduced insulin –resistance, lipid profile, ALT and nonalcoholic fatty liver disease score (Della Corte et al. 2016).

6.2.1 Serum 25(OH)D concentration and visceral adiposity

Although obesity has been previously associated with a lower serum concentration of 25(OH)D (Cabral et al. 2016, Cediél et al. 2016), this thesis did not find any statistically significant relation between serum 25(OH)D and total body fat mass %, BMI, and waist circumference.

Only 14 % of girls and 10% of the boys in this population sample of prepubertal children aged 6-8 years were overweight or obese (Viitasalo et al. 2012). It is possible that children with a slightly higher BMI also had a higher muscular mass. However, in this thesis, BMI was highly correlated with body fat %, even after adjustment for age, sex, physical activity and DASH-score.

Interestingly, the studied children had an important variability in their body fat %, ranging from 5 to 45%, with a median of 19%. This thesis used body fat % as an estimate of the visceral adiposity. Although body fat % has previously been found to correlate with visceral adiposity among children with risk of obesity (Saelens et al. 2007), it is not sure if this correlation would persist among the mostly normal weight children of the PANIC study. Also, the use of body fat % does not measure the possible effects of fat free mass, height, body proportion, height (Weber et al.2012) and fat distribution in metabolic diseases.

Therefore, the use of total body fat % might not be an accurate measurement of visceral adiposity regarding the CMR in this study population of normal weight children.

6.2.2 Serum 25(OH)D concentration and glucose metabolism

This present study found no association between serum 25(OH)D and glucose metabolism and measurements of insulin resistance. This is in contradiction with previous findings. For instance, Cediél et al. (2016) found that higher serum concentration of 25(OH)D was associated with a lower insulin resistance (fasting insulin, HOMA-IR and Quantitative Insulin Sensitivity Check Index among 6-8-year-old children, after adjustment for age, sex, season and BMI. Jang et al. (2013) found a higher FPG in children with a lower 25(OH)D, after adjustment for BMI and physical activity. Johnson et al. (2010) also found an inverse correlation between serum 25(OH)D concentration and FPG in children aged 2-18 years, after adjustment for age, sex, BMI and season.

The reason for discrepancy between the results of this thesis and other studies regarding serum 25(OH)D and glucose metabolism might be partially explained by the low prevalence of overweight and obesity of the participants of the PANIC study. Moreover, the children of the PANIC study were mainly prepuber, therefore, were not yet influenced by the physiological insulin resistance related to puberty.

Physical activity and cardiorespiratory fitness has been previously found to play a protective role regarding measurements of insulin resistance (Jiménez-Pavón D et al. 2013, Väistö et al. 2014, Delisle Nyström et al. 2017) and of fasting glucose (Vaara 2015). In this thesis, 25(OH)D was not associated with markers of glucose metabolism even when the results were adjusted for physical activity. Furthermore, physical activity did not correlate with serum 25(OH)D concentration, despite the fact that physical activity may have been performed outside, and may have been a surrogate value for sun exposure. However, it is important to notice that the variable physical activity used in this thesis did not differentiate between indoor-and outdoor physical activity in different seasons of the year. Interestingly, previously published results from the same study population, showed an inverse association between physical activity and insulin resistance, although did not adjust for vitamin D (Väistö et al. 2014). It is possible that physical activity may be a stronger determinant for glucose metabolism than vitamin D.

Indeed, Belenchia et al. (2013) found that 100 µg daily intake of vitamin D₃ improved the measurements of insulin resistance among obese American adolescents, compared with a group receiving a placebo. Belenchia et al. (2013) proposed that a possible mechanism could be that vitamin D improves peripheral and hepatic reuptake of glucose. Although this thesis did not find significant association between serum 25(OH)D and measures of insulin resistance, it is theoretically possible that vitamin D has a role to play in glucose metabolism and insulin resistance.

For instance, vitamin D receptor has been shown to influence the genes related to HOMA-IR (Carlsberg & Molnár 2015). Vitamin D receptor has been found in the pancreas beta-cells, and it has been suggested that adequate serum 25(OH)D concentration facilitates the biosynthetic capacity of beta-cells of the pancreas, by increasing the conversion rate of proinsulin into insulin (Martini et al. 2006). As previously mentioned, some individuals need a higher serum concentration of 25(OH)D (high-responders) than other individuals (low-responders) in order to have the same indirect activation of the genome (Saksa et al. 2015). In this thesis, the lack of association between serum 25(OH)D and glucose metabolism could be due to individual differences in threshold of vitamin D in order to activate the vitamin D receptor related to glucose metabolism and insulin secretion. This thesis did not include genetic information, although data was available. In this study population of children, it would be therefore interesting to find which threshold of serum 25(OH)D concentration would activate the genetic expression of factors related to insulin secretion and glucose metabolism.

6.2.3 Serum 25(OH)D concentration and lipids

Serum 25(OH)D concentration and TC, LDL-C and TG

This study found an inverse association between serum 25(OH)D concentration and TC, LDL-C and TG. Kelishadi et al. (2014b), Birken et al. (2015) and Petersen et al. (2015) found similar results among children and adolescents. Congruently, Hassan et al. (2014) and Cabral et al. (2016) found an inverse association between serum 25(OH)D and TC and LDL-C and some studies found an inverse association only for TG in children (Rodriguez-Rodriguez et al. 2011, Kwon et al. 2015 and Nwosu et al. 2013). A systematic review with meta-analysis, reported that serum 25(OH)D concentration was associated with a better lipid profile in children and adolescents (Kelishadi et al. 2014a). Similar results were reported in a

systematic review on adults (Jorde et al. 2011). Inversely, a review made by Dolinsky et al. (2012) found a lack of association between serum 25(OH)D and lipids in most of the studies. The possible mechanism on how serum 25(OH)D concentration may be related to lipid syntheses and metabolism is complex. One possible mechanism may possibly be that the circulating level of 25(OH)D and ultraviolet light down-regulates 7-dehydrocholesterol reductase, the enzyme that converts 7-dehydrocholesterol into cholesterol in all tissues, including skin, as suggested by Zou & Porter (2015). According to Zou & Porter (2015), 25(OH)D had a “lesser” effect on 7-dehydrocholesterol reductase, while 1,25(OH)₂D had no effect.

The 7-DCH, stored in the skin, is the substrate for both cholesterol and cholecalciferol (pre-vitamin D₃) (Zou & Porter 2015). Therefore, since both ultraviolet light and DHCR7 compete for the same substrate, it is possible that shifts toward the production of vitamin D₃ instead of cholesterol explains partly why serum 25(OH)D concentration is inversely associated with serum level of TC. In that respect, it would be interesting to know to which extent the skin is contributing to the TC production. It would also be relevant to find if there is a difference between the sources of vitamin D (diet versus UV-radiation) regarding the way it inhibits DHCR7 enzyme in skin and in other tissues, and consequently, down-regulate the synthesis of cholesterol.

Secondly, the role of serum 1,25(OH)₂D and parathormone in the lipid metabolism could be mediated by their action on the lipoprotein lipase (Querfeld et al. 1999). Lipoprotein lipase is found in the adipocytes, skeletal muscle, hearth, capillaries and arteries, and it is an important enzyme, which hydrolyses TG into free fatty acids and lipoproteins, leaving a substrate for HDL-C. Lipoprotein lipase also induces the process of VLDL conversion into LDL-C and helps remove chylomicrons from the circulation. It has been found that adipocytes have both vitamin D receptor and parathormone receptors, but those seem to have opposite effects on the production of lipoprotein lipase; 1,25(OH)₂D directly increases the production of lipoprotein lipase, and parathormone directly suppresses the production of lipoprotein lipase (Querfeld et al. 1999). Since vitamin D deficiency is often accompanied by an increased parathormone and a normal or slightly increased 1,25(OH)₂D (Kennel et al. 2010), it is possible that vitamin D deficiency would favor a lipid profile with higher TG, through a decreased production of lipoprotein lipase in the adipocyte. However, this theory of suppressed action of lipoprotein lipase seen in vitamin D deficiency does not explain the

positive association between serum 25(OH)D and LDL-C and HDL-C observed in this thesis. The study of Querfeld (1999) was made on cultured adipocytes, therefore could not account for the other interactions influencing the lipid synthesis and metabolism in-vivo. Therefore, the previously stated hypothesis between serum 25(OH)D concentration and lipids should be taken with caution.

This is a cross-sectional study; therefore, a causal effect cannot be proven. In this study, adjustment for the score of a healthy diet (DASH score) and other confounding factors age, sex, physical activity and body fat % did not explain the association between 25(OH)D with lipids. However, it is possible that the association between serum 25(OH)D concentration and lipids is due to other factors, for instance unmeasured dietary factors, such as EPA and DHA.

Serum 25(OH)D concentration and HDL-C

Interestingly the children participating in the PANIC study had a positive association between serum 25(OH)D concentration and HDL-C, which is contradictory with what has been previously published in the literature. In their review, Dolinsky et al. (2012) found that, among the 14 cross-sectional studies made on serum 25(OH)D concentration and HDL-C, 13 showed a positive association between HDL-C and serum 25(OH)D concentration, and one, conducted by Kelishadi et al. (2014b), reported non-significant results.

It is possible that vitamin D deficiency (with normal or higher 1,25(OH)₂D) would influence the serum level of HDL-C, through an inhibition of lipolysis in the adipocytes. The 1,25(OH)₂D, but not parathormone, inhibits lipolysis in human adipocytes, in vitro, through an increased entry of calcium into the cells (Zemel et al. 2000). Verghese et al. (2007) found that lipolysis is associated with a conversion and a flowing out of stored cholesterol into HDL-C. Therefore, vitamin D deficiency may contribute to a lower HDL-C, through decreased lipolysis. However, physical activity promotes lipolysis (Polak et al. 2008), and therefore may promote a liberation of HDL-C in blood flow. In a cross-sectional analysis from the same study, it was found that for an increased total physical activity level, there was a higher HDL-C, a lower LDL-C and lower TG (Väistö et al. 2014). However, in the present study, an inverse association between serum 25(OH)D concentration and HDL-C persisted even after adjustment for physical activity.

6.2.4 Serum 25(OH)D concentration and blood pressure

In this study, for a higher serum 25(OH)D concentration, there was a lower SBP, which remained after adjustment for possible confounders. This is congruent with previous studies in children (Xu et al 2017). 1,25(OH)₂D₃ may have a protective effect on blood pressure, and a possible mechanism for that is by a suppression of the renin-angiotensin-aldosterone system, as shown in an experiment with vitamin D receptor-knock-out mice, which became hypertensive (Li 2003). Vitamin D receptor also regulates vascular tone (Vaidya & Forman 2010), through the genomic activation of the endothelial nitric oxide synthase by Vitamin D receptor-bound vitamin D complex (Talmor et al. 2008), leading to a vasodilatory effect on the vascular system.

6.2.5 Serum 25(OH)D concentration and liver enzymes and markers of inflammation

Interestingly, the present study found that for a higher serum 25(OH)D concentration, there was a higher hs-CRP level, a higher ALT and a lower HDL. A previous publication on the same study population by Viitasalo et al. 2012, showed that systemic low-grade inflammation (hs-CRP), obesity, and low HDL-C was associated with a higher level of the liver enzymes, ALT and GGT (Viitasalo et al. 2012). Low-grade inflammation has a central role to play in the pathophysiology of MetS, and of CVD (Devaraj et al. 2009). It seems that in this thesis, serum 25(OH)D concentration is linked with a pro-inflammatory state characterized by higher hs-CRP and ALT, and lower HDL-C, which are also characteristics of the MetS. At least one other study found partly similar results (Ashraf et al. 2011). Ashraf et al (2011) found a positive association between serum 25(OH)D concentration and liver enzyme in children. The reasons for this finding are unclear and need further investigation. One possible explanation could be due to a common pathway for systemic inflammation, inflammation in the liver, and insulin resistance, which would be mediated by vitamin D or by other factors related to vitamin D. However, those findings do not explain why vitamin D had no effects on other components of the MetS like glucose metabolism and total fat body %, an estimate of visceral adiposity.

In this analysis, the reference cut-off value of hs-CRP for adults was chosen due to the lack of reference values for children. Therefore, those results should be interpreted with caution. Those results are also contrary to previous studies. For instance, Rai & Agrawal (2017) stated that vitamin D supplementation decreases inflammation and pro-inflammatory cytokines. Interestingly, in a cross-sectional British study of 4274 children with mean age of 9.9 years, Williams et al. (2012) found that serum 25(OH)D₂ concentration, but not serum 25(OH)D₃

concentration, was associated with a pro-inflammatory state (Interleukine-6 and C-reactive protein). This present study did not distinguish between 25(OH)D₂ and 25(OH)D₃ in the analysis. In a previous publication by Soininen et al. 2016, 78.3% of vitamin D intake of the children participating in the PANIC study came from food fortified with vitamin D₃ (milk and milk products, fat), 10.1 % from fish products. The median amount of vitamin D₃ ingested from supplements was 0.36 µg/d and from food, 5.9 µg/d. Therefore, I assume that the serum 25(OH)D concentration of the PANIC study population most likely reflect 25(OH)D₃ status, which should have had a protective effect in inflammation. It is, therefore, unclear why our results diverge from the Williams et al. (2012) study. It is possible that the association between serum 25(OH)D concentration and inflammation may be due to other unmeasured confounding factors associated with dietary vitamin D sources, for instance fish- or milk consumption.

The effects of EPA, DHA, heavy metals and milk on inflammation

This thesis, although adjusting for many confounders, including age, sex, physical activity, quality of fat in diet, vitamin D intake, skin type, measures of sun exposure (travel to sunny locations, use of sunscreen, length of day), socioeconomic factors and body fat %, did not include in the analysis EPA, DHA or measurements of pollutants. This could be of clinical importance since omega 3 from fish- and plant consumption have been associated with decreased blood pressure and inflammation, with better lipid profile and with a decrease in progression of CVD in adults (Rodriguez Leyva et al.2010, Kris-Etherton et al. 2002). In children, Crowe-White et al. (2018) showed, in a cross-sectional study, that a higher omega 6:3 PUFA's ratio intake was associated with a lower TC and LDL-C, and a higher intake of omega 3 PUFA was associated with a higher HDL-C in children.

The serum level of mercury has been found to be proportional to the amount of fish ingested (Gump et al. 2012). In a cross-sectional study of 9-11-year-old children, the serum level of mercury, was associated with a systemic low-grade inflammation (Gump et al. 2012). EPA and DHA and vitamin D are found in liver of fatty fishes, and heavy metals, like mercury can be found in both fatty and non-fatty fishes, like pike (Karjalainen et al. 2013). Fetus and young children are even more sensitive to the neuro-, nephro and immunotoxic effects of methylmercury (Bose-O'Reilly et al. 2010). Therefore, fish consumption may have both pro- and anti-inflammatory effect, depending of the amount of the omega 3 and of pollutant contained in fish.

Milk consumption may be another confounder for the possible positive association between inflammation and serum 25(OH)D concentration. A previous publication of the same study population has shown that children having higher serum 25(OH)D concentration were also those who drank more milk (Soininen et al. 2016). A review of adults revealed that both fatty and non-fatty dairy products were associated with a pro-inflammatory state, but only in subjects with milk protein allergy (Bordoni et al. 2017). In the present study, the % of children having milk allergy was unknown, but most likely to be low in the Finnish school age children (Ruoka allergia (lapset), Suomalaisen Lääkäriseuran Duodecimin ja Lastenlääkärin yhdistys ry:n asettama työryhmä 2015). However, whole-milk consumption in pre-pubertal children may cause a shift in the growth hormone/insulin-like growth factor-1 axis; a factor associated in adults, with inflammatory and auto-immune diseases, like type 2 diabetes and CVD (Melnik 2009). Milk contains saturated fat, unless non-fat milk is used. The consumption of saturated fat has a pro-inflammatory effect (Fritsche 2015). In the present study, the ratio of unsaturated to saturated fat was taken into account in the backward stepwise analysis, and was not a significant confounder for hs-CRP nor ALT.

6.3 Limitations, strengths and further directions

6.3.1 Limitations of the study

A limitation of this thesis is that it did not include measurements of apolipoprotein B. Apolipoproteins B are transporters of LDL-C. Higher levels of apolipoprotein B, which are of smaller size, are involved into the formation of atherogenic depot in the vascular wall, leading to CVD. Apolipoproteins B are linked to vascular dysfunction, inflammation and a lipidogenic profile, and is considered to be a risk factor for CVD in children (Castro et al. 2018). It would have been interesting to know the relationship between vitamin D and apolipoprotein B in the present study. As mentioned earlier, this study did not include EPA, DHA and mercury level in the statistical analysis, which could have been useful in order to control for those confounding factors.

The results for hs-CRP in children should be interpreted with caution, since adult reference values were used, which were not yet validated for children. However, it seems that a higher serum 25(OH)D concentration is linked with a pro-inflammatory effect in this study

population of children. The reasons for this association need further investigation. The weakness of this study is that it is a cross-sectional, therefore causality cannot be proven. It is possible that the association between serum 25(OH)D concentration and cardiometabolic components may be due to other unknown variables.

6.3.2 Strengths of the study

The strengths of the study are that it is a well-designed study from a representative sample of a homogeneous population with a high participation rate and with a high number of children, ensuring a sufficient statistical power. The study included carefully collected data concerning diet, physical activity, measures of body fat % by the Lunar dual energy X-ray absorptiometry as well as biochemical analysis from fasting blood samples. The PANIC physical activity questionnaire has been reported to have good validity and long-term reproducibility to measure total amount of physical activity (Haapala et al. 2014).

The studied population consisted of mostly pre-pubertal children. This allowed the study of the specific role of serum 25(OH)D concentration on the CMR without the influence of puberty on glucose, lipid and bone metabolism.

The data collected made it possible to do a statistical analysis accounting for a wide range of confounders, including factors affecting the production of vitamin D, like sunscreen use, and measures of daytime light and the dietary intake of vitamin D. The dietary fat intake ratio was selected as a confounder, based on results from some previous literature, which showed that dietary fat affects serum lipids (Hodson et al. 2001, Institute of Medicine 2005, European Food Safety Authority 2010). In the present study, dietary fat intake ratio has been found to be a significant confounder in the multiple regression analysis for SBP (model 3).

Another strength is that it is one of the few studies made on children living in Nordic latitudes. Therefore, it gives specific information on how the serum 25(OH)D concentration affect the cardiometabolic health of children living at the 62°64`N.

6.3.3 Further directions

In Finland, sunlight is inadequate for vitamin D synthesis in the skin approximately half of the year (Wacker & Holick 2013). Children participating to PANIC study had lower risk for low 25(OH)D after summer in autumn, and when daylight time was >13h/d (Soininen et al. 2016). From October to March, children rely on dietary source of vitamin D to maintain

vitamin D sufficiency. Therefore, the results of the present study may reflect more the effects of dietary vitamin D rather than vitamin D synthesis through effect of sun on the CMR. It is theoretically possible that the source of vitamin D (dietary or skin synthesis) affect the lipid and glucose metabolism and inflammation in different ways. The results of this present study may be valuable for further research aiming to assess those issues. For instance, it would be interesting to pursue randomized control trials, regarding the effect of supplementation of vitamin D on the CMR in children in a sun-deprived environment.

Soininen et al. (2018) recently analyzed genetic data from this population sample and found that some of the gene variants that have been previously associated with 25(OH)D were associated with TC and LDL-C, and HDL independently of serum 25(OH)D concentration. A recently published study of the same project showed, that serum 25(OH)D concentration was associated with TC, LDL-C and HDL, independently of the gene variants (Soininen et al. 2018). This finding is interesting and also needs further investigation.

Also, it would be interesting to see if including the consumption of fish, EPA, DHA and mercury as confounders would affect the results on lipids and inflammation in this present study.

7. CONCLUSION

This study presents interesting and controversial results regarding the association of serum 25(OH)D concentration and CMR in children. Serum 25(OH)D concentration was associated with some, but not all, components of CMR in children. Serum 25(OH)D seems to have a protective role in some components of CMR, such as SBP, TC and LDL-C, but a detrimental role in other components such as liver enzyme, HDL-C and some markers of inflammation. Some hypotheses have been stated to explain the possible divergent results. However, randomized-controlled trials are needed to prove a causal role of serum 25(OH)D and the components of CMR in children.

The results of this thesis bring additional information in favor of a possible association between serum 25(OH)D and lipid profile, blood pressure and inflammation. However, the results of this cross-sectional study should be taken with caution, because it was not possible to control for all possible confounders, and because causality cannot be proven. This thesis is a step forward into the process of improving children's health in a Nordic population at risk of vitamin D deficiency. Indeed, if vitamin D sufficiency could promote both skeletal and cardiovascular benefits, we could prevent the development of bone and cardiometabolic diseases, starting already from childhood. It would be of huge public health impact to prevent diseases at their source.

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