1. Introduction

Vitamin D plays an essential role in calcium homeostasis and skeletal mineralization\(^1\) and adequate vitamin D status seems particularly important during childhood growth, where a high calcium absorption is vital\(^2\). In recent years, there has been a growing interest in non-skeletal effects of vitamin D\(^3-7\), but the current evidence is insufficient to base dietary vitamin D recommendations on other health outcomes than the classic skeletal effects\(^8-10\). Immune defence and cardiometabolic health are two non-skeletal areas with importance for public health, which may be influenced by vitamin D\(^3,7,11-17\).

Acute respiratory infections (ARIs) are common in children\(^18\) especially during winter and they impose a major economic burden to the health system\(^19\). Cardiovascular disease (CVD) is a major cause of morbidity and mortality in adults worldwide\(^20\), and risk factors track from childhood into adulthood\(^21\). Hence, strategies to lessen the ARI burden as well as early prevention of CVD are important. In Denmark and other countries in moderate-higher northern latitudes, the risk of vitamin D deficiency in children is substantial during winter, since their vitamin D status (serum 25-hydroxyvitamin D [25(OH)D]) declines at this time of year\(^22-24\). This is due to the negligible exposure to solar ultraviolet B (UVB) radiation during winter\(^25\), which is the primary source of vitamin D\(^26\). Since few foods are natural sources of vitamin D, the dietary vitamin D intake is limited, especially in Denmark where fortification is rare. Therefore, ensuring adequate serum 25(OH)D, for example through supplementation, could be a strategy to reduce ARIs and contribute to early prevention of CVD.

The risk of ARIs in children has been reduced by vitamin D supplementation in previous studies\(^13\). It has frequently been suggested that the mechanism underlying this effect is that vitamin D modulates the innate immune defence\(^27-31\), which is the body’s important first-line defence against invading pathogens. This is supported by studies in vitro\(^32-34\), and the European Food Safety Authority (EFSA) has approved the health claim “vitamin D contributes to normal function of the immune system” for children\(^35\). In contrast, human studies in- and ex vivo, which have investigated the effects of vitamin D on the innate immune system, are scarce and their results are inconsistent, particularly in children. Cardiometabolic risk markers such as blood pressure, blood lipids, and markers of glucose metabolism have been associated with serum 25(OH)D in many studies in children\(^11\). However, randomized controlled trials (RCTs) in children aged 0-18 years have mainly been conducted in overweight adolescents and the effects of vitamin D supplementation on
2) Does children’s oral mucosa express the genes encoding the vitamin D receptor (VDR) and the 1α-hydroxylase (CYP27B1), which converts serum 25(OH)D to the biologically active vitamin D metabolite, 1,25-dihydroxyvitamin D [1,25(OH)₂D]? (paper I)

*Hypothesis 2*: CYP27B1 and VDR are expressed in children’s oral mucosal epithelial cells as a mechanistic link explaining possible effects of vitamin D supplementation on oral mucosal gene expression (*hypothesis 1a*).

3) Does winter vitamin D supplementation affect markers of cardiometabolic risk in young healthy children? (paper II)

*Hypothesis 3*: vitamin D supplementation has dose-dependent and favourable effects on the cardiometabolic risk profile, i.e., decreases systolic and diastolic blood pressure, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides, blood glucose, insulin, and glycated haemoglobin (HbA1c), and increases high-density lipoprotein (HDL-C).

4) Is serum 25(OH)D associated with markers of cardiometabolic risk in cross-sectional baseline analyses in young healthy children? And are potential associations in accordance with effects of vitamin D supplementation on cardiometabolic markers? (paper II)

*Hypothesis 4*: serum 25(OH)D is associated with a more favourable cardiometabolic risk profile, i.e., serum 25(OH)D is inversely associated with systolic and diastolic blood pressure, TC, LDL-C, triglycerides, blood glucose, insulin, and HbA1c; and positively associated with HDL-C.

5) Does vitamin D supplementation affect markers of cardiometabolic risk in children up to 18 years of age? (paper III)

*Hypothesis 5*: vitamin D supplementation has favourable effects on cardiometabolic risk profiles, i.e., decreases systolic and diastolic blood pressure, TC, LDL-C, triglycerides, blood glucose, insulin, insulin resistance (homeostatic model of insulin resistance (HOMA-IR)), HbA1c, and increases HDL-C.