Vitamin D Status and Markers of Cardiometabolic and Liver Disease Risk in Childhood Obesity

by

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Abstract

Vitamin D insufficiency is highly prevalent in children (up to 40%), particularly in northern climates such as Alberta, due to reduced sunlight exposure and low intake. Although suboptimal vitamin D status and metabolic dysregulation are commonly observed in obesity, little is known about the interrelationships between vitamin D and body composition and the prevalence of co-morbid conditions (mental health disorders, cardiometabolic and liver dysfunction) in pediatric obesity. Two studies will be described. The first study is a retrospective chart review (n=217) of obese children attending the Pediatric Centre for Weight and Health (PCWH) at the Misericordia Hospital in Edmonton, Alberta. The second study focuses on two clinical populations of pediatric obesity: children with non-alcoholic fatty liver disease (NAFLD) and Prader-Willi Syndrome (PWS). Study findings indicate that rates of vitamin D insufficiency in obese children in Alberta (30-50%) are similar to levels in the general population, indicating that vitamin D status in children is independent of total body adiposity or the presence of co-morbid conditions such as mental health disorders. Children with PWS showed significantly lower muscle strength/muscle function compared to obese children with NAFLD or lean healthy children, and this was independent of overall vitamin D status. However, vitamin D insufficiency was related to an increased prevalence of hyperinsulinemia, insulin resistance and elevated systolic blood pressure in overweight and obese children, with/without the presence of other co-morbid conditions such as NAFLD or PWS. This has significant potential health policy implications in terms of the prevention and treatment of co-morbid conditions in childhood obesity.

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Although my name may be alone on the front cover of this thesis, I am by no means its sole contributor. Many people, in different ways, helped to make this possible.

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List of Abbreviations
(As they appear)

25(OH)D; serum 25-hydroxyvitamin D
EAR; estimated average requirement
NAFLD; nonalcoholic fatty liver disease
PWS; Prader-Willi Syndrome
NASH; nonalcoholic steatohepatitis
FSV; fat soluble vitamin
UV; ultraviolet
VDBP: vitamin D binding protein
MED; minimal erythemal dose
RDA; recommended dietary allowance
IU; international units
tsp; teaspoon
ANGCY; Alberta Nutrition Guidelines for Children and Youth
USDA; United States Department of Agriculture
CSEM; Canadian Society of Endocrinology and Metabolism
$1,25(OH)_2D$; 1,25-dihydroxyvitamin D
VDR; vitamin D receptor
VitD; vitamin D
BMI; body mass index
IV; intravenous
PTH; parathyroid hormone
24-OHase; 25-hydroxyvitamin D-24-hydroxylase
FGF23; Fibroblast growth factor 23
IR; insulin resistance
DXA; Dual-energy X-ray absorptiometry
EWGSOP; European Working Group on Sarcopenia in Older People
CT; computed tomography
MRI; magnetic resonance imaging
BIA; bioimpedance analysis
SPPB; short physical performance battery
SMM; skeletal muscle mass
ASM; appendicular skeletal muscle mass
LST; lean soft tissue
6MWT; six minute walk test
TG; triglycerides
TC; total cholesterol
LDL-C; low density lipoprotein cholesterol
HDL-C; high density lipoprotein cholesterol
ALT; alanine aminotransferase
AST; aspartate transaminase
GGT; gamma-glutamyl transferase
GI/GL; glycemic index/glycemic load
T2DM; type 2 diabetes mellitus
SNP; single nucleotide polymorphisms
US; ultrasonography
MRS; magnetic resonance spectroscopy
LFT; liver function test
PUFAs; polyunsaturated fatty acids

GH; growth hormone
IGF; insulin like growth factor
UPD; uniparental disomy
FFA; free fatty acid
DB; Downs and Black
NOS; Newcastle-Ottawa scale
LC-MS/MS; liquid chromatography-tandem mass spectrometry
CLIA; chemiluminescence immunoassay
HOMA; homeostatic model assessment
PCWH; Pediatric Centre for Weight and Health
SBP; systolic blood pressure
DBP; diastolic blood pressure
ADHD; attention deficit hyperactivity disorder
WHtR; waist to height ratio
WC; waist circumference
BSA; body surface area
WHO; World Health Organization
DSM-5; Diagnostic and Statistical Manual of Mental Disorders, 5^{th} edition
TSH; thyroid stimulating hormone
AHS; Alberta Health Services
IQR; interquartile range
RAS; renin angiotension system
NACTRC; Northern Alberta Clinical Trials Centre
BMR; basal metabolic rate
IBW; ideal body weight
HC; hip circumference
MAC; mid-arm circumference
FAC; flexed arm circumference
CC; calf circumference
WHR; waist to hip ratio
TER; trunk to extremity ratio
BP; blood pressure
HR; heart rate
SpO_2; pulse oximetry
ALP; alkaline phosphatase
CRP; C-reactive protein
ANA; antinuclear antibody
HAES; Habitual Activity Estimation Scale
SSF; sum of 3 skinfolds
AMDR; acceptable macronutrient distribution range
AI; adequate intake
DRI; dietary reference intakes

Presentation of Work within Thesis

Presentations

MacDonald K*. Interrelationships Between Vitamin D and Insulin Resistance, Liver Function, Cardiometabolic Disease Risk and Body Composition in Obese Children, ADI Research in Progress Seminar, April 2016.

Abstracts

MacDonald K*, Haqq AM, Yap J, Mager DR. Body Composition, Handgrip Strength, Physical Capacity and Markers of Cardiometabolic and Liver Dysfunction in Children with Nonalcoholic fatty liver disease and Prader-Willi Syndrome. WCHRI Research Day, November, 2016. Poster Presentation.

MacDonald K*, Haqq AM, Yap J, Mager DR. Body Composition, Handgrip Strength, Physical Capacity and Markers of Cardiometabolic and Liver Dysfunction in Children with Nonalcoholic fatty liver disease and Prader-Willi Syndrome. ADI Research Day, October, 2016. Poster Presentation.

MacDonald K*, Haqq AM, Godziuk K, LaFrance R, Ansarian, M, Yap J, Mager D.R. Lower vitamin D status is associated with hypertension and abdominal obesity but not mental health comorbidities in pediatric obesity. ASPEN Clinical Nutrition Week, January 16-19, 2016, Austin Texas. Poster Presentation.

MacDonald K*, Haqq AM, Yap J, Mager DR. Influence of adiposity and insulin resistance/hyperinsulinemia on vitamin D status in children with non-alcoholic fatty liver disease: a systematic review. WCHRI Research Day, November, 2015. Poster Presentation.

MacDonald K*, Haqq AM, Godziuk K, LaFrance R, Ansarian, M, Yap J, Mager D.R. Associations between vitamin D status and anthropometric, cardiometabolic, liver function and mental health parameters in childhood obesity. ADI Research Day, October, 2015. Poster Presentation.

CHAPTER 1: Literature Review

1.1 Introduction: Vitamin D and Pediatric Obesity

Vitamin D insufficiency is highly prevalent in children and adults, particularly in northern climates such as Alberta, due to reduced sunlight exposure and low intake [4, 5]. In Northern Alberta, 25-40% of children and adults have deficient serum 25-hydroxyvitamin D (25(OH)D) levels (<50 nmol/L) [6, 7]. Recent evidence indicates that 78% of children (10-11 years) in Alberta have vitamin D intakes that are significantly below the estimated average requirement (EAR) of 400 IU/day [8]. This is consistent with nationally representative data from Health Canada where vitamin D intake is below the EAR in 84.5% of boys and 93.1% of girls aged 9-13 years and 74.7% of boys and 93.5% of girls aged 14-18 years [9]. This finding is likely secondary to reduced intake of vitamin D fortified foods.

Vitamin D insufficiency has been associated with poor bone health, presence of liver disease and indices of muscle function, insulin resistance/insulin sensitivity, inflammation, mental health disorders and other cardiometabolic risk factors, particularly in obese individuals [10-15]. Childhood obesity has been increasing at alarming rates in Canada. In Alberta, approximately 11-14% of children are overweight and 9-11% are obese [5, 8, 16]. Obese individuals tend to have lower levels of vitamin D compared to lean individuals [17, 18]. Obesity has also been associated with chronic diseases such as liver disease and diabetes, where vitamin D status may be further compromised.

Prader-Willi Syndrome (PWS) is a syndromic form of obesity and NAFLD is a complication associated with non-syndromic obesity. NAFLD is a spectrum of liver disease that ranges from simple steatosis to steatosis with inflammation and fibrosis (nonalcoholic steatohepatitis or NASH) to cirrhosis [19, 20]. The development of NAFLD is influenced by

both lifestyle and genetic factors [20]. PWS is multisystem genetic disorder that often leads to obesity [21]. One interesting contrast in both clinical populations, is that children with PWS experience primarily subcutaneous obesity with little or no insulin resistance, whereas children with NAFLD experience primarily visceral adiposity that contributes to significant insulin resistance, hyperinsulinemia and metabolic dysregulation [22-29]. The objective of this literature review is to summarize and evaluate the available literature relating the interrelationships between vitamin D, pediatric obesity, markers of metabolic dysregulation (e.g. insulin resistance/ hyperinsulinemia) and body composition in two pediatric populations of obese children (NAFLD and PWS).

1.2 Vitamin D

Vitamin D is a 9,10-secosteroid [30]. Six forms with similar molecular structures have been identified (**Figure 1.1**) [30, 31]. The two main forms of the fat-soluble vitamin (FSV) relevant to human nutrition are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) [30, 32]. The significant difference between the two is one double bond in the side chain [33].

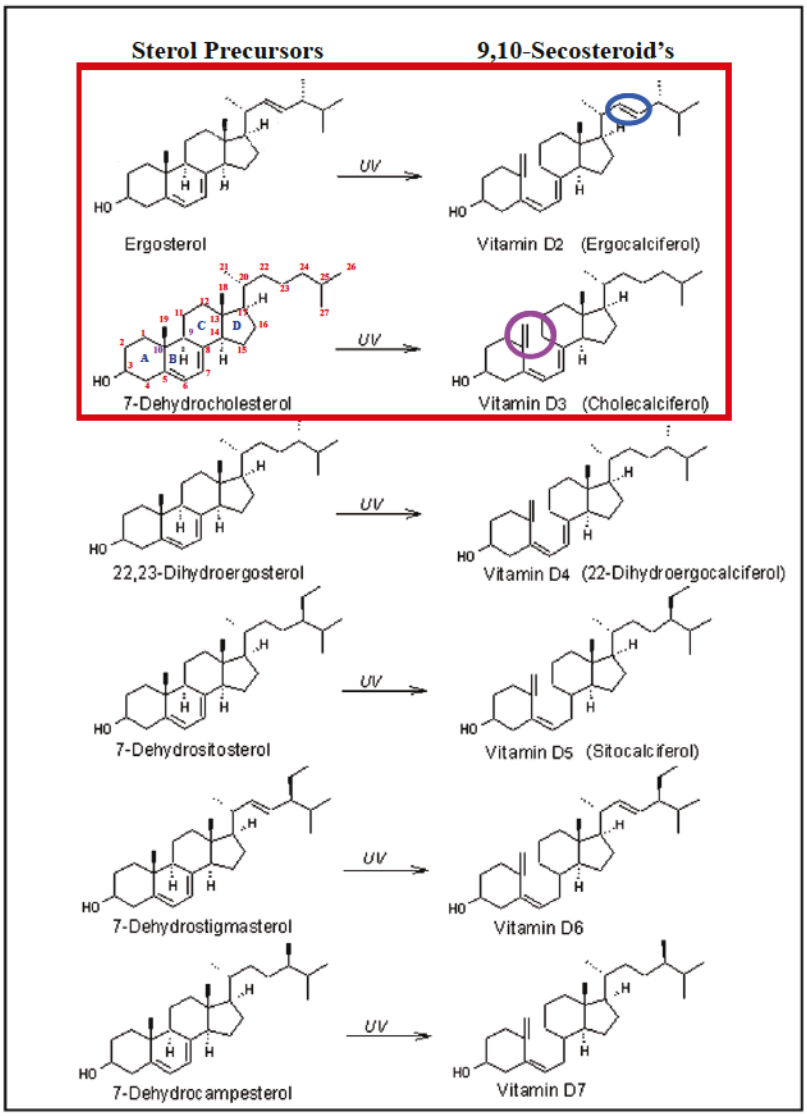

Figure 1.1 *Different Forms of Vitamin D.*
Red box: Two forms relevant to human nutrition (vitamin D_2 and vitamin D_3).
Blue circle: Double bond difference in side chain between vitamin D_2 and vitamin D_3.
Purple circle: Bond between carbon 9 and 10 cleaved in steroid B-ring of 9,10-secosteroid [34].
(Source: [30]).

1.2.1 Skin Synthesis of Vitamin D

The major contributor to vitamin D status is from exposure to ultraviolet (UV) radiation (sunlight) (**Figure 1.2**) [35]. Low UVB (290-315 nm) radiation synthesizes cholecalciferol (vitamin D_3) in a non-enzymatic process [31, 36]. UVB is absorbed by 7-dehydrocholesterol present in the plasma membranes of the epidermis and dermis and is converted to pre-

cholecalciferol (previtamin-D_3) [31, 33, 37]. Previtamin-D_3 is thermodynamically unstable and over several hours isomerizes to vitamin D_3 [31, 34, 38]. Vitamin D_3 is released into circulation bound to the major transport protein vitamin D binding protein (VDBP) [39, 40]. Excessive pre-vitamin D_3 or vitamin D_3 synthesized is converted to inactive photoproducts by UVB radiation [37].

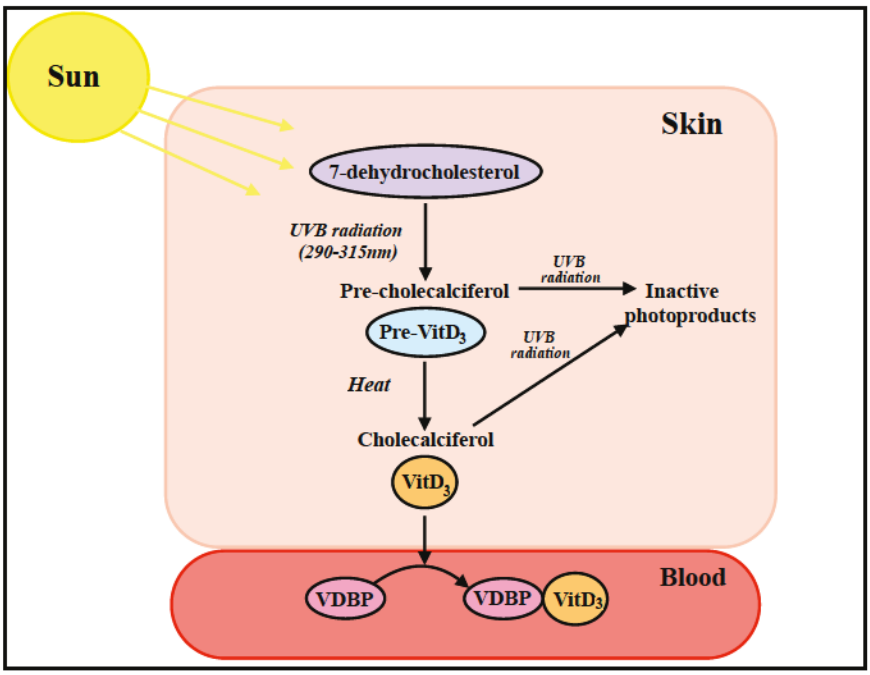

Figure 1.2 *Skin Synthesis of Vitamin D.*
(Source: [31, 33, 37]).

It is difficult to advise a level of sun exposure that will contribute to optimal vitamin D status as many factors influence amount/length of UVB radiation required (**see section 1.2.6**). There is also an important balance between the necessary amount of exposure for vitamin D synthesis and the potential increased risk of skin cancer [33]. Sun exposure of the arms and legs for about 5-30 minutes (between 10 am to 3 pm) twice a week is likely satisfactory during summer months [34, 37]. The minimal erythemal dose (MED) is the amount of sun exposure that

will produce a light pinkness to the skin [34, 41]. Exposure reaching the MED (wearing only a bathing suit), has been shown to be equivalent to ingesting ~ 20,000 IU of vitamin D_2 [37].

1.2.2 Dietary/Supplemental Intake of Vitamin D

Both vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) can be obtained through dietary and/or supplemental intake [31, 33]. For infants (0-12 months), the Health Canada recommended dietary allowance (RDA) is 400 IU/day and the estimated average requirement (EAR) is not determinable [42]. For children/teens (1-18 years), the RDA is 600 IU/day and the EAR is 400 IU/day [42]. Few foods naturally contain vitamin D and most are considered poor sources (contribute <10% of daily requirements) (**Table 1.1**) [33, 36]. Oily fish are an exception and can naturally contain high levels of vitamin D_3 [43, 44]. Irradiation of ergosterol found in plants produces ergocalciferol (vitamin D_2) [33]. Through this process wild mushrooms can contain vitamin D_2 (7 IU – 147 IU/ 100g) [30, 43, 45]. Additionally, the vitamin D_2 content in mushrooms can be increased by deliberate UVB radiation exposure (from non-detectable to 250 IU/g dry weight) [45, 46]. Human milk naturally contains low levels of vitamin D (~20 IU/L) [37]. In vitamin D deficient women, this level can be even lower, highlighting the importance of vitamin D supplementation in breastfeeding women [37]. Some foods have become modest sources of vitamin D through fortification, including: cereals, margarine, juice, and dairy products (milk, cheese and yogurt) (**Table 1.1**) [33, 43]. However, levels of fortification vary from product to product and between countries [33]. Vitamin D supplements are available in either the vitamin D_2 or vitamin D_3 form [43]. Over the counter supplements usually contain vitamin D_3 (although some contain vitamin D_2), while non-hormone vitamin D prescription supplements contain vitamin D_2 [37, 47].

Table 1.1 Dietary and Supplemental Sources of Vitamin D_3 and Vitamin D_2.

Food	Per one serving*	Vitamin D (IU)
Natural Sources		
Salmon fresh [a]	75 g (2 ½ oz)	204-530 IU vitamin D_3
Salmon Canned [a]	75 g (2 ½ oz)	200-644 IU vitamin D_3
Cod liver oil [a]	5 mL (1 tsp)	428 IU vitamin D_3
Snapper fresh [a]	75 g (2 ½ oz)	307-392 IU vitamin D_3
Trout fresh [a]	75 g (2 ½ oz)	117-200 IU vitamin D_3
Tuna fresh [a]	75 g (2 ½ oz)	83-220 IU vitamin D_3
Tuna Canned [a]	75 g (2 ½ oz)	28-60 IU vitamin D_3
Sardines canned [a]	75 g (2 ½ oz)	68-144 IU vitamin D_3
Egg, chicken [a]	2 eggs	52-80 IU vitamin D_3
Beef liver [a]	75g (2 ½ oz)	36-37 IU vitamin D_3
Mushrooms, brown, Italian, portabella or crimini, exposed to UV light, raw [b]	125 mL (1/2 cup)	488-556 vitamin D_2
Mushroom Maitake raw [a]	125 mL (1/2 cup)	408 IU vitamin D_2
Mushroom Morel raw [a]	125 mL (1/2 cup)	72 IU vitamin D_2
Mushroom Chanterelle raw [a]	125 mL (1/2 cup)	60 IU vitamin D_2
Fortified Foods		
Milk (cow/soy/almond/rice)[ab]	250 mL (1 cup)	~90-100 IU vitamin D_3/D_2
Orange juice [a]	125 mL (1/2 cup)	8-48 vitamin D_3
Cereal [a]	30g (1 cup)	12-112 vitamin D_3
Yogurt [a]	175 g (3/4 cup)	40-104 vitamin D_3
Cheese [b]	50g (1.5 oz)	10-126 vitamin D_3
Margarine [a]	5 mL (1 tsp, 4 g)	21-34 IU vitamin D_3
PediaSure® (vanilla)[c]	1 bottle (235mL)	24 IU vitamin D_3
Supplement/ Pharmaceutical sources		
Prescription		
Vitamin D_2	1 capsule	50 000 IU vitamin D_2
Drisdol (liquid vitamin D_2)	1mL	8 000 IU vitamin D_2
Over the counter		
Multivitamin	capsules/ drops	200, 400, 600, 800 IU vitamin D_2/D_3
Vitamin D_3	capsules drops	400, 800, 1000 or 2000 IU vitamin D_3

Abbreviations: IU; international units, tsp; teaspoon,
**Serving sizes of foods based on the Alberta Nutrition Guidelines for Children and Youth (ANGCY) [48, 49]. Food data from: (A) Canadian Nutrient File (2010) [50], (B) USDA release 27 [51], (C) http://pediasure.ca/en/products.html.*
(Source: [37, 43])

1.2.3 Metabolism of Vitamin D

Endogenous skin synthesis (Vitamin D_3) and oral sources (vitamin D_2 or Vitamin D_3) both contribute to vitamin D status [35, 36] (**Figure 1.3**). Dietary sources of Vitamin D_2 and Vitamin D_3 are incorporated into chylomicrons and transported through the lymphatic system into the venous circulation [37, 52]. Vitamin D_3 synthesized in the skin is released directly in to circulation [36]. Vitamin D is transported through the blood via the vitamin D-binding protein (VDBP) (**section 1.2.4**). Once in circulation bound to the VDBP vitamin D has two fates; it can either be stored in adipocytes or transported to the liver (hydroxylation) [37]. The activation of vitamin D requires two hydroxylation steps. In the liver, vitamin D is converted to 25-hyroxyvitamin D (25(OH)D) (major circulating form), by vitamin D-25-hydroxylase [36, 37, 53].

Serum 25(OH)D has a half-life of two to three weeks, making it a good indicator of vitamin D status [43]. Although there is no consensus on what constitutes as "optimal" vitamin D status, the Canadian Society of Endocrinology and Metabolism (CSEM) suggests the following reference ranges: vitamin D deficiency (25(OH)D <50.0 nmol/L or <20.0 ng/mL); suboptimal status (25(OH)D 50.0-74.9 nmol/L or 20.0-29.9 ng/mL); and sufficiency (25(OH)D ≥75.0 nmol/L or ≥30.0 ng/mL) [52, 54, 55].

Although both vitamin D_2 and vitamin D_3 contribute to 25(OH)D levels, there is conflicting research concerning children and adults which suggests that vitamin D_3 may be more effective at increasing 25(OH)D levels compared to vitamin D_2 [56, 57]. Conversely, a study in adults found that vitamin D_2 was just as effective as vitamin D_3 in maintaining 25(OH)D concentrations [58]. A second study in infants and toddlers with vitamin D deficiency found that

2000IU/day of vitamin D_2, 2000IU/day of vitamin D_3 and 50,000 IU/week of vitamin D_2 were equally effective in raising 25(OH)D concentrations [59].

Circulating 25(OH)D is biologically inactive and must undergo a second hydroxylation reaction [43]. In the kidneys, 25(OH)D is converted to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) or calcitriol by 25-hydroxyvitamin D 1-a-hydroxylase [36, 37, 52] (**Figure 1.3**). The active hormone, $1,25(OH)_2D$, acts by binding to the nuclear vitamin D receptor (VDR) in most cells and tissues [31, 36].

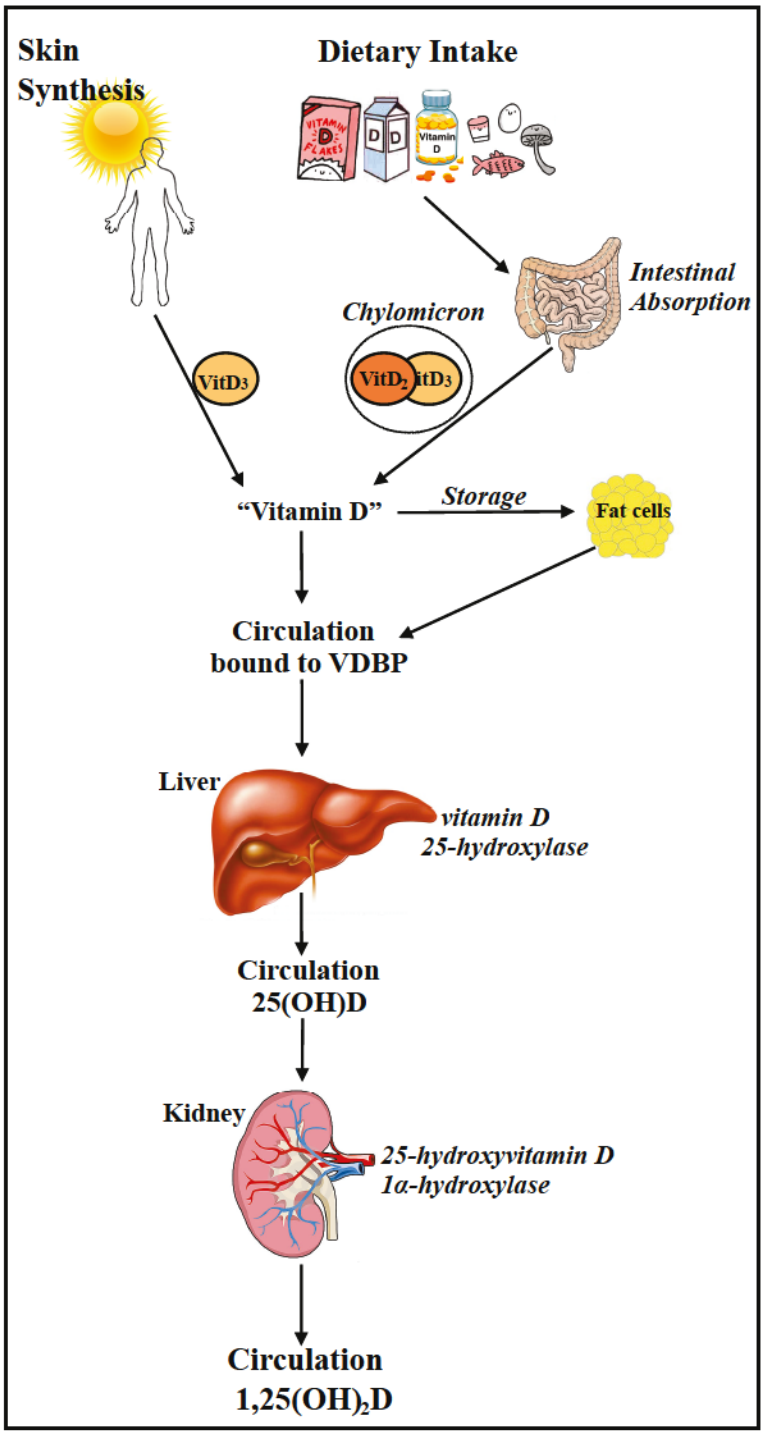

Figure 1.3 *Metabolism of Vitamin D.*
Abbreviations: VitD; vitamin D, VDBP; vitamin D binding protein, 25(OH)D; 25-hyroxyvitamin D, 1,25(OH)$_2$D; 1,25-dihydroxyvitamin D.
(Source: [33, 37]).

1.2.4 Vitamin D Binding Protein (VDBP)

The vitamin D binding protein (VDBP) is a 58 kDa alpha globulin [40]. It is primarily synthesized in the liver and is the major transport protein for vitamin D [39, 40]. VDBP has a high affinity for vitamin D metabolites (25(OH)D, 1,25(OH)D_2, etc.) and binds approximately 85-90% of vitamin D [39, 40]. Circulating albumin has a lower affinity compared to VDBP; nevertheless, due to its higher relative abundance (650 μM albumin vs 5 μM VDBP) approximately 10-12% is bound to albumin [39, 60]. A small proportion (<1%) of vitamin D metabolites circulate unbound/free [39, 60]. This free fraction, as well as those bound to albumin, make up the bioavailable 25(OH)D pool [39, 60]. There are over 120 variants of VDBP [40]. The three main alleles are GC2, GC1S and GC1F which have different affinities for 25(OH)D and correspond to different serum VDBP concentrations [40, 61]. These allele types vary between different geological locations and ethnicities [40]. There is conflicting evidence examining the relationship between VDBP polymorphisms and obesity. Studies have shown that VDBP polymorphisms are associated with both percent fat mass and increased body mass index (BMI) (evidence is stronger in females) [62, 63]. However, a large cohort study (n=5224) did not find any association with obesity [64].

1.2.5 Vitamin D Intoxication

The Pediatric Endocrine Society defines vitamin D toxicity as levels of 25(OH)D greater than 150ng/ml or 375 nmol/L [37, 65]. Vitamin D intoxication (hypervitaminosis D) is rare and occurs due to extremely large oral intakes of vitamin D [65]. Excessive exposure to sunlight does not cause vitamin D_3 toxicity as excess pre-vitamin D_3 or vitamin D_3 is converted to inactive photoproducts by UVB radiation [37]. Vitamin D intoxication can cause hypercalcemia related to increased bone resorption [66]. In children, symptoms of hypercalcemia include: aches and

pains, decreased appetite, weight loss, abdominal pain, vomiting, constipation, lethargy, polyuria or polydipsia [65-67]. In some cases, nephrocalcinosis, which can impair renal function; vascular calcification, which can lead to renal hypertension; or severe dehydration occurs [65, 67]. Since vitamin D is fat soluble and stored in the adipose tissue, it may take time for levels to decline naturally (1-2 months) [65, 67]. Treatment strategies involve 1) removing the source of vitamin D 2) intravenous hydration (IV) with saline to increase glomerular filtration rate and increase calcium excretion (can be combined with diuretics to further increase calcium excretion) 3) if hypercalcemia persists, glucocorticoids which prevent renal absorption of calcium and inhibits calcitriol; or calcitonin which reduces bone resorption can be added 4) alternatively, antiresorptive therapy with oral/IV bisphosphonates (pamidronate and alendronate) can be used 5) if a child is not responding to other treatments or in life-threatening cases, hemodialysis can be used [65, 67, 68].

1.2.6 Factors Influencing Vitamin D Status

Aside from poor dietary and/or supplemental intakes of vitamin D, many environmental, cultural and physiological factors can impair vitamin D status (**Table 1.2**) [33].

Table 1.2 Factors Contributing to Hypovitaminosis D.

Factor	How it impacts vitamin D status
Sunscreen	•Sunscreen absorbs UVB radiation
Skin pigment	•Melanin absorbs UVB radiation
Cloud cover/clothing	•Blocks UVB radiation
Age/skin grafts	•Reduced 7-dehydrocholesterol (~75% less >70 years old)
Season, latitude, altitude and time of day	•Angle of sun effects number of UVB photons that reach earth (>35° N (all of Canada) vitamin D production from November to February is negligible)
Fat malabsorption (celiac disease, cystic fibrosis, medications that reduce cholesterol absorption etc.,)	•Decreased vitamin D absorption
Obesity	•Sequesters vitamin D **(see section 1.3)**
Insulin resistance/hyperinsulinemia	**• (see section 1.4)**
Increased vitamin D catabolism (anticonvulsants, glucocorticoids etc.,)	•Converts 25(OH)D and 1,25(OH)D_2 to calcitroic acid (inactive)
Exclusively breastfeed infants	•Human milk is poor in vitamin D
Liver failure	•Mild to moderate – malabsorption of vitamin D, 25(OH)D production is possible •>90% dysfunction – insufficient production of 25(OH)D
Nephrotic Syndrome	•25(OH)D loss in urine
Chronic kidney disease	•Decreased synthesis of 1,25(OH)D_2
Inherited disorders – leads to rickets	•Mutations: renal 25(OH)D 1α-hydroxylase gene (CYP27B1), VDR, gene for fibroblast growth factor 23
Primary hyperparathyroidism	•Increased metabolism of 25(OH)D to 1,25(OH)D_2
Hyperthyroidism	•Reduced 25(OH)D through enhanced metabolism

Abbreviations: UV; ultraviolet, 1,25(OH)$_2$D; 1,25-dihydroxyvitamin D 25(OH)D; serum 25-hydroxyvitamin D, VDR; vitamin D receptor.
(Source:[33, 36, 37, 69])

1.2.7 Classical Functions of Vitamin D: Mineral Homeostasis

Vitamin D has roles in calcium and phosphorous homeostasis and bone metabolism (**Figure 1.4**) [37]. Vitamin D, in its active hormonal form (calcitriol), protects against calcium and phosphate deficiency through effects on the intestine, kidney, parathyroid gland, and bone

[31]. Maintaining an appropriate level of calcium and phosphate is essential for bone mineralization [31]. In the parathyroid gland, calcium is monitored by the calcium receptor [31]. When plasma calcium levels decrease, parathyroid hormone (PTH) is secreted and stimulates the enzyme 25-hydroxyvitamin D 1-a-hydroxylase in the kidney to convert 25(OH)D to $1,25(OH)_2D$ (calcitriol) [31, 36, 37]. Increased calcitriol increases intestinal absorption and stimulates osteoclasts to mobilize calcium from bone [31, 37]. Without vitamin D, only 10-15% of dietary calcium and approximately 60% of phosphorous would be absorbed [37]. The interaction between calcitriol and the vitamin D receptor increases intestinal absorption of calcium ~30-40% and phosphorous ~80% [31, 37]. $1,25(OH)_2D$ is controlled through negative feedback [37]. $1,25(OH)_2D$ increases the expression of 25-hydroxyvitamin D-24-hydroxylase (24-OHase) which catabolizes $1,25(OH)_2D$ to calcitroic acid (inactive, water soluble, and excreted in the bile) [37].

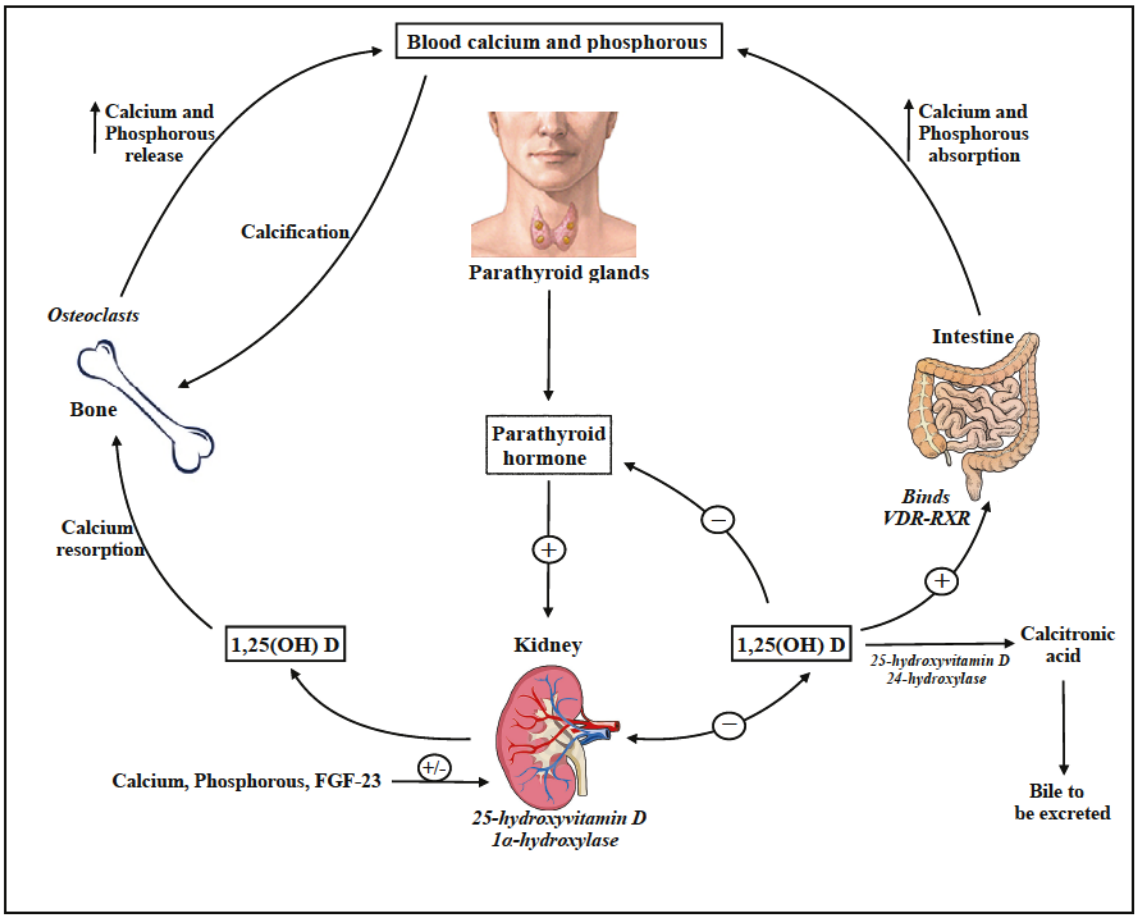

Figure 1.4 *Bone Homeostasis.*
(Source: [37])

1.2.8 Non-classical Functions of Vitamin D

Traditionally, vitamin D status was considered adequate if individuals presented with no clinical or radiographic signs of bone disease, such as rickets in children or osteomalacia and osteoporosis in adults [31, 47]. In recent years, the discovery of the vitamin D receptor (VDR) in most body tissue and cells, has led to the realization that vitamin D has roles beyond skeletal health [31, 70, 71]. The vitamin D receptor (VDR) is part of the nuclear receptor super family and mediates the effects of calcitriol [31].

Findings from both animal and human studies have reported roles of vitamin D in normal growth, puberty, cancer prevention, hormone control (e.g. parathyroid hormone (PTH)),

insulin (**section 1.4**), diabetes, adipose tissue (**section 1.3**), fibroblast growth factor 23 (FGF23), renin, and immune function [31, 70, 72-75]. Vitamin D results in the activation and regulation of 5% of the human genome through the binding of calcitriol (active form of vitamin D) to the nuclear vitamin D receptor (VDR) [31]. Suboptimal vitamin D status has also been associated with increasing severity of metabolic dysregulation (hyperlipidemia, hyperinsulinemia, insulin resistance, liver disease and hypertension) in children and adults [10, 12, 14, 15]. Furthermore, there is evidence that suboptimal vitamin D status may predispose an individual to an increased risk for depression, reduced quality of life and other mental health disorders, particularly in individuals with obesity [73, 74].

1.3 Relationships: Vitamin D and Adiposity

Obese individuals tend to have lower levels of vitamin D compared to lean individuals [17, 18]. Studies have shown that 25(OH)D and $1,25(OH)_2D$ are negatively correlated with both body mass index (BMI) and fat mass [76, 77]. Percent body fat content has also been shown to be inversely related to 25(OH)D concentrations [78]. One study has shown that with a 1 kg/m^2 increase in BMI there was a ~ 1.3 nmol/L reduction in serum 25(OH)D [79]. Furthermore, weight loss has been associated with increases in 25(OH)D [80, 81].

Several hypotheses have been postulated to explain this association. These include: 1) sequestration of vitamin D into the adipose tissue thus reducing its bio-availability, 2) insufficient dietary/supplemental intake of vitamin D and/or 3) increased clothing coverage and reduced sun exposure related to physical inactivity [82, 83]. One of the most prevalent explanations is the sequestration of vitamin D into the adipose tissue [82, 83]. Vitamin D is a fat-soluble vitamin and it readily stored in the adipose tissue [84]. It is thought that the bioavailability of vitamin D from endogenous synthesis and dietary intake is decreased in obese

individuals due to sequestration into the expanded body fat mass [84]. Results from one study showed that after whole-body UVB irradiation, the increase in vitamin D_3 was 57% lower in obese individuals despite a greater body surface area compared to lean individuals [83, 84]. This was observed despite no difference in the content of 7-dehydrocholesterol in the skin or the conversion of pre-vitamin D_3 to vitamin D_3 between the two groups [84].

1.4 Relationships: Vitamin D and Insulin Resistance/Hyperinsulinemia

Hypovitaminosis D has also been associated with insulin resistance (IR) and hyperinsulinemia, particularly in obese individuals, where vitamin D status may be further compromised with comorbid conditions such as liver disease and diabetes [10-13]. The exact mechanisms by which IR/hyperinsulinemia influences 25(OH)D levels is not known, however several hypotheses have been suggested. These include: 1) inhibition of liver 25-hydroxylase, 2) up regulation of 24-hydroxylase (increased turnover of 25(OH)D to 24,25$(OH)_2$D) leading to increased inactive vitamin D levels, 3) increased sequestration into adipose tissue and/or 4) changes in vitamin D binding protein (VDBP) levels which may impact free 25(OH)D levels [39, 85, 86]. Studies have shown that 25(OH)D deficiency may impair insulin release from the pancreas and higher levels are associated with improved beta-cell function [1]. A recent clinical trial found that correcting vitamin D insufficiency with 4000 IU/day for 6 months in obese adolescents, improved insulin sensitivity (decreased fasting insulin and HOMA-IR) [87].

1.5 Vitamin D, Obesity and Muscle Function

The vitamin D receptor (VDR) is present in human muscle tissue and plays important roles in muscle health and function [88-90]. Lower vitamin D levels have been significantly associated with poor physical performance and lower muscle strength [88, 91]. Additionally,

proximal muscle weakness, muscle pain, and gait impairments are symptoms associated with vitamin D deficiency [89]. Results from a recent systematic review of randomized control trials (summarizing the effects of vitamin D supplementation on muscle function) showed that vitamin D supplementation (ranging from 300 IU/day to 100,000 IU/month) had a small but significantly positive impact on muscle strength (specifically lower limb) [14].

It is important to look at muscle function and bone health in addition to low muscle mass as muscle strength is also influenced by these factors [92]. Current guidelines recommend routine dual-energy X-ray absorptiometry (DXA) in children with PWS every 6-12 months for assessment of bone health and body composition, and annually in children with NAFLD [93-96]. New guidelines from the European working group (not yet validated) on sarcopenia in older people (EWGSOP) recommend using both low muscle mass and low muscle function (strength or performance) for the assessment of sarcopenia (**Table 1.3**) [92]. Current pediatric literature on sarcopenia is limited. No official definition in childhood exists, however several studies have used different approaches to evaluate pediatric sarcopenia (**Appendix Table A2.1**)[97-100].

Table 1.3 Diagnostic Criteria/Assessment Methods for Sarcopenia as Suggested by the European Working Group on Sarcopenia in Older People (EWGSOP).

Criterion	Assessment Methods in research
1. Low muscle mass	• Computed tomography (CT) • Magnetic resonance imaging (MRI) • **Dual energy x-ray absorptiometry (DXA)** • Bioimpedance analysis (BIA) • Total/partial potassium per fat free soft tissue
2. Low muscle strength	• **Handgrip strength** • Knee flexion/extension • Peak expiratory flow
3. Low physical performance	• Short physical performance battery (SPPB) • Usual gait speed • Timed get-up-and-go test • Stair climb power test
***Diagnosis of sarcopenia requires criterion 1 in addition to either criterion 2 or 3**	

(Source: [92]).

1.5.1 Calculating Skeletal Muscle Mass (SMM) Z-Scores in Children

Dual X-ray absorptiometry (DXA) measurements can be expressed as age and gender dependent z-scores, which can be used to interpret skeletal muscle mass (SMM) [101]. Measured SMM is calculated using tanner stage specific (above and below stage 5) formulas and appendicular skeletal muscle mass (ASM) measurements [101]. ASM is the sum of the lean soft tissue (LST) from the arms and legs measured by DXA [102]. Predicted SMM is calculated using age and pre-established gender specific constants [101]. SMM z-scores are then calculated using the difference between measured SMM and predicted SMM and age and gender specific constants [101] (**see Appendix A2 for formulas/constants**).

1.5.2 Measures of Muscle Strength and Physical Functioning in Children

Several different validated tests can be used to assess muscle strength and physical functioning in children. An example of muscle strength is grip strength (**section 1.5.3**) and an

example of physical functioning is a six minute walk test (6MWT) (**section 1.5.4**). For additional validated assessment tools, see (**Appendix Table A2.2**).

1.5.3 Grip Strength

Grip strength (measured in pounds/kilograms) is commonly assessed using a Jamar® hydraulic hand dynamometer as this device has the most normative data available [103-105]. It has also been shown to have high validity, reliability, as well as high reproducibility when used in children (4-11 years) [103, 104]. A recent study has shown that in children/adolescents and young adults, there is a strong correlation between grip strength and total muscle strength [106].

1.5.4 Six Minute Walk Test

A self-paced six minute walk test (6MWT) is commonly used and can reflect physical capability [107-109]. In children/adolescents, it is a valid and reliable test for assessing exercise tolerance and endurance [107, 110]. It is easy to administer, better tolerated and is more reflective of daily living than other walk tests as it is performed at a submaximal level of exertion [107-109].

1.6 NAFLD and PWS: Demographics, Etiology and Pathophysiology.

Table 1.4 NAFLD and PWS Comparison.

	Obese child with NAFLD	**Obese child with PWS**
Pathophysiology	-More common in males -Non-syndromic: life style induced and some genetic component	-No gender differences -Syndromic: genetic, hyperphagia
Height	-Normal	- Short stature due to growth hormone deficiency
Body Composition	*Adipose Tissue* ↑Total body fat ↑ Primarily visceral fat/ subcutaneous fat ↑ or within normal range Lean Mass -Lean mass normal/↓lean mass possible	*Adipose Tissue* ↑ Total body fat ↑ Primarily subcutaneous fat/visceral fat likely in normal range Lean Mass ↓Lean mass
Lipid Panel	↑Blood lipids (TG, TC, LDL) ↓HDL -Could be normal	-Could be normal ↑Blood lipids (TG, TC, LDL) possible
Liver Dysfunction	↑Liver enzymes (ALT, AST, GGT)	↓Prevalence of NAFLD in PWS ↑Liver enzymes possible
Insulin resistance /hyperinsulinemia	↑Insulin resistance/ hyperinsulinemia	-Possible insulin resistance/ hyperinsulinemia -Literature to suggest children with PWS are more insulin sensitive compare to obese controls with similar BMI-z scores

Abbreviations: NAFLD, nonalcoholic fatty liver disease; PWS, Prader-Willi Syndrome; TG, triglycerides; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase.
(Source: [20, 111-114]).

1.6.1 Nonalcoholic Fatty Liver Disease (NAFLD)

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in children, with an estimated prevalence of 8-13% in children and adolescents and 38-57% in obese children and adolescents [115-117]. NAFLD is a chronic liver disease that encompasses a spectrum of

disease that ranges from simple steatosis to steatosis with inflammation and fibrosis (nonalcoholic steatohepatitis or NASH) and ultimately cirrhosis (**Table 1.5**) [19, 20]. Although NAFLD is typically observed in overweight and obese children, a small subset (~15%) of normal weight children have be shown to have NAFLD [118]. As the disease advances the potential to reverse the condition diminishes [20]. The term "NAFLD" can refer to the entire spectrum of the disease or a specific sub-group [20]. Hyperinsulinemia and insulin resistance with liver dysfunction and visceral adiposity are hallmark features of this disease in childhood. Body composition is characterized predominantly by visceral adiposity, compared to subcutaneous adiposity [23].

Table 1.5 Spectrum of NAFLD.

Simple steatosis	>5% of hepatocytes with microvesicular and macrovesicular fat infiltration
Nonalcoholic steatohepatitis	Biopsy proven inflammatory activity associated with steatosis with or without fibrosis
Cirrhosis	Advanced fibrosis with disruption of hepatic architecture and regenerative nodules

(Source: [20]).

1.6.1.1 Risk Factors and Pathophysiology

NAFLD has both modifiable and non-modifiable risk factors (**Table 1.6**) [20]. The etiology of NAFLD is likely multifactorial with both environmental (dietary and sedentary activity patterns) and genetic influences. The factors that determine the progression of simple steatosis to NASH or cirrhosis are yet undetermined [20, 119]. NAFLD is often considered the hepatic component of the metabolic syndrome and is generally related to at least one metabolic characteristic such as abdominal obesity, dyslipidemia, hyperinsulinemia or IR [2, 120].

Table 1.6 Factors that Increase Risk of Pediatric NAFLD.

Modifiable Risk factors	Non-modifiable Risk Factors
• Obesity/Overweight • Waist circumference >95th percentile • Sedentary lifestyle • Diets high in simple sugars (eg. fructose) and high GI/GL • Not breastfed	• Male sex • Hispanic ethnicity • Age (risk increases with age) • Family history of NAFLD or T2DM • Parental (maternal) obesity • Low birth weight and early catch up growth • Genetic polymorphisms (SNP)

Abbreviations: GI/GL, glycemic index/glycemic load; NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphisms.
(Source: [20, 22, 121])

1.6.1.2 Diagnosing, Grading and Staging NAFLD

NAFLD is usually clinically silent but has been associated with non-specific symptoms such as fatigue or malaise [19, 121]. Individuals have reported vague abdominal pain or discomfort, especially in the right upper quadrant (often associated with more progressive NASH) [19, 121]. Before a diagnosis of NAFLD can be made, patients should be screened for: excessive alcohol consumption, genetic conditions (i.e. Cystic fibrosis, Hemochromatosis, Wilson's disease), hepatotoxic drugs (i.e. corticosteroids, chemotherapy), prolonged parenteral nutrition, Hepatitis B or C infections or other known causes of hepatosteatosis (**Table 1.7**) [19, 20].

Table 1.7 Causes of Fatty Liver Disease Unrelated to NAFLD.

General or Systemic	Genetic-metabolic	Rare hereditary genetic disorders	Drugs that can induce hepatotoxicity
-Acute systemic disease	-Cystic fibrosis	-Alström syndrome	-Ethanol
-Acute starvation	-Shwachman syndrome	-Bardet-Biedl syndrome	-Ecstasy, cocaine
-Protein energy malnutrition	-Wilson disease	-Prader-Willi syndrome	-Nifedipine
-Total parenteral nutrition	- α_1-Antitrypsin deficiency	-Cohen syndrome	-Diltiazem
-Polycystic ovary syndrome	-Galactosemia	-Cantu syndrome	-Estrogens
-Obstructive sleep apnea	-Fructosemia	(1p36 deletion)	-Corticosteroids
-Rapid weight loss	-Cholesteryl ester storage disease	-Weber-Christian disease	-Amiodarone
-Anorexia nervosa	-Glycogen storage disease		-Perhexiline
-Cachexia	(types I and VI)		-Coralgil
-Inflammatory bowel disease	-Mitochondrial and peroxisomal		-Tamoxifen
-Celiac disease	defects of fatty acid oxidation		-Methotrexate
-Hepatitis C	-Madelung lipomatosis		-Prednisolone
-Nephrotic syndrome	-Lipodystrophies		-Valproate
-Type 1 or 2 diabetes mellitus	-Dorfman-Chanarin syndrome		-Vitamin L-asparaginase
-Mauriac syndrome	-Abeta or hypobetalipoproteinemia		-Zidovudine and HIV
-Thyroid disorders	-α- and β-oxidation defects		treatments
-Hypothalamo-pituitary	-Porphyria cutanea tarda		-Solvents
disorders	-Homocystinuria		-Pesticides
-Blind loop	-Familial hyperlipoproteinemias		
(bacterial overgrowth)	-Tyrosinemia type 1		
	-Bile acids synthesis defects		
	-Congenital disorders of glycosylation		
	-Turner syndrome		
	-Organic acidosis		
	-Citrin deficiency		
	-HFE (hemochromatosis)		

(Source: [121]).

Liver Biopsy is considered the gold standard for the diagnosis and grading of NAFLD [116, 122]. Due to its invasive nature, other non-invasive methods are more commonly used [123]. These include: ultrasonography (US), fibroscans, computerized tomography (CT),

magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) (**Table 1.8**) [121, 123]. Although these tools are able to detect significant steatosis, they are unable to distinguish between NASH and other forms of NAFLD and are less sensitive in detecting low levels of steatosis [122, 123]. Liver ultrasound (US) or fibroscan and liver function tests (LFTs) (elevated serum hepatobiliary enzymes: alanine transaminase (ALT) and gamma-glutamyl transferase (GGT)) are usually the first investigation techniques used to diagnose NAFLD [20, 121, 124]. Liver ultrasound has lower sensitivity when steatosis is milder (<30%) [117, 125]. In additional to detecting steatosis, fibroscans can give a measurement of liver stiffness, which correlates with degree of fibrosis [126]. ALT is known to have a low sensitivity for NAFLD discrimination as adult and pediatric patients often present with ALT levels in the normal range [20]. Although the cut off levels of ALT for diagnosing NAFLD are controversial, in children ALT levels >20 U/L are considered abnormal (ALT cut-offs lower for children and are shown to be closer to 20 U/L) [121, 127]. CT, MRI and MRS are considered second-line due their higher cost, lower availability and radiation exposure (CT) [123].

Table 1.8 Sensitivity and Specificity of ALT and Non-Invasive Imagining Techniques.

Noninvasive imagining techniques*					
ALT[Ŧ]	**US**	**CT**	**MRI**	**MRS**	**Fibroscan**
Sensitivity 80%-92%	Sensitivity 60%-96%	Sensitivity 82%	Sensitivity 100%	Sensitivity 87%-100%	Sensitivity 97%-100%
Specificity 79%-85%	Specificity 84%-100%	Specificity 100%	Specificity 90.4%	Diagnostic precision 80-85%	Specificity 91%-100%

**None of these methods distinguishes NAFLD from NASH (only liver biopsy).*
[Ŧ] 95[th] percentile for ALT levels in NHANES pediatric participants (normal weight, metabolically healthy, no liver disease), boys (25.8 U/L) and girls (22.1 U/L) [127].
Abbreviations: ALT, alanine aminotransferase; US, ultrasonography; CT, computed tomography; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy.
(Source: [121, 127, 128]).

1.6.1.3 Mental Health, Quality of Life and NAFLD

Although most of the studies examining this relationship have primarily been done in adults, there is preliminary data to suggest that children with NAFLD have increased psychosocial concerns when compared to their obese non-NAFLD counterparts. It has been shown that children with NAFLD have reduced quality of life (influenced predominately by fatigue, insomnia, and sadness), as well as increased emotional and behavioural problems compared to healthy children [129, 130]. Further, children with NAFLD have also been shown to have higher levels of depression compared to obese controls [131].

1.6.2 Prader-Willi Syndrome (PWS)

Prader-Willi Syndrome (PWS) is a genetic multisystem disorder with a prevalence of 1 in 10,000 to 1 in 30,000 [21, 132]. The prevalence is similar between male and females and there are no ethnic differences [132, 133]. Currently, there are approximately 400,000 people worldwide with PWS [133].

1.6.2.1 Risk Factors and Pathophysiology

PWS has been described as having two distinct nutritional phases: failure to thrive, followed by hyperphagia, which can lead to obesity [21]. Recently it has been suggested that the progression and development of the nutritional phases are more complex and as many as seven different phases have been reported [21, 132]. In infancy, PWS is characterized by difficulty feeding due to poor suckling, hypotonia and growth retardation [21, 133]. In early childhood (usually between 2-6 years of age) the onset of hyperphagia (insatiable appetite) can result in the gradual development of morbid obesity [21, 132]. In late childhood and adolescence, patients experience delayed puberty, morbid obesity, and other related complications [133]. However

with strict diet and activity regimes, children with PWS can stay within normal body weight ranges. Motor milestones and language development are delayed and all individuals have some degree of cognitive impairment [133]. Patients often present with distinctive behavioral patterns such as tantrums, stubbornness and manipulative and compulsive behaviours [21]. Both males and females experience hypogonadism, which expresses as genital hypoplasia and incomplete pubertal development (often with infertility) [21]. Many also experience short stature in part due to growth hormone (GH) deficiency [21]. Patients with PWS also have lower energy requirements compared to healthy age matched controls. For both adults and children, the energy requirements have been found to be 20-30% lower, potentially due to a reduced metabolic rate and lower muscle mass [132].

In patients with PWS, body composition is abnormal [134]. Patients have increased body fat (primarily subcutaneous) and decreased lean body mass [134]. As a result, at any given BMI (healthy weight to obese), patients with PWS will have a higher body fat percentage with a lower ratio of visceral fat to subcutaneous fat compared to age and gender matched healthy children [134]. These differences in body composition are thought to be due to the impaired activity of the GH-IGF (growth hormone-insulin like growth factor) system and partial hypogonadism [134]. There are some protective effects of reduced visceral fat on obesity complications such as insulin resistance (IR), whereby children with PWS have less severe IR than children with visceral adiposity (as in NAFLD) [134]. However, metabolic complications such as Type 2 diabetes, dyslipidemia and cardiovascular disease have been reported in PWS later in life [134].

Results from a study by Lindmark et al., found that the diets of children with PWS were low in several nutrients, including vitamin D [132]. They also found that some parents/caregivers of children with PWS restrict fat intake too much resulting in the child consuming inadequate

intakes of fat (<20% of the daily energy requirement) [132]. This could result in children consuming insufficient amounts of polyunsaturated fatty acids (PUFAs) as well as impacting fat soluble vitamin (FSV) absorption (i.e. vitamin D) [132]. Children with PWS experience growth issues and poor bone health [93]. As a result, bone health and body composition are routinely monitored. Current guidelines recommend routine DXA in children with PWS every 6-12 months due to the high risk for suboptimal linear and bone growth [93].

1.6.2.2 Diagnosing PWS

PWS occurs as a result of the lack of expression of genes from an imprinted region of the paternally inherited chromosome 15q11.2-q13 [133]. This absence of expression can occur by one of three different mechanisms: deletion of a region from the paternally contributed chromosome 15 (65-75% of cases), maternal uniparental disomy (UPD) 15 (20-30% of cases) and a defect in the genomic region that controls the imprinting process (1-3% of cases) [21].

1.6.2.3 Management of PWS

To prevent complications and improve life quality and expectancy, PWS must be managed through a multidisciplinary lifelong approach [132]. Obesity can be prevented through strict supervision: restriction to food access while providing regular well balanced and low energy meals and adhering to regular exercise [132]. However, while it is important to prevent excessive energy intake, too much restriction could result in inadequate energy intake and nutrient insufficiencies/deficiencies which may negatively affect growth and development [132].

1.9 Vitamin D, Insulin Resistance/Hyperinsulinemia and Body Composition in NAFLD

Several studies have shown that adults with NAFLD have lower serum 25(OH)D, but the mechanism for this is largely unknown [15, 120, 122]. Several potential factors for their suboptimal vitamin D status include reduced vitamin D intake/supplementation, altered body composition and elevated IR (**Figure 1.5**).

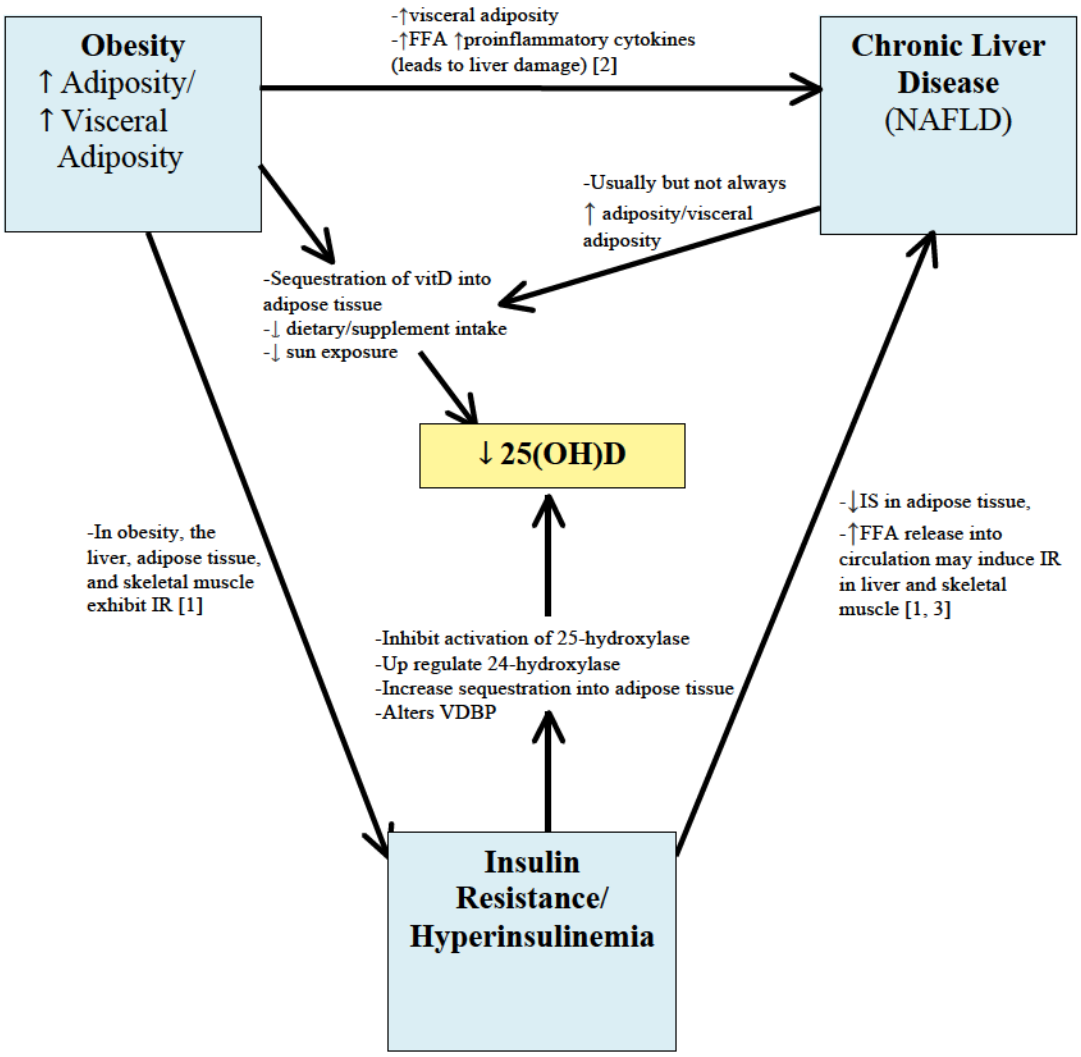

Figure 1.5 *A Proposed Mechanism for how Alterations in Body Composition (Visceral Adiposity), Insulin Resistance and Hyperinsulinemia Influence Vitamin D Status in Adults and Children with NAFLD.*

Abbreviations: IR, insulin resistance; IS, insulin sensitivity; vitD, vitamin D; VDBP, vitamin D binding protein; FFA, free fatty acid.

In NAFLD, the presence of adiposity (visceral and total body) and IR/hyperinsulinemia may negatively influence vitamin D status. Suboptimal vitamin D status may also influence the severity and onset of IR/hyperinsulinemia in obese children and therefore potentially influence risk for NAFLD. The current evidence relating the influence of adiposity, IR and hyperinsulinemia on vitamin D status in children and adults with NAFLD are presented in (**Appendix 2 Tables A2.4-A2.6**). Currently there are no studies in children or adults with PWS that have looked at the relationship between vitamin D, IR/hyperinsulinemia and body composition.

1.9.1 Summary of Scoping Review

Several studies have shown that individuals with NAFLD are at risk for suboptimal vitamin D status [15, 120, 122]. NAFLD is often accompanied with increased adiposity (visceral and total body) and IR/hyperinsulinemia, both of which may negatively influence vitamin D status. To evaluate these relationships, a scoping review was conducted (March 2015) examining the literature relating IR/hyperinsulinemia, body composition and vitamin D status in individuals with NAFLD (**Appendix 2 Figure A2.1**). A second search was performed November 2016, yielding three new articles. Currently (as of November 2016) the evidence consists of 14 observational studies and 2 interventional studies that examined the impact of adiposity (central and total) and insulin resistance/hyperinsulinemia on vitamin D status in obese children and adults with NAFLD (**Appendix 2 Tables A2.4-A2.6**).

Although there is some data to support a relationship between 25(OH)D and body composition, most studies showed that the relationship between NAFLD and 25(OH)D is independent from adiposity or no relationship was observed. Similar findings were observed for the relationships between insulin resistance/hyperinsulinemia and 25(OH)D. Some studies

showed a relationship between insulin resistance/hyperinsulinemia and 25(OH)D while others found that the relationship between 25(OH)D and NAFLD was independent from insulin resistance/hyperinsulinemia or no relationship was observed.

Six studies [124, 135-139] found a relationship between 25(OH)D and adiposity. Four of these studies [135, 137-139] showed on additional analysis the relationship between NAFLD and 25(OH)D was actually independent from adiposity. Three additional studies [116, 140, 141] found that the relationship between 25(OH)D and NAFLD was independent from adiposity. Five studies [142-146] showed no relationship between 25(OH)D and adiposity. Results from the two RCT's trial show that vitamin D supplementation had no effect on adiposity [147, 148]. Two studies found that insulin resistance (IR)/hyperinsulinemia was independently associated with hypovitaminosis D [137, 143]. Conversely, three studies [135, 139, 141] found that the relationship between 25(OH)D and NAFLD was independent from IR/hyperinsulinemia. Six studies found no relationship between 25(OH)D and IR/hyperinsulinemia [136, 140, 142, 144-146]. Finally, vitamin D supplementation had no effect on IR/hyperinsulinemia [147, 148].

Using the Downs and Black (DB) checklist with the Silverman scoring system (adjusted for observational studies), studies ranged in overall methodological quality from fair to good (**Appendix 2 Table A2.3**) and demonstrated inconsistent findings relating these variables to vitamin D status. The observational studies scored higher (very good-excellent) on the Newcastle-Ottawa scale (NOS). This may be due to fewer questions on the NOS or that studies score lower on the DB checklist even with the adjusted Silverman scoring system. These studies achieved an overall level B (moderate quality) of evidence) [149]. To note, the three additional studies added November 2016 were not ranked.

The variability in study findings might be due to inconsistent study populations (of varying age and gender), failing to exclude patients taking vitamin D supplements [135-138, 140-142, 144, 145] or medications known to affect vitamin D metabolism [124, 135, 136, 140-142, 144, 148] or failing to control for seasonal variation [124, 136, 144-146]. Finally, the method used to measure 25(OH)D levels varied and only one of the included studies used the gold standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) [145, 150]. This may affect results as other methods such as chemiluminescence immunoassay (CLIA) tend to report significantly lower 25(OH)D levels compared to LC-MS/MS [151]. Absolute levels of 25(OH)D might have an impact on the results observed since more extreme lowered values of vitamin D might yield more significant results.

The methods used to diagnose NAFLD were also inconsistent. Half of the included studies used liver ultrasound (US) to diagnose NAFLD while the other half used liver biopsy. Although liver US is more readily available and less invasive, it is not as accurate compared to the gold standard liver biopsy [152]. Despite the inconsistencies in tools used for diagnosis, fourteen out of the sixteen studies excluded patients with other known causes of steatosis. This is important because other forms of hepatic disease (Hepatitis C, Hepatitis B, autoimmune liver disease, hemochromatosis, Wilson's disease, etc.), excessive alcohol intake or hepatotoxic medications can cause steatosis.

The methods of body composition assessment also varied. Most studies used anthropometric measurements (mostly waist circumference) to assess body composition rather than DXA, CT or MRI, which would give a more accurate estimate of body composition. This idea is highlighted by a study which found that the inverse association between total body fat and 25(OH)D levels was weaker with anthropometric measurements as compared to more precise

measurements using DXA [153]. Finally, most studies used the homeostatic model assessment (HOMA) to assess insulin resistance, which is considered a surrogate measure of insulin resistance rather than the gold standard euglycemic insulin clamp technique [140]. One study showed that although there is moderate agreement between these two methods, the correlation was smaller in those with lower BMI (<25kg/m^2), lower HOMA-beta cell function and a higher fasting glucose level (>5.7 mmol/L) [154].

In NAFLD patients specifically, there is a limited number of studies examining the relationship between body composition and vitamin D status, especially as a primary outcome. It is important to understand the influence of both total and visceral adiposity and insulin resistance/hyperinsulinemia in those with NAFLD. Not only do these factors potentially impact vitamin D status, but suboptimal vitamin D status may also influence the severity and onset of insulin resistance/hyperinsulinemia. More studies are needed using gold standard methods for the evaluation of NAFLD and measurements of body composition, insulin resistance and vitamin D status.

1.9 Conclusion

Both vitamin D deficiency and obesity are a concern in children living in Alberta. Vitamin D insufficiency has been associated with poor bone health, presence of liver disease and indices of muscle function, insulin resistance/insulin sensitivity, inflammation and other cardiometabolic risk factors, particularly in obese individuals [10-15]. Obese individuals tend to have lower levels of vitamin D compared to lean individuals [17, 18]. Additionally, obesity has been associated with chronic diseases such as liver disease and diabetes, where vitamin D status may be further compromised.

In those with NAFLD, the presence of adiposity (visceral and total body) and IR/ hyperinsulinemia may negatively influence vitamin D status. Suboptimal vitamin D status may also influence the severity and onset of IR/hyperinsulinemia in obese children and hence potentially influence risk for NAFLD. In PWS, little is known about vitamin D status and how this influences these factors.

The contrast in insulin sensitivity and body composition between PWS (primarily subcutaneous obesity with little or no insulin resistance) and NAFLD (primarily visceral adiposity that contributes to significant insulin resistance, hyperinsulinemia and metabolic dysregulation) enables an interesting comparison on how these and other factors including inflammatory and cardiometabolic parameters and muscle function/functional capacity influence vitamin D status [22-29].

CHAPTER 2: Research Plan

2.1 Study Rationale

Vitamin D deficiency is highly prevalent in Canada; particularly in obese individuals [4, 5, 155]. This has important public health implications in childhood, as more than 25% of Canadian children are reported to be overweight or obese [8]. Vitamin D plays an important role in bone growth and body composition, and has also been shown to have important roles in the immune system. Several studies show that vitamin D may play a role in the expression of cardiometabolic dysregulation, hypertension, insulin resistance, depression and risk for sarcopenia; all common comorbid conditions in obesity. However, little work has been done in obese children in Canada to examine the interrelationships between these factors and vitamin D.

Vitamin D status is influenced by both endogenous cutaneous synthesis and dietary intake of vitamin D. In northern climates like Canada, cutaneous synthesis tends to be quite low due to reduced sunlight in the winter months and vitamin D intake has been reported to be consistently low in the general population [155]. Vitamin D status can also be influenced by the presence of either liver disease and/or renal disease due to impairments in the conversion of vitamin D to its active form ($1,25(OH)_2D$). Both total body fat and body fat distribution (visceral vs subcutaneous adiposity) are also factors that have been related to overall vitamin D status. Hence, understanding the factors that may contribute to suboptimal vitamin D status in obese children (body composition, dietary intake, seasonal effects, presence of comorbid conditions) is important.

The purpose of this thesis was to study the factors influencing vitamin D status in pediatric obesity (Chapter 1, 3 & 4). To enable a comprehensive evaluation of this topic we conducted two studies: a) Retrospective review of obese children with and without comorbid

conditions (e.g insulin resistance, mental health disorders) attending a Pediatric Weight Management Centre (Chapter 3) and b) Prospective study examining vitamin D status, body composition, markers of metabolic dysregulation in obese children with nonalcoholic fatty liver disease (NAFLD) and Prader-Willi Syndrome (PWS) (Chapter 4). NAFLD is highly prevalent in obese children (up to 25% of the population); while PWS is a rare genetic disorder causing hyperphagia and obesity. Examination of these two pediatric populations affords the unique opportunity to examine differences in body fat distribution (NAFLD: visceral, PWS: subcutaneous), liver, cardiometabolic dysregulation and how this may be related to overall vitamin D status in pediatric obesity.

2.2 Hypothesis and Objectives

2.2.1 Study 1: *Interrelationships between vitamin D status and anthropometric, cardiometabolic, liver function and mental health parameters in children (2-18 years) attending a Pediatric Centre for Weight and Health (PCWH). (Chapter 3).*

Objective #1: To describe the prevalence of vitamin D deficiency, metabolic dysregulation (insulin resistance, hypertension, hyperinsulinemia, dyslipidemia), mental health disorders and comorbidities in obese children attending a multidisciplinary specialty care clinic that specializes in the treatment and management of obese children.

Objective #2: To examine the interrelationships between vitamin D status and anthropometric, cardiometabolic, liver and mental health parameters in obese children attending the PWCH.

Hypothesis #1: Prevalence of vitamin D deficiency, cardiometabolic and liver dysfunction, total and mental health comorbidities with be higher in obese children attending the PCWH compared to that reported in the general pediatric population.

Hypothesis #2: Suboptimal vitamin D status will contribute to metabolic dysregulation (insulin resistance, hypertension, hyperinsulinemia, dyslipidemia and liver dysfunction) and mental health disorders in obese children.

2.2.2 Study 2: *Is vitamin D status influenced by insulin resistance, liver function, cardiometabolic disease risk, body composition, muscle strength in obese children with Nonalcoholic fatty liver disease and Prader-Willi Syndrome (7-18 years)? (Chapter 4).*

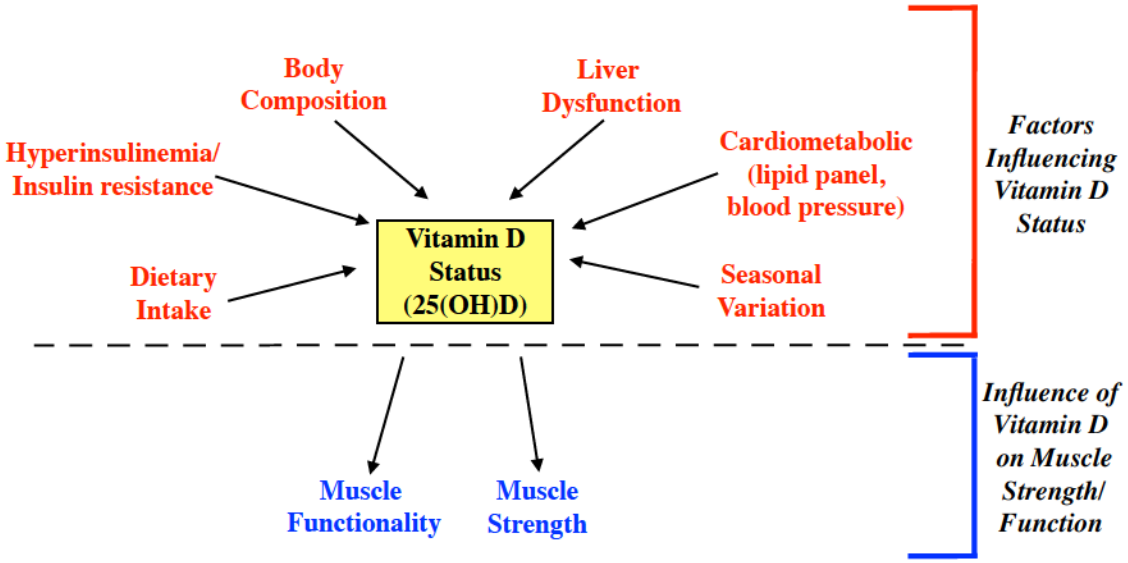

Figure 2.1 *Study 2 Objectives.*

Objective 1: To describe the prevalence of vitamin D deficiency, and factors which may influence vitamin D status, including hyperinsulinemia, insulin resistance, cardiometabolic risk factors, dietary intake, season and body composition in obese children with nonalcoholic fatty liver disease and Prader-Willi Syndrome (7-18 yrs).

Objective 2: To examine the influence of vitamin D status on muscle strength and muscle functionality.

Hypothesis #1: Children with NAFLD and PWS will have significantly lower vitamin D status compared to healthy children with body weights within normal reference ranges.

Hypothesis #2: Suboptimal vitamin D status in obese children with NAFLD and PWS will be related to diet, season, increased adiposity and a higher prevalence of cardiometabolic dysfunction, dyslipidemia, insulin resistance, hyperinsulinemia, and liver dysfunction.

Hypothesis #3: Higher vitamin D status will be associated with increased muscle strength and muscle functionality.

CHAPTER 3: Vitamin D Status, Cardiometabolic, Liver and Mental Health Status in Obese Youth Attending a Pediatric Weight Management Centre in Northern Alberta.

Abstract

Background: Metabolic dysregulation, suboptimal vitamin D status and mental health comorbidities are commonly observed in childhood obesity. The study objective was to describe vitamin D status and associations with anthropometric, cardiometabolic, liver and mental health parameters in obese children living in a northern community. ***Methods****:* A retrospective chart review was conducted in children aged 2-18 years referred to a pediatric obesity management clinic (n=217). Variables assessed included: anthropometric (weight, height, BMI, WC), vitamin D (serum (25(OH)D), cardiometabolic (SBP, DBP, glucose, insulin, HOMA-IR, TG, HDL, LDL, TC), liver function (ALT, GGT) and mental health (number, diagnosis) parameters. ***Results:*** Obese children (12.0 ± 2.9yrs; 112M/105F) had a median BMI percentile of 99.9 (99.4 – 99.9) and mean WC of 99.1 ± 15.1cm. Vitamin D insufficiency was 29% (mean: 62 ± 19nmol/L). Prevalence of hypertension: 14% had pre-hypertension, 25% had stage I hypertension and 7% had stage II. Mental health diagnoses included anxiety, ADHD, mood disorders and learning disabilities/developmental delays in 18%, 17%, 10% and 15%, of patients respectively. Waist circumferences >100cm were associated with lower vitamin D (58 ± 18 nmol/L vs. 65 ± 17 nmol/L; p=0.01). Vitamin D status ≥50nmol/L was associated with lower insulin (P<0.01) and HOMA-IR (P<0.01) values and lower SBP percentiles (p=0.04). No relationships between mental health and vitamin D status were observed. ***Conclusions:*** Obese children attending a pediatric weight management clinic had a high prevalence of vitamin D deficiency, liver and cardiometabolic dysfunction and mental health disorders. Vitamin D status was related to reduced insulin sensitivity, higher blood pressure, and central obesity; but not mental health disorders.

3.1 Introduction

Vitamin D has roles in calcium and phosphorous homeostasis and bone metabolism [37]. Vitamin D is essential for adequate growth and development. In recent years, the discovery of the vitamin D receptor (VDR) in most body tissue and cells, has lead to the realization that vitamin D has many roles beyond skeletal health [37]. These roles include those related to immune function, muscle function, cardiometabolic regulation and mental health [73-75].

Vitamin D insufficiency is highly prevalent in children and adults, particularly in northern climates, due to reduced sunlight exposure and low intake [156]. In northern communities up to 40% [6, 7] of children and adults have deficient/insufficient serum 25-hydroxyvitamin D (25(OH)D<50nmol/L) levels and up to 78% of children have vitamin D intakes that are significantly below the estimated average requirement (EAR) of 400 IU/day [5, 8]. This suggests that routine vitamin D supplementation in children may be warranted within the general population in Canada. Suboptimal vitamin D status has also been reported in up to 75% and 90% of obese youth [157, 158]. This is of particular concern as 15-25% of Canadian children have been reported as overweight or obese and hence are potentially at increased risk for vitamin D deficiency compared to lean children [5, 8].

Suboptimal vitamin D status has also been associated with increasing severity of metabolic dysregulation (insulin resistance, hyperlipidemia, liver disease and hypertension) in children and adults with obesity [12, 15]. Furthermore, there is evidence that suboptimal vitamin D status may predispose an individual to an increased risk for depression, reduced quality of life and other mental health disorders, particularly in individuals with obesity [73, 74]. Therefore, understanding vitamin D needs and the underlying contribution of suboptimal vitamin D status to the expression of metabolic dysregulation and mental health disorders in obese children is important. This is particularly relevant in a population already at high risk for suboptimal

vitamin D status, as treatment and prevention of vitamin D deficiency may improve overall outcomes in overweight and obese youth at high risk for comorbid conditions.

The study objective was to describe vitamin D status and the associations with anthropometrics and markers of cardiometabolic, liver and mental health status in a cohort of children attending a multidisciplinary specialty care clinic that specializes in the treatment of obese children. We hypothesized that suboptimal vitamin D status is highly prevalent in obese children and is associated with increased expression of metabolic dysregulation (insulin resistance, hyperinsulinemia, dyslipidemia, liver dysfunction) and mental health disorders in the children attending this clinic.

3.2 Methods

3.2.1 Subjects

This retrospective medical chart review (2011-2014) included overweight and obese children (n=217) between the ages of 2-18 years attending the Pediatric Centre for Weight and Health (PCWH) at the Misericordia Hospital in Edmonton, Alberta. The PCWH's interdisciplinary health care team includes a pediatric psychiatrist, assessment psychologist, specialty pediatrician, exercise specialist, registered nurse and registered dietitian and follows a family/patient centred care model.

3.2.2 Anthropometric Variables

Weight (kg) and height (cm) were measured using standard methodologies by trained personnel. Height was measured using a Seca wall mounted stadiometer (model 240, Chino, USA) and weight was measured using a Seca scale (model 644, Chino, USA). Body mass index (BMI) was calculated as weight (kg) / height (m^2). Waist to height ratio (WHtR) was calculated

as waist circumference (WC) / height. Body surface area (BSA) was calculated using Mosteller's formulae [159]. Weight, height, BMI, WC and WHtR were converted into z-scores/percentiles using the World Health Organization (WHO) growth charts for Canada (2014 revision) [160]. Obesity was defined according to WHO criteria as a BMI above the 97th percentile and overweight as a BMI between the 85th and 97th percentile. Waist circumference was measured in triplicate to the nearest 0.1 cm, according to the National Institutes of Health (NIH) criteria (highest point of iliac crest) using a Gulick II Plus measuring tape with a tension meter attached.

3.2.3 Mental Health

Previous mental health diagnoses, family history of mental health conditions and mental health conditions diagnosed after assessment in clinic (including any queries) were evaluated from the psychiatrist's and psychologist's notes. Mental health diagnoses were stratified into five groups: mood disorders, anxiety, attention deficit-hyperactivity disorder (ADHD), learning disabilities/developmental delays and any other diagnoses. Mental health diagnoses were evaluated using the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria.

3.2.4 Cardiometabolic & Laboratory Variables

Fasting blood work included triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC)), insulin, glucose, thyroid stimulating hormone (TSH), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and 25-hydroxyvitamin D (25(OH)D). These were performed in the Core Laboratory at Alberta Health Services (AHS) according to standard methodologies [102].

Vitamin D status was classified as: deficient 25(OH)D <50nmol/L, suboptimal 25(OH)D 50-75 nmol/L and sufficient 25(OH)D >75 nmol/L according to the Canadian Society of Endocrinology & Metabolism [52]. ALT values >20 U/L were considered abnormal [127]. The homeostasis model assessment for insulin resistance (HOMA-IR) (glucose mmol/L x insulin mU/L /22.5) was used as an index of insulin resistance [161]. To assess for the overall risk for liver disease (primarily nonalcoholic fatty liver disease or NAFLD), any obese child with an ALT > 20 U/L with positive echogenic finding (fatty liver) on ultrasound was deemed at high risk for NAFLD [121].

Blood pressure was measured at the initial clinic visit using an automatic blood pressure machine (Welch Allyn Vital Signs Monitor 300 series). Blood pressure was converted to z-scores/percentiles and classified as normal, pre-hypertensive, stage I hypertension and stage II hypertension according to the National High Blood Pressure Education Program Working group standards [162].

3.2.5 Comorbidities, Symptomology and Lifestyle Variables

Lifestyle variables including sleep duration and screen time were evaluated from a self-reported questionnaire that was routinely administered at each clinic visit. Comorbidities were evaluated from the physician exam/patient history. Pre-existing comorbidities were assessed using established categories: endocrine disorders (e.g. polycystic ovarian syndrome, type 2 diabetes mellitus), respiratory (e.g. asthma, obstructive sleep apnea), bone/joint (e.g. delayed bone growth, juvenile idiopathic arthritis), gastrointestinal (e.g. celiac disease, gastroesophageal reflux disease), nonalcoholic fatty liver disease, hypertension, hyperlipidemia and mental health (e.g. anxiety, depression).

3.2.6 Statistical Analysis

Data analysis was completed using the SAS 9.0 statistical software (SAS, Version 9.4; SAS Institute Inc., Cary, NC, USA). Data was expressed as mean ± standard deviation. Data was assessed for normality. Non-parametric variables were log transformed and expressed as median (interquartile range). Laboratory values were expressed as both continuous and categorical variables (normal/abnormal). Variables such as serum 25(OH)D (> and < 50 nmol/L), mental health diagnoses (mood disorders, anxiety, attention deficit-hyperactivity disorder, learning disabilities and other), and comorbid conditions were expressed as categorical variables. Primary outcome variables included vitamin D status (25(OH)D), anthropometric variables (weight, weight-z scores, height, height-z scores BMI, BMI-z scores, WC, WC-z scores), markers of cardiometabolic (lipid panel, hypertension, insulin, HOMA-IR) and liver (ALT, GGT) dysfunction and mental health comorbidities. Univariate and multivariate analyses were performed to assess the interrelationships between vitamin D status with anthropometric, cardiometabolic, liver and mental health comorbidities. Where needed, primary outcome variables were adjusted for potential confounders (age, gender, season, changes in weight > or < 5% over six months). A p-value <0.05 was considered significant.

3.3 Results

3.3.1 Demographic, Anthropometric and Lifestyle Variables

Anthropometric, demographic and lifestyle variables data are presented in (**Table 3.1**). At the initial clinic visit, 9% (n=19) of children were overweight and 91% (n=196) of children were obese. Waist circumference (WC) was >100cm in 47.5% (n=84) of children. Median time between referral and the initial clinic visit was 143 (104 – 188) days.

Table 3.1 Anthropometric, Demographic and Lifestyle Variables.

Variable	Value* (n=217)
Male n (%)	112 (52%)
Age (years)	12.0 ± 2.9
Weight (kg)	76.8 ± 29.5
Weight Z-score	2.9 (2.2 – 3.0)
Weight Percentile	99.8 (98.5 – 99.9)
Height (cm)	154.8 ± 15.8
Height Z-score	1.0 ± 1.3
Height Percentile	72.7 ± 27.8
BMI (kg m^{-2})	30.9 ± 7.1
BMI Z-score	3.0 (2.5 – 3.0)
BMI Percentile	99.9 (99.4 – 99.9)
WC (cm)	99.1 ± 15.1
WC Z-score	2.0 ± 0.4
WC percentile	98.0 (96.0 – 99.0)
WHtR	0.64 (0.60 – 0.69)
WHtR Z-score	2.0 (1.7 – 2.2)
WHtR Percentile	98.0 (96.0 – 99.0)
BSA (m^2)	1.8 ± 0.4
SBP (mmHg)	117 ± 12
SBP Z-score	0.9 (0.3 – 1.6)
SBP Percentile	82.1 (62.4 – 94.6)
DBP (mmHg)	65 ± 9
DBP Z-score	0.1 ± 0.8
DBP percentile	53.1 ± 24.1
Average Sleep (hrs/night)	9.6 (9.0 – 10.0)
Weekday Screen Time (hrs/day)	3.0 (2.3 – 5.0)
Weekend Screen Time (hrs/day)	5.5 (4.0 – 8.0)

**Values expressed as mean ± standard deviation or median (interquartile range). There were missing data for weight (n=1), weight z-score (n=1), weight percentile (n=1), BMI (n=1), BMI z-score (n=1), BMI percentile (n=1), WC (n=40), WC z-score (n=42), WC percentile (n=42), WHtR (n=42), WHtR z-score (n=42), WHtR percentile (n=42), BSA (n=1), SBP (n=4), SBP z-score (n=5), SBP percentile (n=5), DBP (n=4), DBP z-score (n=5), DBP percentile (n=5), Sleep (n=8), weekday screen time (n=10), weekend screen time (n=20). Abbreviations: BMI, body mass index; WC, waist circumference; WHtR, waist to height ratio; BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure.*

3.3.2 Vitamin D Status, Markers of Cardiometabolic, and Liver Dysfunction

Biochemical and cardiometabolic data are presented in (**Table 3.2**). The prevalence of vitamin D deficiency (<50nmol/L) was 29% and insufficiency (50-75 nmol/L) 47% (mean 62 ± 19 nmol/L). Risk of pre-hypertension, stage I hypertension and stage II hypertension was 14%, 25% and 7% respectively. In general 40-70% of children presented with elevated laboratory variables that were indicative of cardiometabolic and liver dysfunction (41% had insulin values >20 mU/L, 68% had HOMA-IR values ≥ 3, 41% had TG ≥ 1.5 mmol/L and 42% had total cholesterol values ≥ 4.4 mmol/L). ALT values were ≥ 20 U/L in 70% of children (median: 24 (18 – 34) U/L). Ten percent (n=22) of children had liver ultrasounds indicative of fatty infiltration coinciding with a mean serum ALT of 64 ± 29 (13 – 133) U/L. Ninety-five percent (n=21) of these children had serum ALT levels consistent with risk for NAFLD (≥ 20 U/L) [127].

Table 3.2 Vitamin D Status Above and Below 50 nmol/L.

Variable	25(OH)D < 50 nmol/L[1]	25(OH)D ≥ 50 nmol/L[1]	P-value
Age (years)	12.3 ± 2.5	11.8 ± 2.9	0.30
Weight (kg)	84.1 ± 33.0	73.8 ± 27.4	0.03*
Weight z-score	3.0 (2.2 – 3.0)	2.8 (2.1 – 3.0)	0.22
Weight percentile	99.8 (98.4 – 99.9)	99.7 (98.4 – 99.9)	0.21
Height (cm)	156.7 ± 14.2	153.9 ± 16.0	0.26
Height Z-score	1.0 ± 1.5	1.0 ± 1.2	0.87
Height Percentile	69.5 ± 30.3	73.4 ± 26.6	0.38
BMI (kg/m^2)	33.0 ± 8.1	30.1 ± 6.6	0.01*
BMI z-score	2.8 ± 0.4	2.6 ± 0.5	0.04*
BMI percentile	99.9 (99.6 – 99.9)	99.9 (99.2 – 99.9)	0.06
WC (cm)	101.7 ± 15.2	98.5 ± 15.3	0.23
WC z-score	2.0 ± 0.4	2.0 ± 0.4	0.54
WC percentile	98.0 (97.0 – 99.0)	98.0 (96.0 – 99.0)	0.90
WHtR	0.66 (0.61 – 0.70)	0.6 (0.6 – 0.7)	0.16
WHtR z-score	2.1 (1.8 – 2.3)	2.0 (1.6 – 2.2)	0.35
WHtR percentile	98.0 (97.0 – 99.0)	97.0 (95.0 – 99.0)	0.55
BSA (m^2)	1.9 ± 0.4	1.8 ± 0.4	0.045*
SBP (mmHg)	120 ± 10	117 ± 13	0.08
SBP z-score	0.9 (0.6 – 1.7)	0.9 (0.2 – 1.6)	0.80
SBP percentile	80.6 ± 17.0	73.0 ± 25.8	0.04*
DBP (mmHg)	67 ± 9	64 ± 9	0.13
DBP z-score	0.2 ± 0.8	0.1 ± 0.8	0.23
DBP percentile	57.2 ± 24.8	52.3 ± 23.8	0.20
ALT (U/L)	24 (19 – 40)	23 (18 – 31)	0.40
GGT (U/L)	17 (12 – 28)	15 (11 – 22)	0.40
Glucose (mmol/L)	4.9 ± 0.5	4.9 ± 0.4	0.54
Insulin (mU/L)	21.1 (14.3 – 34.2)	15.8 (11.7 – 23.1)	<0.01*

Table 3.2 Vitamin D Status Above and Below 50 nmol/L Continued.

Variable	25(OH)D < 50 nmol/L[1]	25(OH)D ≥ 50 nmol/L[1]	P-value
HOMA-IR	4.8 (3.1 – 6.9)	3.5 (2.5 – 4.9)	<0.01*
TG (mmol/L)	1.3 (1.0 -1.9)	1.3 (0.9 – 1.8)	0.29
TC (mmol/L)	4.3 ± 0.9	4.4 ± 0.8	0.38
HDL-C (mmol/L)	1.1 ± 0.3	1.2 ± 0.3	0.53
LDL-C (mmol/L)	2.4 ± 0.7	2.6 ± 0.7	0.22
TSH (mU/L)	2.0 (1.5 – 3.3)	2.2 (1.7 – 3.2)	0.77
Sleep (hrs/night)	9.7 (9.0 – 10.1)	9.5 (9.0 – 10.0)	0.53
Screen Weekday (hrs/day)	3.0 (2.0 – 5.0)	3.2 (2.5 – 5.0)	0.17
Screen Weekend (hrs/day)	5.8 (4.0 – 8.0)	6.0 (4.0 – 8.0)	0.65
Total Comorbidities	2.3 ± 1.7	2.2 ± 1.7	0.84

**P-values <0.05 are statistically significant. [1]Values expressed as mean ± standard deviation or median (interquartile range). There were missing data for weight (n=1), weight z-score (n=1), weight percentile (n=1), BMI (n=1), BMI z-score (n=1), BMI percentile (n=1), WC (n=40), WC z-score (n=42), WC percentile (n=42), WHtR (n=42), WHtR z-score (n=42), WHtR percentile (n=42), BSA (n=1), SBP (n=4), SBP z-score (n=5), SBP percentile (n=5), DBP (n=4), DBP z-score (n=5), DBP percentile (n=5), ALT (n=2), GGT (n=15), glucose (n=5), insulin (n=16), HOMA (n=19), TG (n=2), TC (n=2), HDL (n=2), LDL (n=2), TSH (n=12) and 25(OH)D (n=21). Abbreviations: 25(OH)D, 25 hydroxyvitamin D; BMI, body mass index; WC, waist circumference; WHtR; waist to height ratio; BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HOMA-IR, homeostatic model assessment of insulin resistance; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TSH, thyroid stimulating hormone.*

3.3.3 Mental Health Disorders and Other Comorbid conditions

Sixty children (28%) presented with a mental health diagnosis and one hundred twenty-one (56%) with a positive family history (parent, grandparent). The prevalence of anxiety, ADHD, mood disorders, and learning disabilities/developmental delays was 18% (n=26), 17% (n=24), 10% (n=14) and 15% (n=21) respectively.

The median (interquartile range; IQR) number of total comorbid conditions in youth attending the clinic was 2 (1-3). Physician exam indicates that 91% (n=198) of children presented with defined comorbidities (2% endocrine, 11% respiratory, 1% bone/joint 2% gastrointestinal, 4% Nonalcoholic fatty liver disease, 19% hypertension, 30% hyperlipidemia, 29% mental health and 3% other out of a total of n=500 comorbidities).

3.3.4 Interrelationships between Vitamin D status, Anthropometric, Lifestyle Factors, Cardiometabolic and Liver Dysfunction and Mental Health Disorders

Serum 25(OH)D was higher in summer months (Aug 1 – Oct 31; 68 ± 22 nmol/L months) vs winter months (Feb 1 – April 30; 57 ± 18 nmol/L) ($p=0.03$). Vitamin D status was negatively correlated with age ($p=0.02$) and waist circumference ($p<0.01$). These relationships were independent from gender and BMI-z scores. When adjusted for seasonal effects, these relationships were no longer significant. In contrast, WC > 100 cm was associated with lower serum 25(OH)D concentrations (58 ± 18 nmol/L vs. 65 ± 17 nmol/L; $p=0.01$), independent from age and BMI-z. Changes in body in weight (above or below 5%) also did not influence serum 25(OH)D ($p>0.05$). No interrelationships between sleep duration and screen time and vitamin D status were observed ($p>0.05$).

Vitamin D showed negative correlations with systolic blood pressure (SBP) ($p=0.01$), insulin ($p<0.01$) and HOMA-IR ($p<0.01$) that were independent from age and BMI-z. When

adjusted for the potential confounding effects of gender and season, only HOMA-IR remained significant. Serum 25(OH)D concentrations ≥ 50 nmol/L were associated with lower insulin ($p<0.01$), HOMA-IR ($p<0.01$) and SBP percentile ranges ($p=0.04$). When adjusted for the potential confounding effects of age, gender, season, and BMI-z scores, only HOMA-IR and serum insulin levels remained significant (**Table 3.2**).

No relationships between the total number of comorbid conditions and vitamin D status were observed. No relationships between mental health parameters (type or total) and vitamin D status were observed ($p>0.05$).

3.4 Discussion

In Canada, the prevalence of obesity is increasing leading to an increased risk for comorbid conditions such as hypertension, depression, and liver disease in early childhood [143, 163, 164]. In addition, suboptimal vitamin D status is highly prevalent within northern communities [165]. The study purpose was to examine vitamin D status in an ambulatory population of obese children attending a pediatric weight management clinic and to assess the potential interrelationships between vitamin D status and markers of cardiometabolic, liver disease, and mental health.

The major study findings included a high prevalence of vitamin D deficiency (29%) and insufficiency (47%) in the children attending an obesity management clinic. Interestingly these findings were very similar to what is reported in children with healthy body weights within Canada; suggesting that vitamin D needs in obese children may not differ from the general population [4, 5, 165]. These findings are in contrast to other studies, where the presence of obesity in childhood has been associated with higher rates of suboptimal vitamin D status when compared to age-matched lean children [18]. Factors such as vitamin D intake/supplement use,

weight loss and endogenous cutaneous synthesis are important factors of overall vitamin D status. It is unlikely that a history of weight changes influenced study findings as we did not find any differences in serum 25(OH)D concentrations between those children with recent weight loss change (> or < 5%). While vitamin D intake/supplement use may have been a determinant factor, recent evidence suggests that both vitamin D intake/vitamin D supplement use are uniformly low in youth and adults within our region [156]. The prevalence of cardiometabolic risk factors (insulin resistance, dyslipidemia) and liver dysfunction were similar to what has been reported in other pediatric obesity centers in North America [166, 167]. Of interest, was the high proportion of elevated systolic blood pressure present; with 46% of the children in this cohort classified as either having pre-hypertension, stage I or stage II hypertension. This finding has not been consistently reported within the literature [166, 167]. Another important finding in this study was the relatively high prevalence of diagnosed mental health disorders and positive family histories of mental illness in the cohort. While there is some research to suggest a higher prevalence of mental health disorders in obese children, a comprehensive examination of mental health diagnoses using DSM-V criteria has not been well described in obese children attending weight management centres [166, 168]. These findings have important implications for treatment and management of obesity in childhood.

Relationships between vitamin D and liver biochemistries, lipid panel or glucose were not evident. Vitamin D status was inversely related with age, WC, systolic blood pressure, hyperinsulinemia, HOMA-IR, but not to other markers of cardiometabolic, liver dysfunction or total comorbid burden. Several hypotheses have been postulated that could explain associations between blood pressure, waist circumference, insulin sensitivity, and vitamin D status. The active hormonal form of vitamin D (1,25 dihydroxyvitamin D or $1,25(OH)_2D$) has been shown to

be a negative regulator of the renin angiotension system (RAS) [169]. Vitamin D receptor (VDR) knockout mice or mice with 1-alpha hydroxylase deficiency have elevated renin, angiotension II and hypertension [169]. Lower 25(OH)D levels has been associated with increased arterial stiffness and endothelial dysfunction [170]. Increased visceral adiposity may influence vitamin D status through increased sequestration of vitamin D and/or potentially exacerbation of insulin resistance. Vitamin D deficiency is associated with impaired beta-cell function and insulin release [1], while insulin resistance has been associated with 25-hydroxylase inhibition, up regulation of 24-hydroxylase and/or changes in vitamin D binding protein (VDBP) levels; all of which impact vitamin D status [39, 86].

No associations between overall vitamin D status and mental health comorbidities were evident. The data relating vitamin D and mental health is equivocal within the literature; particularly in childhood. Recent studies in children have shown little to no interrelationships between vitamin D status and mental health disorders in children, while other studies in obese adults have shown associations between an increased risk for depression and suboptimal vitamin D status [73, 157, 171]. Factors that may have influenced the ability to detect potential interrelationships in this study included the tools used to categorize mental health parameters in many studies. Many studies use self-reported mental health diagnosis (angriness, sadness or worry), rather than specific DSM-V criteria to categorize mental health. This study used DSM-V mental health standards [73]. While this should have increased rigour in the categorization of mental health, this may have influenced the overall inability to detect associations between suboptimal vitamin D status and mental health. The influence of vitamin D status on mental health may also extend beyond mental health disease type to disease severity, as disease expression may change with overall child development [172].

In summary, children attending a pediatric weight management centre had a high prevalence of suboptimal vitamin D status and cardiometabolic (particularly hypertension) and liver dysfunction, along with mental health disorders. Expression of these comorbid conditions was largely independent of vitamin D status. One important finding was that vitamin D status in obese children was not different from that reported within the general Canadian population, indicating that vitamin D needs may not be increased by the presence of obesity in childhood. Given that the majority of children had suboptimal vitamin D status, routine vitamin D supplementation is warranted in obese children attending weight management centres to prevent nutrient deficiency. Whether this would minimize the risk for comorbid disease expression in childhood obesity remains unclear. Longitudinal data would confer increased strength related to the assessments of the interrelationships between vitamin D status, weight management, metabolic dysregulation and mental health disorders in obese children.

CHAPTER 4: Vitamin D and Body Composition in Children and Adolescents with Nonalcoholic Fatty Liver Disease and Prader-Willi Syndrome

Abstract

Introduction: The study objective to determine factors influencing vitamin D status (season, diet, body composition, metabolic, biochemical) and to examine the influence of vitamin D status on muscle strength and muscle functionality in obese children with nonalcoholic fatty liver disease (NAFLD) and Prader-Willi Syndrome (PWS). **Methods:** Children aged 7-18 years with NAFLD (n=8), PWS (n=9) and healthy lean controls (n=16) were recruited from the Stollery Children's Hospital and the community. Anthropometrics (weight, height, circumferences, skinfolds), body composition (DXA), handgrip (measure of muscle strength), 6 minute walk test (6MWT), dietary (3-day food record), cardiometabolic and biochemical (25-hydroxyvitamin D, blood pressure, triglyceride (TG), total-cholesterol (TC), HDL-and-LDL-cholesterol, glucose, insulin) measures were assessed. **Results:** The prevalence of vitamin D deficiency (<50nmol/L) was 50% in the NAFLD and control group and 11% in the PWS group. Vitamin D status was higher in those taking vitamin D supplements (81 ± 30 nmol/L vs. 53 ± 15 nmol/L; p=0.002). SBP was significantly lower in children with vitamin D status ≥ 50 nmol/L compared to those <50nmol/L (114 ± 11mmHg vs. 124 ± 10mmHg; p=0.01). No other significant interrelationships between vitamin D status and markers of cardiometabolic, liver dysfunction and muscle strength/functionality were found (p>0.05). **Conclusion:** Vitamin D status was influenced by vitamin D supplementation, but not by body composition, cardiometabolic and liver dysfunction or season. Significant unique reductions in markers of muscle strength/physical capacity were observed in the children with PWS, despite adequate vitamin D status. Further investigations elucidating the potential mechanism/lifestyle factors influencing these findings are warranted.

4.1 Introduction

Nonalcoholic fatty liver disease (NAFLD) is a complication of non-syndromic obesity and Prader-Willi Syndrome (PWS) is a syndromic form of obesity. Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in children, with an estimated prevalence of 8-13% in children and adolescents and 38-57% in obese children and adolescents [115-117]. NAFLD is a chronic liver disease that encompasses a spectrum of disease that ranges from simple steatosis to steatosis with inflammation and fibrosis (nonalcoholic steatohepatitis or NASH) and potentially cirrhosis [19, 20]. The development of NAFLD is influenced by both lifestyle and genetic factors [20]. However, the specific genetic influences on disease expression are not clearly defined. In contrast, PWS is a condition that arises from a well-described genetic defect that often leads to obesity due to hyperphagia [21]. The etiology of PWS is due to the lack of expression of genes from an imprinted region of the paternally inherited chromosome 15q11.2-q13 [133]. Children with PWS experience primarily subcutaneous obesity with little or no insulin resistance. In contrast, children with NAFLD experience primarily visceral adiposity that contributes to significant insulin resistance, hyperinsulinemia and metabolic dysregulation; these features are thought to be primary determinants of disease expression [22-29].

Although many studies have reported reduced vitamin D levels with obesity (sequestered into the adipose tissue [82, 83]), few studies have examined the influence of adipose tissue distribution (subcutaneous vs visceral) and lean tissue mass on vitamin D status, particularly in children with NAFLD and PWS [17, 18]. Vitamin D insufficiency has been associated with poor bone health, presence of liver disease and indices of muscle function, insulin resistance/insulin sensitivity, inflammation and other cardiometabolic risk factors, particularly in obese individuals [10-15]. It is important to understand this as the main focus of treatment in both populations are

lifestyle modifications to promote weight loss and alterations in body composition; this treatment may influence both vitamin D intake, vitamin D status, and long term disease risk.

The study objective is to determine factors influencing vitamin D status, including hyperinsulinemia, insulin resistance, cardiometabolic risk factors, dietary intake, season and body composition and to examine the influence of vitamin D status on muscle strength and muscle functionality in obese children with NAFLD and PWS. We hypothesized that vitamin D deficiency would be related to diet, season, increased adiposity, and a higher prevalence of cardiometabolic dysfunction, dyslipidemia, insulin resistance, hyperinsulinemia, and liver dysfunction; along with decreased muscle strength and functionality in children with NAFLD and PWS.

4.2 Methods

Obese children with NAFLD and PWS were prospectively recruited from the Liver Clinics and the Endocrine Clinics at the Stollery Children's Hospital (October 2015-October 2016), Alberta Health Services. Healthy lean controls with body weights within normal reference ranges were recruited from the community using recruitment flyers. Informed consent was obtained from the legal guardian(s) of participants and informed consent or assent (depending on age) was obtained from all participants prior to subject enrolment (Appendix 1). Ethics approval was obtained from the Human Research Ethics Board, University of Alberta (Pro: 00056649). Operational and Administrative approval was obtained from Alberta Health Services through the Northern Alberta Clinical Trials Centre (NACTRC).

4.2.1 Inclusion and Exclusion Criteria

Children aged 7-17 years with clinically diagnosed NAFLD or PWS were included. The diagnosis of NAFLD was made in overweight/obese children with elevated liver enzymes (alanine aminotransferase; ALT and gamma-glutamyl transferase; GGT), the presence of hyperinsulinemia and hyperlipidemia, evidence of steatosis on liver ultrasound and confirmation that no other known causes of steatosis existed. This was done by the completion of routine blood work to rule out potential diagnosis of Wilson's disease, viral and autoimmune liver diseases, etc. The diagnosis of PWS was made through genetic testing (methylation studies and looking for the deleted region (q11-q13) of chromosome 15) [173]. Exclusion Criteria included: 1) children with a history of a known primary liver disease associated with steatohepatitis (e.g. Wilson disease, various metabolic disorders, viral hepatitis, etc.); 2) children with a known primary diagnosis of Type 2 Diabetes or those on insulin; 3) children on medications known to cause hepatic steatosis or interfere with vitamin D metabolism (e.g., corticosteroids, statins, Orlistat, etc.) or 4) children with a history of comorbid conditions known to affect vitamin D metabolism including other liver disorders or gastrointestinal disorders such as inflammatory bowel disease or celiac disease. Healthy controls were asked to fill out a health history questionnaire, to exclude the potential for metabolic dysregulation (Appendix 1). Healthy controls were excluded from the study if fasting metabolic blood work (triglycerides, cholesterol: total, LDL or HDL), liver enzymes (ALT, AST), insulin or glucose were outside the normal reference range.

4.2.2 Metabolic and Anthropometric Measurements

4.2.2.1 Height and Weight

Weight (kg) and height (cm) were measured to the nearest 0.1 kg and 0.1 cm. Height was measured without shoes, using a Digital Stadiometer (Measurement concepts and QuickMedical, Washington, USA). Weight was measured without shoes, using a Health o meter® Professional digital scale (Illinois, USA). Body mass index (BMI) was calculated as weight (kg) / height (m^2). Body surface area (BSA) was calculated using Mosteller's formulae [159]. Obesity was defined according to WHO criteria as a BMI above the 97^{th} percentile and overweight as a BMI between the 85^{th} and 97^{th} percentile [174]. Ideal body weight (IBW) was determined using the Moore Method [175]. Weight, height and body mass index (BMI) were converted into z-scores/percentiles using the World Health Organization (WHO) growth charts for Canada (2014 revision) [160].

4.2.2.2 Body Circumferences

Body circumferences were measured to the nearest 0.1 cm using a steel flexible tape (Rosscraft Innovations Incorporated, USA). All applicable circumferences were taken from the right side. Waist circumference (WC) was measured following the WHO criteria (midpoint between the highest point of the iliac crest and the bottom of the rib cage) [176]. Waist circumference (WC) and waist to height ratio (WHtR) were converted into z-scores/percentiles using the World Health Organization (WHO) growth charts for Canada (2014 revision) [160]. Hip circumference (HC) was measured at the maximum posterior protuberance of the buttocks (International Standards for Anthropometric Assessment, 2001). Mid-arm circumference (MAC) was measured at the midpoint between the top margin and most lateral aspect of the acromion bone and the proximal and lateral boarder of the head of the radius bone (International Standards

for Anthropometric Assessment, 2001). Flexed arm circumference (FAC) was measured at the highest peak of the biceps muscle with the forearm supinated and flexed at about 45-90 degrees to the arm (International Standards for Anthropometric Assessment, 2001). Calf circumference (CC) was measured at the maximum circumference of the calf (International Standards for Anthropometric Assessment, 2001). Waist to height ratio (WHtR) was calculated as WC / height. Waist to hip ratio (WHR) was calculated as WC / HC.

4.2.2.3 Bone Breadths

Biepicondylar bone breadths of the femur and humerus were measured to the nearest 0.1 cm using a Campbell small bone caliper (Rosscraft Innovations Incorporated, USA) with firm pressure to compress the subcutaneous tissue. All bone breadths were taken from the right side. Humerus bone breath was measured as the width between the medial and lateral epicondyles of the humerus with the elbow flexed at 90 degrees (Heath and Carter, 2003). Femur bone breath was measured seated, with the knee at a right angle, as the width between the medial and lateral epicondyles of the femur (Heath and Carter, 2003).

4.2.2.4 Skin Folds

Skinfolds were measured to the nearest 0.5 mm using a Lange skinfold caliper (Beta technology, Santa Cruz, California, USA). All skinfolds were taken from the right side. Biceps skinfolds were measured at the most anterior part of the biceps at the midpoint between the acromion bone and the top of radius bone (see MAC) (International Standards for Anthropometric Assessment, 2001). Triceps skinfolds were measured at the most posterior part of the triceps at the midpoint between the acromion bone and the top of radius bone (see MAC) (International Standards for Anthropometric Assessment, 2001). Subscapular skinfolds were

measured 2 cm laterally and obliquely downward at a 45° angle from the undermost tip of the inferior angle of the scapula (International Standards for Anthropometric Assessment, 2001). Iliac crest skinfolds were measured immediately above the most lateral edge of the iliac crest (International Standards for Anthropometric Assessment, 2001). Supraspinal skinfolds were measured at the intersecting point between two lines (1) a line from the anterior axillary boarder to the most inferior part of the tip of the anterior superior iliac spine and (2) a horizontal line from the most lateral edge of the iliac crest (International Standards for Anthropometric Assessment, 2001). Abdominal skinfolds were measured 5 cm to the right of the navel (International Standards for Anthropometric Assessment, 2001). Medial calf skinfolds were measured on the most medial aspect of the calf at the level of maximum circumference (International Standards for Anthropometric Assessment, 2001). Trunk to extremity ratio (TER), an estimate of regional subcutaneous fat distribution, was calculated as the sum of 4 trunk skinfolds (subscapular, supraspinal, iliac and abdominal) / sum of 3 extremity skinfolds (bicep, triceps and calf) [177, 178].

4.2.2.5 Body Somatotyping

The Heath-Carter anthropometric somatotyping method was used (Heath and Carter, 2003). This method uses ten different anthropometric measurements in its calculation: height, weight, two circumferences (flexed arm and calf), two bone breadths (humerus and femur) and four skinfolds (triceps, subscapular, supraspinal and medial calf) (Heath and Carter, 2003). If there was no obvious peak of the biceps muscle for the flexed arm circumference measurement, MAC was used (International Standards for Anthropometric Assessment, 2001). Measurements were recorded in the Somatotype 1.2.5 software (Sweat Technologies, Australia). The following

equations were used to calculate the magnitude of the *endomorphy*, *mesomorphy* and *ectomorphy* components (Heath and Carter, 2003):

Endomorphy
= -0.718 + 0.1451 (**X**) – 0.00068 ($\mathbf{X}^2$) + 0.0000014 ($\mathbf{X}^3$) **X** = (**Σ** triceps, subscapular and supraspinal skinfolds) x 170.18 / height (cm)
Mesomorphy
= 0.858 x humerus breadth + 0.601 x femur breadth + 0.188 x corrected arm girth + 0.161 x corrected calf girth – height 0.131 + 4.5
Ectomorphy
Depending on the height to weight ratio (HWR), there are three different equations to calculate ectomorphy If HWR ≥ 40.75, then ectomorphy = 0.732 (HWR) – 28.58 If HWR < 40.75 but > 38.25, then ectomorphy = 0.463 (HWR) – 17.63 If HWR ≤ 38.25, then ectomorphy = 0.1

Figure 4.1 *Somatotyping Equations.*

4.2.2.6 Metabolic Measurements

Blood pressure (BP), Heart rate (HR) and pulse oximetry (SpO_2) were measured using an Adview®9000 modular diagnostic station (American Diagnostic Corporation (ADC), NY, USA). Measurements were taken prior to (after 10 minutes of rest) and after the six minute walk test (6MWT). Blood pressure was converted to z-scores/percentiles and classified as normal, pre-hypertensive, stage I hypertension or stage II hypertension according to the National High Blood Pressure Education Program Working group standards [162]. As only one measurement of resting blood pressure was taken, percentile categorization indicating pre-hypertension /hypertension will be referred to as "risk of hypertension".

4.2.3 Biochemical Variables

Study blood work was collected at time of routine clinical blood work. Routine clinical blood work included triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (TC), insulin, glucose, thyroid

stimulating hormone (TSH), alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), creatinine, ferritin, C-reactive protein (CRP), antinuclear antibody (ANA) screen and 25-hydroxyvitamin D (25(OH)D). These were performed in the Core Laboratory at Alberta Health Services (AHS) according to standard methodologies [102]. Vitamin D status was classified as: deficient 25(OH)D <50nmol/L, suboptimal 25(OH)D 50-75 nmol/L and sufficient 25(OH)D >75 nmol/L according to the Canadian Society of Endocrinology & Metabolism [52]. ALT values >20 U/L were considered abnormal [127]. The homeostasis model assessment for insulin resistance (HOMA-IR) (glucose mmol/L x insulin mU/L /22.5) was used as an index of insulin resistance [161].

4.2.4 Physical Capacity

4.2.4.1 Hand Grip Strength

Hand grip strength was assessed using a Jamar® Hydraulic Hand dynamometer (Patterson Medical, Mississauga, ON, Canada) (**Protocol Appendix A2**). The handle was set to the second position (first handle position if child's hand too small). Scores were recorded for three successive trials for each hand, starting with the dominant hand [179]. Average scores of three trials for each hand were compared to Jamar Hydrolic Hand Dynamometer© normative values. Scores below 2 standard deviations of the average value for age and gender were considered abnormal [103-105].

4.2.4.2 Six Minute Walk Test

The six minute walk test (6MWT) was preformed indoors on a long flat surface 15 meters in length (30-m one lap) (**Protocol Appendix 2**). Blood pressure, heart rate and pulse

oximetry were measured immediately before (after 10 minutes rest) and after the 6 minute walk. Prior to the 6MWT, children were instructed to sit and relax in a chair for 10 minutes. Children were also asked to rate their overall level of fatigue before and after the 6 minute walk using a Borg scale (appendix 2). Results from the 6MWT were compared to reference values [110]. Scores below 2 standard deviations of the average value for age and gender were considered abnormal.

4.2.5 Dietary Intake Analysis

Dietary intake was assessed using a three-day food record (2 weekdays and 1 weekend day) (**Appendix 1 Form O**). Micronutrient (emphasis on vitamin D and calcium intake) and macronutrient intake in the three-day food record was analyzed using Food Processor (2015 ESHA® Research, version 10.15.4, Salem, OR, USA). When necessary, nutrient information for (e.g yogourt) was obtained from product labels or product websites. This was done to ensure accuracy of vitamin D and calcium intake. Vitamin K content in the diet was determined using the United States Department of Agriculture (USDA) nutrient database (release 28) [180]. These nutrients were assessed as vitamin K and calcium are factors known to influence vitamin D absorption [37, 54]. Dietary intake was categorized into food groups and the number of servings based on the Alberta Nutrition Guidelines for Children and Youth (ANGCY) for age and gender [2, 48]. This was done to assess the major contributors to vitamin D intake in the diet.

Average energy intake, determined from the 3-day food record was divided by an estimate of basal metabolic rate (BMR) to assess for the potential underreporting of intake (Energy intake/BMR <1.06 is indicative of underreporting of energy intake) [22]. Estimates of BMR were calculated using Schofield-(WH) equations [181]. Ideal body weight (IBW) was used

if body weight was <90% or >120% of IBW [175]. This was done to assess whether under reporting was a factor in the estimation of vitamin D intake in the children studied.

4.2.6 Physical Activity

Physical activity was assessed using the Habitual Activity Estimation Scale (HAES) questionnaire (**Appendix 1 Form N**). Children were asked to report on two days; a Tuesday, Wednesday or Thursday and a Saturday within two weeks of the study visit. HAES results are presented as percentage of hours for each day spent at four different activity levels: inactive, somewhat inactive, somewhat active and active (Hay, 2006).

4.2.7 Statistical Analysis

Data analysis was completed using the SAS 9.0 statistical software (SAS, Version 9.4; SAS Institute Inc., Cary, NC, USA). Data was expressed as mean ± standard deviation. Data was assessed for normality using the Shapiro-Wilks test. Non-parametric variables were log transformed and expressed as median (interquartile range). Laboratory values were expressed as both continuous and categorical variables (normal/abnormal). Primary outcome variables included vitamin D status (25(OH)D), anthropometric variables (weight, height, circumference, skinfolds), markers of cardiometabolic (lipid panel, hypertension, insulin, HOMA-IR) and liver dysfunction (ALT, GGT), muscle function and body composition. Univariate and multivariate analyses were performed to assess the interrelationships between vitamin D status with anthropometric, cardiometabolic, liver, muscle function and body composition. Where needed, primary outcome variables were adjusted for potential confounders (age, gender, season). A p-value <0.05 was considered significant.

4.3 Results

In total, nine children with PWS, eight children with NAFLD and 18 control children were recruited. Two female controls were excluded from analysis after blood work revealed elevated ALT (12 year old) and elevated total cholesterol (16 year old). These results were reported to the responsible health care provider. There was no positive history of metabolic dysregulation prior to assessment for these healthy controls.

4.3.1 Demographic and Anthropometric Measurements

Demographic and anthropometric variables are presented in (**Table 4.1**). In the control group, 25% (n=4) of children had a BMI between the 85th-97th percentile and no children were >97th percentile. In the PWS group, 44% (n=4) of children had a BMI between the 85th-97th percentile and 33% (n=3) >97th percentile. In the NAFLD group, 100% (n=8) of children had a BMI >97th percentile. Circumferences, skinfolds and bone breadths are presented in (**Table 4.2**). In the control group, 6% (n=1) of children had a WC ≥85th percentile and no children were ≥95th percentile. In the PWS group, 33% (n=3) of children had a WC ≥85th percentile and 11% (n=1) ≥95th percentile. In the NAFLD group, 38% (n=3) of children had a WC ≥85th percentile and 62% (n=5) ≥95th percentile.

Somatotype data is presented in (**Figure 4.2**). Children with PWS and NAFLD had significantly higher values for endomorphy, mesomorphy and lower values for ectomorphy compared to controls ($p<0.05$). Children with NAFLD had significantly higher values for endomorphy and mesomorphy compared to children with PWS ($p<0.05$). The somatoplots for children with PWS were categorized by those who had healthy body distributions (n=5) and those who were outside of healthy reference ranges (n=4). Children outside of a healthy body distribution, had lower handgrip strength, 6MWT distance and vitamin D status (reduced vitamin

D intakes). They also had increased cardiometabolic and liver dysfunction including increased blood pressure, insulin, HOMA-IR and ALT.

For the entire cohort, older children (≥12.8 yrs) had increased weight, height, BMI, BSA, circumferences and bone breadths compared to younger children (<12.8 yrs). No other gender or age differences existed.

Table 4.1 Demographic and Anthropometric Measurements.

	Healthy Controls (n=16)[1]	PWS (n=9)[1]	NAFLD (n=8)[1]	P-value[2]
Gender (M:F)	9:7	2:7	3:5	
Age (years)	12.6 ± 3.6 (7.2 – 18.0)	12.4 ± 3.4 (7.5 – 18.7)	12.4 ± 3.5 (8.4 – 17.5)	NS
Weight (kg)	46.5 ± 17.3 (22.4 – 77.8)	48.2 ± 19.5 (22.5 – 86.9)	70.6 ± 22.4 (36.6 – 96.7)	0.008ϕ 0.04*
Height (cm)	155.8 ± 21.5 (124.4 – 190.1)	141.5 ± 16.6 (112.3 – 164.1)	155.4 ± 14.7 (127.6 – 170.7)	NS
Ht-to-Wt[6]	44.2 ± 2.5 (40.7 – 48.9)	39.7 ± 2.7 (34.0 – 43.2)	38.1 ± 1.9 (36.0 – 40.8)	<.0001ϕ 0.0003Ŧ
BMI (kg/m^2)	18.4 ± 3.1 (14.3– 24.6)	23.3 ± 6.3 (17.9 – 38.2)	28.4 ± 5.1 (21.6 – 34.2)	<.0001ϕ 0.01Ŧ
Weight z-score[3]	0.3 ± 1.0 (-1.3 – 2.0)	0.6 ± 1.1 (-1.1 – 2.5)	2.5 ± 0.6 (1.6 – 3.0)	<.0001ϕ 0.0008*
Weight Percentile[3]	60.3 ± 31.0 (10.0 – 98.0)	65.8 ± 29.4 (13.0 – 99.0)	98.0 ± 1.9 (94.0 – 99.0)	0.003ϕ 0.008*
Height z-score[3]	0.8 ± 1.2 (-1.0 – 3.2)	-1.1 ± 1.0 (-2.0 – 0.8)	1.1 ± 1.5 (-0.7 – 3.0)	0.0007Ŧ 0.002*
Height Percentile[3]	66.5 ± 27.7 (17.0 – 99.0)	20.4 ± 25.9 (3.0 – 80.0)	71.4 ± 32.3 (25.0 – 99.0)	0.0005Ŧ 0.003*
BMI z-score[3]	-0.1 ± 1.2 (-1.5 – 1.6)	1.5 ± 1.0 (-0.1 – 3.0)	2.6 ± 0.4 (2.0 – 3.0)	<.0001ϕ 0.004Ŧ 0.009*
BMI Percentile[3]	47.2 ± 35.7 (6.0 – 95.0)	84.7 ± 17.8 (46.0 – 99.0)	98.9 ± 0.4 (98.0 – 99.0)	0.0005ϕ 0.007Ŧ 0.04*
BSA (m^2)[4]	1.4 ± 0.4 (0.9 – 2.0)	1.4 ± 0.3 (0.8 – 1.9)	1.7 ± 0.4 (1.1 – 2.1)	0.047ϕ 0.04*
WHtR[3]	0.4 ± 0.04 (0.4 – 0.5)	0.5 ± 0.1 (0.5 – 0.7)	0.6 ± 0.04 (0.5 – 0.6)	<.0001ϕ <.0001Ŧ
WHtR z-score[3]	-0.6 ± 0.9 (-1.9 – 1.0)	1.0 ± 0.7 (0.1 – 2.1)	1.5 ± 0.4 (0.9 – 2.0)	<.0001ϕ <.0001Ŧ
WHtR Percentile[3]	33.1 ± 27.3 (3.0 – 83.0)	81.1 ± 14.9 (54.0 – 98.0)	91.6 ± 5.5 (81.0 – 98.0)	<.0001ϕ <.0001Ŧ
WHR[5]	0.8 ± 0.1 (0.7 – 0.9)	0.9 ± 0.1 (0.8 – 1.0)	0.9 ± 0.1 (0.8 – 1.0)	0.004ϕ

**between PWS and NAFLD; Ŧbetween PWS and Control; ϕbetween NAFLD and Control. [1]Values are expressed as mean ± SD (range) or median (IQR). [2]p-values <0.05 are considered statistically significant. [3]Determined using World Health Organization (WHO) anthropometric calculator (Canada, 2014 revision) [160]. [4]Calculated using Mosteller's formulae [159]. WHtR calculated as waist circumference (cm)/height (cm). [5]WHR calculated as waist circumference (cm)/hip circumference (cm). [6]Calculated using Somatotype 1.2.5 software (Sweat Technologies, Australia). Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease; Ht-to-Wt, height to weight ratio; BMI, body mass index; BSA, body surface area; WHtR, waist to height ratio; WHR, waist to hip ratio.*

Table 4.2 Circumferences, Skinfolds and Bone Breadths.

	Healthy Controls (n=16)[1]	PWS (n=9)[1]	NAFLD (n=7)[1]	P-value[2]
Circumferences				
Waist (cm)	66.7± 8.8 (53.9 – 85.9)	77.0 ± 13.8 (58.6 – 102.9)	89.5 ± 11.0 (69.0 – 101.3)	<.0001ϕ 0.03Ŧ
Waist z-score[3]	-0.1 ± 0.7 (-1.1 – 1.2)	0.8 ± 0.7 (0.0 – 1.8)	1.6 ± 0.4 (1.1 – 2.2)	<.0001ϕ 0.005Ŧ 0.009*
Waist Percentile[3]	45.3 ± 26.2 (14.0 – 88.0)	75.2 ± 17.7 (50.0 – 96.0)	93.5 ± 5.3 (85.0 – 99.0)	<.0001ϕ 0.006Ŧ 0.01*
Hip (cm)	83.5 ± 12.2 (64.3 – 99.5)	89.5 ± 15.1 (67.1 – 119.5)	100.6 ± 14.1 (81.5 – 116.9)	0.006ϕ
Mid arm (cm)	23.2 ± 4.5 (17.3 – 30.5)	25.2 ± 4.9 (19.5 – 35.3)	31.8 ± 5.9 (23.5 – 38.8)	0.0007ϕ 0.02*
Calf (cm)	31.4 ± 4.4 (24.5 – 38.1)	31.3 ± 6.2 (25.1 – 43.2)	36.4 ± 8.0 (22.3 – 43.9)	0.04*
Skinfolds				
Subscapular (mm)	9.2 ± 4.5 (5.0 – 23.7)	14.1 ± 3.6 (9.0 – 20.3)	19.0 ± 5.2 (9.7 – 26.3)	<.0001ϕ 0.01Ŧ 0.04*
Iliac Crest (mm)	11.3 ± 3.9 (6.0 – 20.0)	16.3 ± 4.1 (10.0 – 23.3)	20.4 ± 4.2 (14.0 – 25.7)	<.0001ϕ 0.005Ŧ
Supraspinal (mm)	9.1 ± 4.3 (4.8 – 20.0)	14.9 ± 3.2 (11.0 – 20.3)	23.1 ± 5.6 (13.3 – 30.3)	<.0001ϕ 0.002Ŧ 0.002*
Abdominal (mm)	10.8 ± 4.9 (4.5 – 23.0)	15.3 ± 3.1 (11.0 ± 21.0)	22.0 ± 5.6 (14.0 – 30.2)	<.0001ϕ 0.02Ŧ 0.009*
Bicep (mm)	6.8 ± 3.8 (2.2 – 13.3)	11.2 ± 3.7 (6.0 – 18.2)	17.5 ± 4.5 (9.0 – 21.7)	<.0001ϕ 0.01Ŧ 0.006*
Tricep (mm)	13.8 ± 5.3 (6.0 – 25.3)	18.4 ± 4.1 (15.3 – 28.2)	22.7 ± 6.1 (15.0 – 31.0)	0.001ϕ 0.03Ŧ
Medial Calf (mm)	10.7 ± 4.3 (5.0 – 19.0)	16.4 ± 3.8 (9.0 – 21.5)	19.4 ± 7.7 (13.3 – 36.8)	0.001ϕ 0.004Ŧ
SSF[4,5] (mm)	32.0 ± 13.1 (16.8 – 69.0)	47.4 ± 7.6 (38.5 – 63.8)	65.5 ± 13.5 (45.3 – 80.3)	<.0001ϕ 0.004Ŧ 0.004*
TER[6]	1.3 ± 0.3 (1.0– 2.0)	1.3 ± 0.2 (1.1– 1.5)	1.4 ± 0.3 (1.1– 1.9)	NS

Table 4.2 Circumferences, Skinfolds and Bone Breadths Continued.

	Healthy Controls (n=16)[1]	PWS (n=9)[1]	NAFLD (n=7)[1]	P-value[2]
Bone Breadths				
Humerus (cm)	6.0 ± 0.8 (4.7 – 7.4)	5.5 ± 0.7 (4.5 – 6.9)	6.1 ± 0.5 (5.4 – 7.0)	NS
Femur (cm)	8.7 ± 0.8 (7.1 – 10.0)	8.7 ± 1.3 (7.1 – 11.0)	9.9 ± 1.2 (7.9 – 11.2)	0.008ϕ
Somatoplot				
Endomorphy[4]	3.5 ± 1.2 (2.0– 6.6)	5.6 ± 0.8 (4.6– 6.7)	6.6 ± 0.9 (5.0– 7.3)	<.0001ϕ 0.0001Ŧ 0.03*
Mesomorphy[4]	3.5 ± 1.2 (1.2– 5.4)	5.1 ± 2.1 (2.8– 9.6)	6.6 ± 2.8 (1.5 – 9.9)	0.001ϕ 0.03Ŧ
Ectomorphy[4]	3.8 ± 1.8 (1.2– 7.2)	1.1 ± 1.0 (0.1– 3.0)	0.4 ± 0.5 (0.1– 1.2)	<.0001ϕ 0.0005Ŧ

**between PWS and NAFLD; Ŧbetween PWS and Control; ϕbetween NAFLD and Control.[1]Values are expressed as mean ± SD (range) or median (IQR). [2]p-values <0.05 are considered statistically significant. There were missing values for iliac crest (n=1), supraspinal (n=1), abdominal (n=1), SSF (n=1), endomorphy (n=1), mesomorphy (n=1) and ectomorphy (n=1). [3]Determined using World Health Organization (WHO) anthropometric calculator (Canada, 2014 revision) [160]. [4]Calculated using Somatotype 1.2.5 software (Sweat Technologies, Australia). [5]Sum of 3 skinfolds calculated as the sum of triceps, subscapular and supraspinal skin folds measurements. [6]TER calculated as the sum of 4 trunk skinfolds (subscapular, supraspinal, iliac and abdominal) / sum of 3 extremity skinfolds (bicep, triceps and calf) [177, 178].Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease; SSF; sum of three skinfolds; TER, trunk to extremity ratio.*

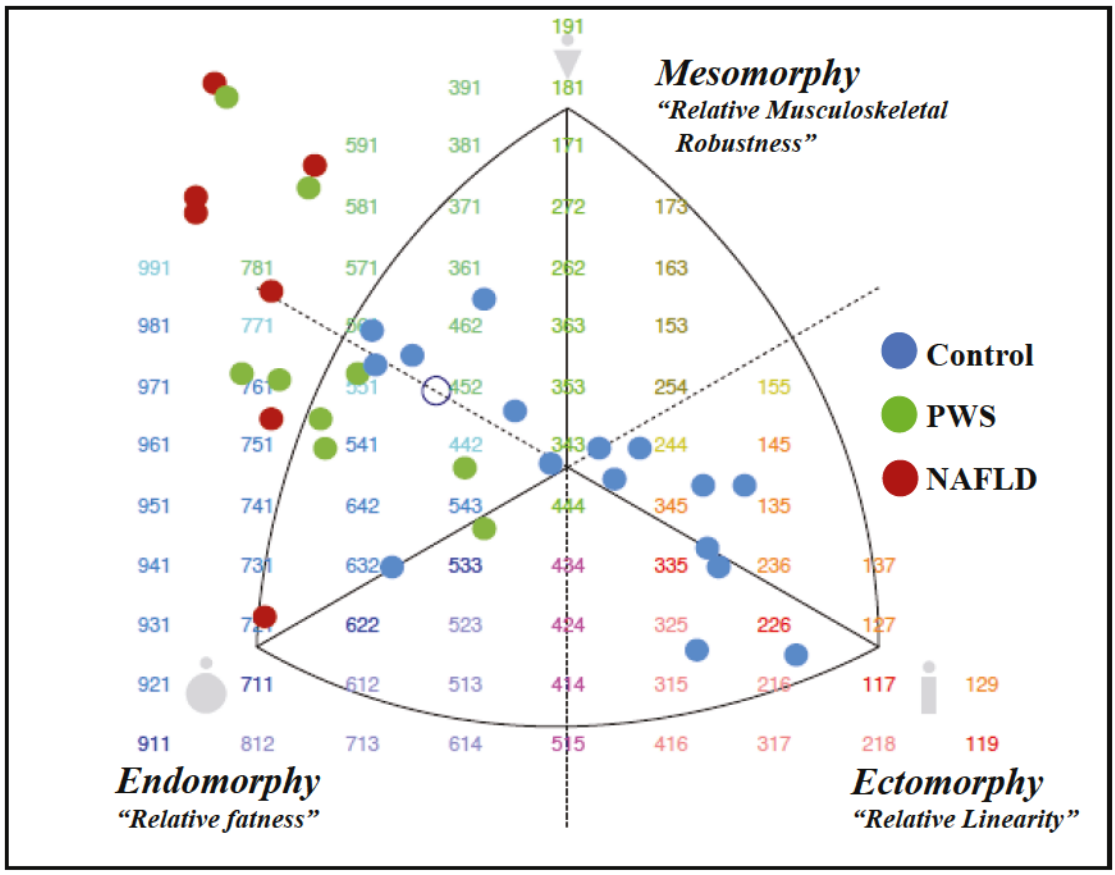

Figure 4.2 *Somatoplot.*

Ten different anthropometric measurements: height, weight, two circumferences (flexed arm and calf), two bone breadths (humerus and femur) and four skinfolds (triceps, subscapular, supraspinal and medial calf) entered into the Somatotype 1.2.5 software (Sweat Technologies, Australia) (Heath and Carter, 2003). Endomorphy describes relative fatness, mesomorphy, relative musculoskeletal robustness and ectomorphy, relative linearity (Heath and Carter, 2003). The magnitude of the endomorphy, mesomorphy and ectomorphy were plotted for controls (n=16), PWS (n=9) and NAFLD (n=7).

4.3.2 Vitamin D Status and Biochemical Markers

Biochemical data is presented in (**Table 4.3**). The prevalence of vitamin D deficiency (<50nmol/L) in controls, PWS and NAFLD was 50% (n=8), 11% (n=1) and 50% (n=4) respectively. Vitamin D supplementation in the control group was 25% (673 ± 460 (25-1000) IU; average 25(OH)D: 78 nmol/L), in PWS 75% (600 ± 490 (200-1400) IU; average 25(OH)D: 91 nmol/L) and in NAFLD 43% (467 ± 346 (68-667) IU; average 25(OH)D: 52 nmol/L). Younger children (<12.8 years (median)) were more likely to have vitamin D levels ≥ 50 nmol/L (p=0.03).

None of the healthy children had elevated levels of serum insulin. In contrast, serum insulin values were elevated (>20 mU/L) in 22% (n=2) of PWS and 75% (n=6) of NAFLD. HOMA-IR values were >3 in 6% (n=1) of controls, 33% (n=3) of PWS and 75% (n=6) of NAFLD. None of the healthy children had serum ALT in excess of 20 U/L. In contrast, 22% (n=2) of PWS and 100% of NAFLD had elevated ALT (>20 U/L).

Table 4.3 Biochemical Measurements.

	Healthy Control (n=16)[1]	PWS (n=9)[1]	NAFLD (n=8)[1]	P-value[2]	Reference Values**
ALT (U/L)	15 ± 3 (13 – 20)	20 ± 10 (7 – 44)	56 ± 45 (25 – 155)	<0.001ϕ 0.004*	<20 [127]
AST	24 ± 5 (16 – 34)	27 ± 8 (17 – 44)	35 ± 17 (23 – 73)	0.009ϕ	2-9 yrs: <50 ≥10 yrs: <40
ALP	227 ± 88 (71 – 418)	174 ± 64 (73 – 299)	185 ± 108 (46 – 359)	NS	2-9 yrs: 130-420 10-17 yrs: 100-500 ≥18 yrs: 30-130
GGT (U/L)	5 ± 0 (5 – 5)	6 ± 3 (5 – 15)	13 ± 12 (5 – 35)	0.001ϕ	Male: <70 Female: <55
Glucose (mmol/L)	5.0 ± 0.4 (4.2 – 5.5)	4.9 ± 0.3 (4.5 – 5.2)	4.9 ± 0.3 (4.2 – 5.2)	NS	3.3-6.0
Insulin (mU/L)	6.9 ± 4.9 (1.6 – 19.4)	15.6 ± 7.5 (6.9 – 32.3)	29.6 ± 18.6 (8.2 – 65.1)	<.0001ϕ 0.002Ŧ	5.0-20.0
HOMA-IR	1.5 ± 1.1 (0.3 – 4.5)	3.4 ± 1.8 (1.6 – 7.5)	6.5 ± 4.3 (1.8 – 14.8)	0.0002ϕ 0.003Ŧ	<3
TG (mmol/L)	0.8 ± 0.5 (0.3 – 2.2)	1.1 ± 0.7 (0.2 – 2.2)	1.6 ± 0.8 (0.4 – 3.0)	0.01ϕ	<1.5
TC (mmol/L)	3.9 ± 0.6 (2.9 – 4.9)	4.5 ± 1.0 (3.3 – 6.2)	4.4 ± 0.9 (2.5 – 5.2)	NS	<4.4
HDL-C (mmol/L)	1.4 ± 0.3 (0.9 – 2.0)	1.4 ± 0.4 (1.1 – 2.2)	1.2 ± 0.2 (1.0 – 1.6)	NS	>1.0
LDL-C (mmol/L)	2.1 ± 0.5 (1.2 – 3.1)	2.6 ± 1.0 (1.2 – 4.3)	2.5 ± 0.7 (1.1 – 3.1)	NS	<2.8
TC/HDL ratio	2.8 ± 0.6 (1.9 – 4.3)	3.5 ± 1.2 (1.6 – 5.3)	3.8 ± 0.9 (2.1 – 4.8)	0.007ϕ	-
Albumin	46.7 ± 2.5 (43.0 – 52.0)	45.2 ± 2.8 (40.0 – 49.0)	44.5 ± 3.5 (40.0 – 50.0)	NS	35-50
TSH (mU/L)	2.4 ± 0.9 (0.9 – 3.8)	1.9 ± 0.9 (0.6 – 3.2)	3.2 ± 1.1 (2.1 – 5.0)	0.02*	5-13 yrs: 0.3-5.0 ≥14 yrs: 0.2-4.0

Table 4.3 Biochemical Measurements Continued.

	Healthy Control (n=16)[1]	PWS (n=9)[1]	NAFLD (n=8)[1]	P-value[2]	Reference Values**
Urate (umol/L)	253 ± 75 (137 – 409)	304 ± 79 (158 – 395)	328 ± 45 (263 – 407)	0.02ϕ	≤9 yrs 100-300 10-17 yrs: Male: 135-510 Female: 180-450 ≥18 yrs: Male: 180-500 Female: 150-400
Ferritin	31 ± 14 (9 – 58)	53 ± 23 (23 – 87)	44 ± 24 (18 – 97)	0.01Ŧ	Male: 25-465 Female: 15-200
CRP (mg/L)	0.4 ± 0.6 (0.2 – 2.6)	3.7 ± 4.1 (0.2 – 11.4)	2.6 ± 1.8 (0.5 – 6.2)	<.0001ϕ 0.0003Ŧ	≤10
Creatinine (umol/L)	49 ± 15 (31 – 77)	44 ± 13 (28– 70)	51 ± 11 (33 – 65)	NS	2-9 yrs 25-90 10-17 yrs: 40-110 ≥18 yrs: Male: 45-125 Female: 40-115
25(OH)D (nmol/L)	57 ± 20 (29 –103)	84 ± 31 (36 – 135)	55 ± 19 (33 – 91)	0.02Ŧ 0.04*	Deficiency: <50 Suboptimal: 50-74.9 Sufficiency: ≥ 75 [52]

**between PWS and NAFLD; Ŧbetween PWS and Control; ϕbetween NAFLD and Control.*

***Pediatric reference ranges obtained from Alberta Health Services: http://www.albertahealthservices.ca/assets/wf/lab/wf-lab-chemistry-reference-intervals.pdf,*

[1]Values are expressed as mean ± SD (range) or median (IQR). [2] p-values <0.05 are considered statistically significant. There were missing values for urate in the control group (n=1) and GGT in the NAFLD group (n=1). Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease; ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; HOMA-IR, homeostatic model assessment of insulin resistance; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TSH, thyroid stimulating hormone; CRP, C-reactive protein; 25(OH)D, 25 hydroxyvitamin D.

4.3.3 Handgrip Strength, Physical Capacity, Metabolic Measures and Physical Activity

Handgrip strength, metabolic and functional capacity measurements are presented in (**Table 4.4**). The prevalence of hypertension was 63% in the both the NAFLD and PWS group. Six percent (n=1) of healthy controls, 89% (n=8) of children with PWS and 25% (n=2) of children with NAFLD were not within the normal limits for handgrip strength (**Figure 4.3**). Thirteen percent (n=2) of healthy controls, 100% (n=9) of children with PWS and 62.5% (n=5) of children with NAFLD were not within the normal limits for 6MWT distance (**Figure 4.3**). Results from the HAES questionnaire are presented in (**Figure 4.4**). Children with PWS slept longer on Saturdays (10.4 ± 0.9 hours) compared to both control children (8.6 ± 1.3 hours; p=0.0008) and children with NAFLD (9.6 ± 1.2 hours; p=0.03).

Boys walked farther during the 6MWT compared to girls (577 ± 100 m vs. 493 ± 79 m; p=0.01). Older children (≥12.8 yrs) had higher handgrip strength (23.2 ± 11.7 kg vs. 13.3 ± 8.9 kg; p=0.01) and resting DBP (73 ± 8 mmHg vs. 66 ± 8 mmHg; p=0.01) compared to younger children. Older children (≥12.8 yrs) spent less time on weekdays inactive (37.2 % vs. 42.4%; p=0.006) and slept less on weekdays (8.9 ± 1.5 vs. 10.0 ± 0.9 hours; p=0.02) compared to younger children. No other gender or age differences existed.

Table 4.4 Hand Grip Strength, Physical Functioning and Metabolic Measures.

	Healthy Control (n=15)[1]	PWS (n=9)[1]	NAFLD (n=7)[1]	P-Value[2]
Handgrip				
Dominant Hand (kg)	22.9 ± 11.7 (9.3 – 46.0)	8.4 ± 5.8 (2.3 – 20.3)	20.7 ± 9.4 (6.3 – 38.0)	0.002Ŧ 0.005*
Non-dominant Hand (kg)	22.1 ± 11.1 (9.3 – 46.0)	8.1 ± 5.8 (1.7 – 19.0)	19.0 ± 8.8 (6.0 – 34.7)	0.002Ŧ 0.008*
Pre-6 min walk test (at rest)				
SBP (mmHg)	115 ± 9 (94 – 131)	116 ± 16 (99 – 139)	125 ± 8 (120 – 143)	0.01ϕ
SBP z-score	0.5 ± 0.9 (-1.7 – 1.9)	1.4 ± 1.3 (-0.3 – 3.3)	1.6 ± 0.7 (0.9 – 2.8)	0.009ϕ
SBP percentile	66.9 ± 27.9 (4.0 – 97.0)	80.3 ± 22.1 (38.0 – 99.0)	91.1 ± 8.0 (81.0 – 99.0)	NS
DBP (mmHg)	66 ± 9 (49 – 85)	72 ± 7 (66 – 85)	73 ± 4 (65 – 80)	0.048ϕ
DBP z-score	0.2 ± 0.7 (-1.0 – 1.6)	1.1 ± 0.6 (0.3 – 1.9)	0.8 ± 0.3 (0.2 – 1.2)	0.02ϕ 0.005Ŧ
DBP percentile	56.0 ± 22.4 (15.0 – 95.0)	82.3 ± 12.9 (63.0 – 97.0)	78.8 ± 10.3 (56.0 – 87.0)	0.01ϕ 0.006Ŧ
HR (beats/min)	77 ± 10 (58 – 88)	83 ± 11 (57 – 93)	81 ± 13 (67 – 104)	NS
Pulse Oximetry	97 ± 1 (95 – 100)	99 ± 3 (90 – 98)	96 ± 1 (95 – 98)	NS
6MWT Distance (m)	601.4 ± 60.8 (494.1 – 736.6)	419.1 ± 52.1 (352.3 – 500.1)	504.9 ± 56.3 (420.0 – 570.0)	0.001ϕ <.0001Ŧ 0.005*
Post-6 min walk test				
Difference SBP percentile (mmHg)	+27.8 ± 24.5 (2.0 – 84.0)	+14.6 ± 21.8 (-2.0 – 61.0)	+7.9 ± 8.0 (0.0 – 18.0)	0.02ϕ
Difference DBP percentile (mmHg)	+24.7 ± 16.5 (-10.0 – 49.0)	+10.0 ± 12.6 (-3.0 – 33.0)	+17.9 ± 9.5 (8.0 – 37.0)	0.04Ŧ
Difference HR (beats/min)	+23 ± 19 (-2 – 61)	+18 ± 14 (-3 – 35)	+36 ± 15 (11 – 56)	0.02*

**between PWS and NAFLD; Ŧbetween PWS and Control; ϕbetween NAFLD and Control.*
[1]Values are expressed as mean ± SD (range) or median (IQR). [2]p-values <0.05 are considered statistically significant. Measurements were taking prior to and after the six minute walk test. Blood pressure was converted to z-scores/percentiles according to the National High Blood Pressure Education Program Working group standards [162]. There were missing values for SBP z-score (n=1), SBP percentile (n=1), DBP z-score (n=1) and DBP percentile (n=1) Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease; SBP, systolic blood pressure; DBP, diastolic blood; HR, heart rate; 6MWT, 6 minute walk test.

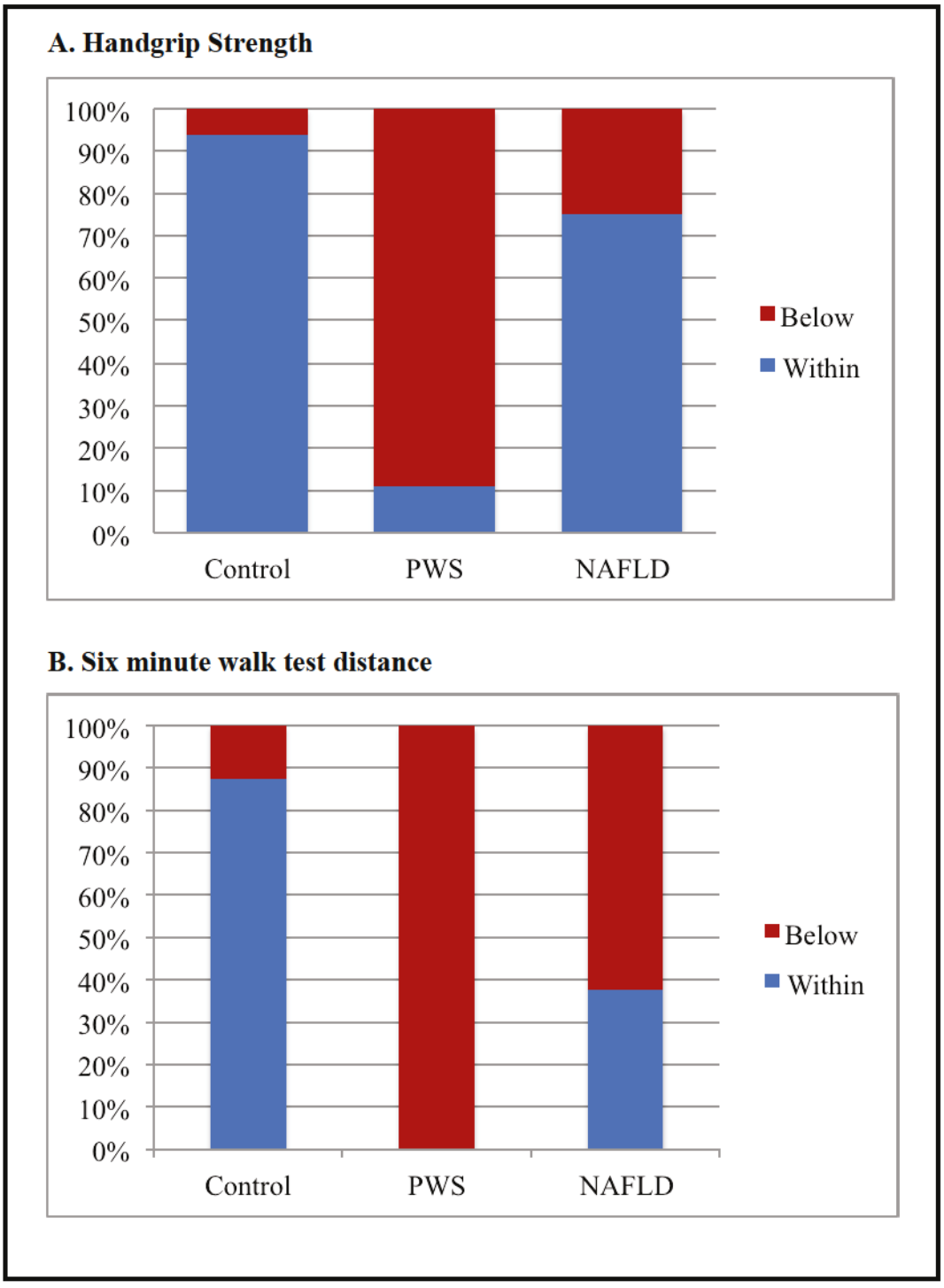

Figure 4.3 *Handgrip Strength and 6 Minute walk Test Distance.*

Figure 2A Handgrip strength, average scores of three trials for each hand were compared to Jamar Hydrolic Hand Dynamometer© normative values. Scores below 2 standard deviations of the average value for age and gender were considered abnormal. Figure 2B 6MWT distance, results were compared to reference values [110]. Scores below 2 standard deviations of the average value for age and gender were considered abnormal.

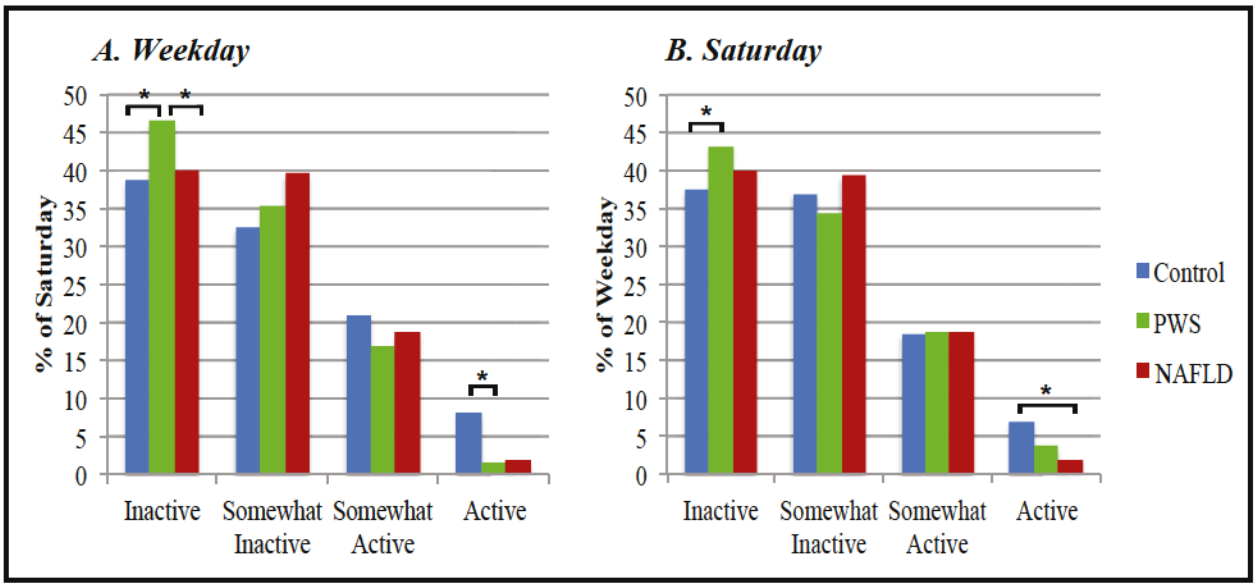

Figure 4.4. *Habitual Activity Estimation Scale (HAES).*

Children were asked to report on two days; a Tuesday, Wednesday or Thursday (Figure 3A) and a Saturday (Figure 3B) within two weeks of the study visit. HAES results are presented as percentage of hours for each day spent at four different activity levels: inactive (lying down), somewhat inactive (sitting), somewhat active (walking) and active (running) (Hay, 2006). Children with PWS had significantly higher values for % of Saturday spent inactive compared to controls (p=0.007) and children with NAFLD (p=0.003). Children with PWS had higher values for % of weekdays spent inactive compared to controls (p=0.02). Control children has a higher % of Saturday spent active compared to children with PWS (p=0.04) and a higher % of weekday spent active compared to NAFLD (p=0.04)

4.3.4 Dietary Intake

Dietary intake of each study group is presented in (**Table 4.5**) and food group servings in (**Table 4.6**). Children ≥12.8 years had more servings of fruit and vegetables (6.3 ± 2.5 vs. 4.2 ± 1.6; p=0.009) and a higher vitamin K intake (158 ± 125 vs. 74 ± 47; p=0.04) compared to children <12.8 years. Boys had a higher grain intake (7.3 ± 1.9 vs. 5.3 ± 1.9; p=0.008), a higher GL (144.5 ± 38.0 vs. 114.6 ± 33.1; p=0.03) and a higher % fat (33.6 ± 6.8 vs. 27.5 ± 5.4; p=0.009) intake compared girls. EI/BMR values were <1.06 in 13% (n=2) of controls, 25% (n=2) of PWS and 14% (n=1) of NAFLD. However, there were no differences in dietary vitamin D intake between those that underreported energy intake and those children that accurately reported intake (183 ± 92 IU/day vs. 203 ± 119 IU/day; p>0.05).

Table 4.5 Dietary Intake (3-Day Food record).

	Healthy Control (n=16)[1]	PWS (n=8)[1]	NAFLD (n=7)[1]	P-value[2]	DRI
Protein (g)	85.8 ± 26.3 (31.8 – 148.3)	72.6 ± 20.9 (42.7 – 97.3)	68.9 ± 12.8 (49.6 – 88.0)	NS	13-52[3]
% Protein	16.9 ± 3.8 (9.3 – 24.0)	18.0 ± 3.2 (13.9 – 23.3)	17.0 ± 4.0 (13.2 – 25.5)	NS	5-30%[4]
Carbohydrate (g)	267.1 ± 64.9 (189.0 – 398.2)	230.4 ± 67.9 (157.6 – 371.1)	218.1 ± 31.9 (179.8 – 260.2)	NS	130[3]
%Carbohydrate	53.0 ± 8.1 (36.7 – 67.2)	56.9 ± 5.2 (50.1– 66.5)	53.7 ± 8.2 (38.6 – 63.8)	NS	45-65%[4]
Fat (g)	75.2 ± 32.3 (24.9 – 155.7)	50.0 ± 16.2 (20.5 – 69.6)	56.0 ± 21.7 (35.5 – 99.6)	NS	-
% Fat	31.7 ± 6.5 (19.3 – 44.2)	27.3 ± 4.5 (19.5 – 33.3)	30.3 ± 8.8 (20.5 – 47.2)	NS	25-40%[4]
% Saturated Fat	11.8 ± 2.8 (7.8 – 17.6)	8.1 ± 1.4 (6.3 – 10.1)	10.6 ± 3.8 (3.5 – 15.0)	0.002Ŧ	<10%[3]
Vitamin D (IU) (Food alone)	209.5 ± 117.2 (23.2 – 471.3)	209.3 ± 84.6 (96.5 – 363.8)	192.7 ± 181.3 (8.6 – 524.9)	NS	600[3]
Vitamin D (IU) (Total)	377.7 ± 355.4 (23.2 – 1187.9)	659.3 ± 530.5 (144.7 – 1641.1)	392.7 ± 302.3 (17.8 – 852.3)	NS	600[3]
Vitamin K (µg)	95.6 ± 61.2 (21.9 – 248.1)	135.8 ± 72.8 (43.7 – 268.7)	144.9 ± 188.2 (15.1 – 434.9)	NS	30-75[5]
Calcium (mg)	1154.2 ± 417.7 (435.8 – 2048.6)	917.6 ± 415.9 (505.5 – 1510.3)	1057.9 ± 386.6 (455.7 – 1563.4)	NS	700-1300[3]

**between PWS and NAFLD; Ŧbetween PWS and Control; ϕbetween NAFLD and Control.*

[1]Values are expressed as mean ± SD (range) or median (IQR). [2] p-values <0.05 are considered statistically significant.[3]Recommended Daily Allowance (RDA). [4]Acceptable Macronutrient Distribution Range (AMDR). [5]Adequate Intake (AI). Vitamin K content was analyzed using the USDA online nutrient database[180]. Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease; DRI, Dietary Reference Intakes; supp, supplementation.

Table 4.6 Food Group Servings (ANGCY).

	Healthy Control (n=15)[1]	PWS (n=7)[1]	NAFLD (n=6)[1]	P-value[2]	Recommended Servings[3]
Servings of Grain Products	7.2 ± 2.2 (4.0 – 11.2)	5.0 ± 0.7 (3.8 – 6.1)	5.5 ± 2.2 (2.8 – 9.0)	0.01Ŧ	3-7
Servings of Vegetables & Fruit	4.6 ± 1.4 (1.8 – 7.9)	6.3 ± 1.2 (4.7 – 8.3)	5.5 ± 4.2 (1.0 – 13.9)	0.01Ŧ	4-8
Servings of Milk & Alternatives	2.9 ± 1.3 (0.7 – 5.5)	2.1 ± 1.1 (0.9 – 3.8)	2.6 ± 1.4 (0.7 – 4.7)	NS	2-3.5
Servings of Meat & Alternatives	2.4 ± 1.8 (0.4 – 8.2)	2.2 ± 0.6 (1.3 – 3.1)	2.1 ± 1.2 (0.6 – 4.2)	NS	1-3

**between PWS and NAFLD; Ŧbetween PWS and Control; ϕbetween NAFLD and Control.*
[1]Values are expressed as mean ± SD (range) or median (IQR). [2]p-values <0.05 are considered statistically significant. [3]Servings sizes where determined from the Alberta Nutrition Guidelines for Children and Youth (ANGCY)[2, 48]. Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease.

4.3.5 Factors Influencing Vitamin D Status: Seasonal, Dietary Intake, Vitamin D supplementation, Markers of Cardiometabolic and Liver Dysfunction.

No differences in vitamin D status between seasons (Fall, Winter, Spring, Summer) were noted (54 ± 17 vs. 67± 34 vs. 64± 32 vs. 65± 20; p>0.05). Vitamin D status was higher in those taking vitamin D supplements (81 ± 30 nmol/L vs. 53 ± 15 nmol/L; p=0.002). However, no relationship between dietary vitamin D and overall vitamin D status was noted. SBP measured at rest, was significantly lower in children with vitamin D status ≥ 50 nmol/L compared to those <50nmol/L (114 ± 11 mmHg vs. 124 ± 10 mmHg; p= 0.01). No other factors were found to be associated with vitamin D status including body composition, liver dysfunction, insulin, HOMA-IR or lipid panel.

4.3.6 Relationships between Vitamin D and Muscle Strength and Physical Capacity

No relationships existed between vitamin D and handgrip strength or physical capacity measurements.

4.4 Discussion

Obesity has been associated with vitamin D deficiency. This is a public health concern, particularly in children, as there is a high prevalence of both obesity and suboptimal vitamin D status within Canada. Few studies have examined the influence of body composition/fat distribution on overall vitamin D status in healthy children and/or whether the presence of comorbid conditions (such as liver disease) may impact overall vitamin D status. Vitamin D has also been related to muscle strength/muscle functionality in adult populations, all of which may be compromised in pediatric obesity [182]. This study examines factors influencing vitamin D status (hyperinsulinemia, insulin resistance, cardiometabolic risk factors, dietary intake, season, and body composition) and the influences of vitamin D status on muscle strength and muscle functionality in obese children with NAFLD and PWS.

The prevalence of vitamin D deficiency (25(OH)D<50nmol/L) in children with NAFLD and control children was 50% and is generally what has been reported for healthy children living in northern Alberta [6, 7]. This is likely due to the fact the majority of the healthy children and NAFLD children in this cohort had minimal vitamin D intakes and did not routinely consume vitamin D supplements [183-185]. Studies in children have shown that vitamin D status is lower in obese populations with NAFLD compared to obese children without NAFLD (31 vs 41 nmol/L; 52 vs 105 nmol/L; 50 vs 72 nmol/L) [116, 143, 146]. It is difficult to determine whether these findings are due to impairments in hepatic synthesis or due to other lifestyle factors such as diet or sunlight exposure. In studies with children there is some limited evidence to support reduced vitamin D levels in those with biopsy proven NASH (more severe form of NAFLD), but these studies have not clearly described vitamin D status differences between obese children and obese children with NAFLD (diagnosed with liver biopsy) [142, 143]. Despite the lack of

evidence in children, it has been shown that adults with biopsy proven NASH have significantly lower vitamin D levels compared to those with biopsy proven steatosis (45 vs. 62 nmol/L; 37 vs. 59 nmol/L) [137, 139].

In this study, it appears that total vitamin D intake, rather than body composition was the major determinant of vitamin D status since the majority of children with PWS had much higher total vitamin D intakes (due to routine vitamin D supplementation) and had body weights within normal reference ranges. Although an interrelationship between vitamin D status and systolic blood pressure was found, other factors such as season, gender, markers of insulin sensitivity, cardiometabolic and liver dysfunction, and anthropometric measures were not shown to influence vitamin D status. Some research has supported the relationship between vitamin D status and blood pressure in animal models where by $1,25(OH)_2D$ has been shown to be a negative regulator of the renin angiotensin system (RAS) [169]. We are not able to determine if this could be a potential contributing mechanism in this study, as we did not measure any markers of renal function. Overall study results suggest that in these pediatric populations, the major determinant of vitamin D status is total vitamin D intake via routine supplementation, rather than body size or sunlight exposure. However, more work is warranted to validate these findings.

One interesting finding in this study was that children with PWS had reduced handgrip strength and shorter 6-minute walk test distances compared to healthy controls and children with NAFLD. While this study was not powered for determining the risk for sarcopenia, we also found that children with PWS had lower SMM-z scores on DXA scan (**Appendix 3 Table A3.2**) compared to children with NAFLD. This is consistent with recent findings which show that children with PWS often have reduced lean mass compared to age/gender matched healthy

children and reduced maximal jump power [134]. Height z–scores were also significantly lower in children with PWS compared to children with NAFLD and healthy children. This finding is not surprising as children with PWS are on average shorter partly due to growth hormone deficiency [111]. As these findings of reduced muscle strength and functionality in the PWS group occurred in the presence of vitamin D adequacy, it suggests that these findings occurred independently from vitamin D status and is more related to other underlying physiological determinants of muscle functionality in this population.

Although this study is unique it has several limitations. The sample size was limited in the two clinical populations. This made it difficult to detect relationships between children with PWS and NAFLD. At the time of the study visit, many children with PWS were on vitamin D supplementation and significantly fewer children with NAFLD or healthy controls were supplemented. This influenced vitamin D status and made it difficult to examine the effect of inherent differences in body composition on vitamin D status. Although adults with PWS have been shown to have a reduced risk of NAFLD compared to other obese adults [113], one or two children with PWS may have had a coinciding diagnosis of NAFLD (based on evaluation of waist circumference z-scores, ALT, insulin). All of this would have directly influenced our ability to determine whether factors such as insulin sensitivity and other measures of cardiometabolic dysfunction were influenced by vitamin D status in our clinical populations. While a variety of methods (DXA, multiple skinfold measures and somatotyping) were utilized to assess body composition, a major limitation was that the use of DXA for body composition was restricted to the clinical populations. This was predominantly related to ethical concerns with the radiation exposure from the DXA in healthy children. In contrast, children with PWS and NAFLD have routine DXA as part of clinical practice for assessment of bone health and

body composition and this data was readily available to review. DXA also has the potential to overestimate lean body mass [186], which could have led to an underestimation of lean body mass in both clinical groups. A larger sample size would be needed to explore these associations, particularly in relation to vitamin D and the markers of muscle strength and functionality. While the findings related to blood pressure and vitamin D status is consistent with our earlier findings in the retrospective study, it would be important to explore these associations more carefully. It is possible that the overall level of adiposity in children with NAFLD skews this finding. Nevertheless, when NAFLD children were removed from the analysis, this relationship was still significant. Expanding the sample size of this current study would help confirm this preliminary finding. Another important confounding variable is that the level of vitamin D supplementation was variable across the patient populations, ranging between 25-1400 IU/D. Only those children with total vitamin D intakes in excess of the RDA, achieved vitamin D adequacy. This highlights the need for consistent approaches to vitamin D supplementation in these populations.

In conclusion, vitamin D status is largely influenced by vitamin D supplementation, rather than body composition, cardiometabolic and liver dysfunction, or season in the children studied in this cohort. While some interrelationships may exist between blood pressure and vitamin D status, this data does not conclusively demonstrate the mechanisms responsible. Children with PWS are unique in that they have lower lean body mass coinciding with reduced muscle strength and muscle functionality. This appears to be independent of vitamin D as these children had adequate levels of vitamin D supplementation (>600 IU/day). Further work to explore the associations between vitamin D, body composition and markers of muscle strength/muscle functionality are needed in children with PWS to assess the factors related to this unique finding.

CHAPTER 5: Conclusions and General Discussion

This thesis examined factors (diet, seasonal, body composition, markers of metabolic dysregulation) influencing vitamin D status in obese children living in northern Alberta. In contrast to work in obese adults, we have shown that obese children (with and without a comorbid condition such as NAFLD), have similar rates of vitamin D deficiency as children with body weights within normal healthy reference ranges (Chapter 3). In addition, the major factor influencing overall vitamin D status in our cohorts is the use of routine vitamin D supplementation or lack thereof (Chapter 4). Both studies (Chapter 3 & 4) consistently showed that vitamin D adequacy was associated with lower systolic blood pressure; this is an important finding since pre-hypertension and hypertension was highly prevalent in obese participants in these studies. Current data suggests that school aged children in Alberta have low intakes of vitamin D and that routine vitamin D supplementation is not commonly implemented [156].

Another important finding in this study was that children with PWS have reduced muscle strength and muscle functionality when compared to both lean and obese children with NAFLD, even in the presence of vitamin D adequacy. While the current sample size (Chapter 4), makes it difficult to assess the major factors influencing these findings, it appears that this finding was related largely to the reduced lean body mass in PWS compared to healthy children. This might also explain why children with PWS choose to spend a significantly greater time in sedentary activity compared to their obese and lean counter parts. A more in-depth analysis relating body composition, muscle functionality and muscle strength to vitamin D status would be needed to further explore the associations observed in this study.

5.1 Clinical Relevance and Clinical Implications

This study illustrates that suboptimal vitamin D status is due to insufficient dietary intake of vitamin D; this has potential implications for cardiovascular health in obese children since we have shown that vitamin D adequacy is associated with lower systolic blood pressure. While hypertension in children with healthy body weights is not reported to be highly prevalent, increasing evidence shows that obese children are at high risk for hypertension [187]. In Alberta, this is important to consider as ~%10 of children are obese and ~20% are overweight and there is a high prevalence of vitamin D deficiency (25-40%) within the general population [5-8, 156]. Additionally, studies have shown that obesity often persists/increases in severity from childhood into adulthood and this includes the risk for obesity related comorbidities (e.g. hypertension, NAFLD, T2DM) [188-190]. Independent from obesity, one study found that pre-pubertal and pubertal hypertension increased the risk of adult hypertension by 34% and 50% respectively [191]. This has important public health policy implications for developing programming to promote improved vitamin D status in our population and to explore in more detail the potential impact that this may have on long-term disease risk for hypertension in obese children. While children with PWS had adequate vitamin D status, study results also highlight that children with PWS had significant limitations in muscle strength/function when compared to obese children with NAFLD and healthy controls. This suggests that the development of intervention strategies such as exercise or diet may be needed in children with PWS as part of routine clinical care.

5.2 Future Directions

The low dietary intake level of vitamin D (Chapter 4) has important implications for fortification levels in food. In Canada, the current level of vitamin D fortification in a limited number of foods is not enough to help children reach the recommended dietary allowance (RDA)

(600 IU/day) requirements (even with sufficient intake of milk and alternative servings). While programming to support increased intake of vitamin D supplements would be beneficial to the Canadian population, a major limitation in this approach is the low adherence rates for micronutrient supplementation in both healthy and clinical populations [156]. A more practical and effective approach to influence the entire Canadian population may be to increase fortification levels and/or increase the range of foods that are fortified with vitamin D. Considerations in this approach should include an evaluation of the other nutrients (e.g. calcium, vitamin K, protein) present in the proposed foods as these nutrients influence both vitamin D bioavailability and overall bone health and lean muscle mass. For children in particular, identification of commonly consumed food items should be done to ensure adequate levels of intake.

Examples of relevant future studies might include dietary intervention trials where vitamin D levels in traditionally fortified foods (e.g. yogourt) are increased to reach RDA levels. Factors such as vitamin D status, bone health and muscle functionality should be studied to determine how these may be improved by increasing vitamin D fortification in foods commonly consumed by children. There is recent evidence that vitamin D levels can be improved in children by increasing vitamin D intake in the diet (through increased fortification) to estimated average requirement (EAR)/(RDA) levels [192]. This indicates that increasing vitamin D fortification can help children reach RDA levels and improve vitamin D status, without supplementation. Longitudinally studies would allow for the assessment of long term effects on body composition, growth, muscle strength/functionality, cardiovascular (blood pressure) and liver health and the onset of comorbid conditions. It would be important in these studies to ensure that children met the adequate intake (AI) for vitamin K and the RDA for calcium as well.

Future studies in PWS populations are necessary to follow up on the low muscle mass and function observed in this population. Intervention studies using strategies to improve muscle mass, strength and function should be considered. It is unlikely that dietary intake influenced these findings, as intake of macronutrients and micronutrients was similar between groups (**Table 4.5**). Further, diet quality, assessed using the Canadian Healthy Eating Index (HEI-C) (data not shown, **Appendix 3**), was higher in the PWS group compared to control children and children with NAFLD (data not shown, **Table A3.3**). Exercise intervention studies, aimed at reducing sedentary activity and introducing simple resistance exercise (RE) may help to increase lean mass and strength and improve body composition in children with PWS. RE has been shown to be an effective strategy in improving sarcopenia in the elderly, as well as in obese children with insulin resistance [193-195]. Many of these studies indicate that RE training in excess of eight weeks, along with consistent support by caregivers to promote adherence, result in significant improvements in lean body mass in obese children [193-195]. Further work examining the effectiveness of these strategies in children with PWS is warranted before further conclusions might be made.

5.3 Final Conclusions

Overall, results from this study show that vitamin D is a nutrient at risk in children in northern Alberta. This is largely independent from body size or body fat distribution and appears to be related to poor dietary intake of vitamin D rich foods. This finding is consistent with the larger population within Canada and has important public health policy implications. While reduced sunlight exposure could potentially be a factor in this finding, there is conflicting evidence regarding a seasonal influence on overall vitamin D status (Chapter 3 vs. 4). Apart from systolic blood pressure, no other associations between body composition, markers of insulin

sensitivity, cardiometabolic and liver dysfunction or muscle strength/functionality, and vitamin D status were found to be consistent between both studies (Chapter 3&4). Although study 1 (Chapter 3) did find a relationship between vitamin D and hyperinsulinemia/insulin resistance, we did not find any associations in the second study. These inconsistencies are likely due to the smaller sample size, which affected the ability to detect differences in the variables (HOMA-IR, body composition) (Chapter 4). A post hoc power calculation was completed for study 2 (sufficient power > 80%). The current sample size does have enough power to detect differences in handgrip strength and 6MWT distance between all three groups, as well as differences in biochemical variables between the control group and each clinical group. However, there was insufficient power to detect differences between clinical groups for biochemical variables and vitamin D status for all group comparisons. In addition, a major difference is that the children with PWS were routinely supplemented with vitamin D, while the other study populations (children with NAFLD and healthy lean children) were not. This potentially resulted in a selection bias that limited our ability to study the differences between the three populations and the potential associations between body composition, metabolic dysregulation, and vitamin D status. Ongoing subject recruitment to ensure sufficient power is available to assess these variables is warranted.

Vitamin D deficiency is a concern in all children living in Alberta (likely independent from adiposity) and strategies to increase vitamin D intake should be considered. Improving vitamin D status in children is important for healthy growth and development. In obese populations, improving vitamin D deficiency may play additional roles in ameliorating the negative consequences associated with the metabolic syndrome (hypertension) to promote cardiovascular health. Although study results suggest reduced muscle functionality/ muscle

strength in the children with PWS, interventions aimed at optimizing lean body mass and muscle strength is warranted in all obese children. Future strategies may include the evaluation of different physical activity regimens; particularly resistance exercise as there is evidence that this may result in significant improvements in lean body mass and insulin resistance in pediatric obesity.

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APPENDIX 1: Study Forms

A1. Form A (Healthy Recruitment Flyer)

UNIVERSITY OF ALBERTA

RESEARCH PARTICIPANTS NEEDED

We are looking for **HEALTHY** volunteer children and teens aged 8-18 years

Would you like to learn about your child's:

- ✔ **Vitamin D levels?**
- ✔ **Diet quality?**
- ✔ **Body composition?**

FOR MORE INFORMATION, PLEASE CONTACT US

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A1. Form B (NAFLD Recruitment Flyer)

UNIVERSITY OF ALBERTA

RESEARCH PARTICIPANTS NEEDED

Does your child have
Non-alcoholic fatty liver disease (NAFLD)?

Would you like to learn about his/her:

- ✔ **Vitamin D levels?**
- ✔ **Diet quality?**
- ✔ **Body composition?**

We would like to talk to you if your child: has a fatty liver, is between 8-18 years of age and does not have any other medical conditions

FOR MORE INFORMATION, PLEASE CONTACT US

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July 17, 2015
Version 2

A1. Form C (NAFLD Recruitment Letter)

UNIVERSITY OF ALBERTA

Dear Mr and Mrs

We are writing you to let you know that we are conducting a research study that is focused in children and adolescents who have fatty livers. You are asked to consider taking part in this study because your child has a fatty liver. We know that low vitamin D levels are common in Alberta and that it is important that your child eats enough vitamin D to make his/her bones and muscles healthy. We would like to study how your child's muscles work and how the vitamin D your child eats affects his/her liver and body composition. We hope that results from this study will tell us how much vitamin D your child needs to eat and how this affects your child's body composition. We would like to ask you to consider having your child participate in our study. We are enclosing a copy of the information letter that describes the kinds of things that occur in our study. We would like to call you on the telephone to talk to you about our study to see if you might be interested in having your child/adolescent participate in our research study. This study is voluntary. It is okay for you to say that you don't want your child to participate in the study or that you are not interested in hearing about this when we call. We will understand.

Sincerely,

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UNIVERSITY OF ALBERTA

Dear Mr and Mrs

We are writing you to let you know that we are conducting a research study that is focused in children and adolescents who have Prader-Willi syndrome (PWS). You are asked to consider taking part in this study because your child has PWS. We know that low vitamin D levels are common in Alberta and that it is important that your child eats enough vitamin D to make his/her bones and muscles healthy. We would like to study how your child's muscles work and how the vitamin D your child eats affects his/her body composition. We hope that results from this study will tell us how much vitamin D your child needs to eat and how this affects your child's body composition. We would like to ask you to consider having your child participate in our study. We are enclosing a copy of the information letter that describes the kinds of things that occur in our study. We would like to call you on the telephone to talk to you about our study to see if you might be interested in having your child/adolescent participate in our research study. This study is voluntary. It is okay for you to say that you don't want your child to participate in the study or that you are not interested in hearing about this when we call. We will understand.

Sincerely,

Diana Mager PhD RD
Associate Professor
Tel: 780-492-7687
mager@ualberta.ca

Krista MacDonald
MSc Candidate
Tel: 780-298-8442
km3@ualberta.ca

A1. Form E (Healthy Control Consent)

UNIVERSITY OF ALBERTA

Information Form & Consent for teenagers and parents of children/teenagers (Healthy Control)

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome

Principal Investigator:	Diana Mager PhD RD	Telephone: 780-492-7687
Co-Investigator:	Andrea Haqq MD, FRCPC	Telephone: 780-248-5488
	Jason Yap, MD, FRACP	Telephone: 780-248-5420
Research Coordinator:	Krista MacDonald	Telephone: 780-298-8442

This information and consent form is for the study participant. When parents/guardians are consenting on behalf of a minor child, "you" should be read as "your child" who is the study participant

Why am I being asked to take part in this study?

You are being asked to take part in this study because we know that low vitamin D levels are common in Alberta and that it is important that you eat enough vitamin D to make your bones and muscles healthy. We would like to study how your muscles work and how the vitamin D you eat affects your liver and body composition. We hope that results from this study will tell us how much vitamin D you need to eat and how this affects your body composition. In total we would like to recruit 45 children/adolescents for this study.

What will I be asked to do?

We will ask you to come in once to the Clinical Research Unit (CRU) at the University of Alberta to have different measurements and tests done. These include blood work, body measurements and muscle tests (~1.5 hours).

Study Procedures

Tests in the Clinical Research Unit (CRU) at the University of Alberta

We will ask you to come to the University of Alberta for one study day. The visit will take about 1.5 hours to complete. These tests are extra to normal clinical care your doctor will ask for. We will pay you back the money for parking your car.

1. Anthropometric Measurements

We will measure your weight and height and take some other body measurements at the beginning of the study. We will measure around your waist, hip and arm with a tape measure. We will also take measurements of your skin from the back of your arm, calf, behind the back and on the side of the waist with a measuring tool called a caliper. Your knee and elbow diameter will also be measured with a small caliper. Calipers look like tongs. It will look like a little pinch but it does not hurt. You should be fasted for these measurements (nothing to eat from midnight before the test). These measurements will take about 15 minutes to complete.

2. Blood Work

You will be asked to give a fasting blood sample. We will use this blood work to measure markers of inflammation, fat and sugar, liver function, vitamin D and bone health in your blood. This will take approximately 15-30 minutes.

3. Food Intake

We will ask you to fill out a food record for three days (including one weekend and two weekdays). You will be provided with instructions on how to do this during your visit. These food records will take about 20-30 minutes to review with you in your visit to the CRU.

4. Physical Activity

We will ask you to answer some questions from a list of physical activity questions called the Habitual Estimation Activity Scale (HAES). This list of questions will take about 15 minutes to review with you in your visit to the CRU. You do not have to be fasted for this.

5. Functional Capacity Measurements

We would like to find out how strong your muscles are and how they work. We will ask you to squeeze a "hand grip" with your hand and we will ask you to walk as far and as fast as you can for 6 minutes. These tests are like squeezing a sponge ball for a few seconds and like taking a very short walk like you do when you go shopping. These measurements will take about 10 minutes to complete.

What are the risks and discomforts? You may experience mild discomfort during the skin fold measurements and/or when your blood is taken. We will try to reduce the pain associated with blood work by only taking blood when your doctors order your regular clinical blood work. If you or the researcher is worried about your safety at **ANY** point during the test, the test will be stopped. If your blood work indicates you have a low level of vitamin D or any other abnormal blood work in your blood we will notify your physician.

What are the benefits to me? There are no direct benefits to you. However, we will be able to tell you if you are meeting all of your vitamin D needs from your diet. The information we learn from the study will help us understand how vitamin D helps with your growth.

Do I have to take part in the study? Being in this study is your choice. If you decide to be in the study, you can change your mind and stop at any time. Stopping the study will **NOT** affect the care or treatment that you are entitled to. If at any time you wish to leave the study, please let the researcher know and we will not collect any more information about you. The researcher will need to keep the information we have already collected. However if you wish us to remove your information completely, all you have to do is let a member of the researcher team know this.

What happens if I am injured because of this research? If you become ill or injured as a result of being in this study, you will receive necessary medical treatment, at no additional cost to you. By signing this consent form you are not releasing the investigator(s) and/or institution(s) from their legal and professional responsibilities.

Will my information be kept private? If you like, we will share the results about your diet with your doctor. We will not share any other information in your study record with anyone and will keep it private. Sometimes, by law, we may have to release your information. However we will make every legal effort to make sure that your health information is kept private. Any research data collected about you during the study will not identify you by name, only by initals and a coded number. Your name will not be shared with anyone outside the research team and your name will not be in any reports published from this research.

During research studies it is important that the data we get is accurate. For this reason your health information, including your name, may be looked at by members of the Health Research Ethics Board (HREB) or auditors at the University of Alberta.

By signing the consent form you give permission for the study doctor/researchers to collect, use and share information from your medical records as described above. After the study is done, we will still securely store your health data that was collected as part of the study. At the Universty of Alberta, study information is required to be kept for 5 years.

What if I have questions?
You can ask the doctor or nurse about anything you don't understand. You can also talk to Dr. Diana Mager, Dr. Andrea Haqq or Dr. Jason Yap. If you have any problems or concerns about any part of this study please call the Research Ethics Office at 780-492-2615. This office has no connection with the study researchers.

Principal Investigator:	Diana Mager PhD RD	Telephone: 780-492-7687
Co-Investigator:	Andrea Haqq MD, FRCPC	Telephone: 780-248-5488
	Jason Yap, MD, FRACP	Telephone: 780-248-5420
Research Coordinator:	Krista MacDonald	Telephone: 780-298-8442

UNIVERSITY OF ALBERTA

PARENT CONSENT FORM

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome.

Principal Investigator(s): Dr Diana Mager PhD RD **Phone Number:** 780-492-7687
Co-Investigator (s): Dr. Andrea Haqq MD, FRCPC **Phone Number:** 780-248-5488
Dr. Jason Yap MD, FRACP **Phone Number:** 780-248-5420

	Yes	No
1. Do you understand that your child has been asked to participate in a research study?	☐	☐
2. Have you read and received a copy of the attached Information Sheet?	☐	☐
3. Do you understand the benefits and risks involved for your child in taking part in this research study?	☐	☐
4. Have you had an opportunity to ask questions and discuss this study?	☐	☐
5. Do you understand that you are free to withdraw your child from the study at any time, without having to give a reason and without affecting your child's future medical care?	☐	☐
6. Do you understand who will have access to your child's records, including personally identifiable health information?	☐	☐
7. Do you want the investigator(s) to inform your child's family doctor or pediatrician that your child is participating in this research study?	☐	☐

Doctor's name: _____

Who explained this study to you? _____

Child's Name _____

I agree for my child to take part in this study: ☐ YES ☐ NO

Signature of Parent or Guardian _____ Date & Time _____

(Printed Name) _____

Signature of Parent or Guardian _____ Date & Time _____

(Printed Name) _____

Signature of Witness _____ Date & Time _____

Signature of Investigator or Designee _____ Date & Time _____

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH SUBJECT

A1. Form F (Healthy Control Assent)

UNIVERSITY OF ALBERTA

Assent Form (Healthy Controls)

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome.

Principal Investigator: Diana Mager PhD RD Telephone: 492-7687

Co-Investigators: Andrea Haqq, MD Telephone: 248-5488
Jason Yap, MD FRACP Telephone: 248-5420

We would like you to take part in a research study that will help us understand how vitamin D helps you to grow.

What will you have to do?

If you and your parents say that it is okay to take part in this study we will ask you to:

1. Let us measure your weight, height and do some measurements on your arm, leg, stomach and back
2. Let us take some of your blood
3. Squeeze a "hand grip" with your hand (It is like squeezing a sponge)
4. Walk as fast and as far as you can for 6 minutes
5. Write down what you had to eat for three days (Your parents can help)
6. Answer some questions about how much you move during the day (Your parents can help)

Will it help?

We know that some children do not get enough vitamin D. You get vitamin D from the sun and from eating things like milk and fish. We want to find out how much vitamin D you eat and have in your body so we can find out if children should have more.

Will it hurt?

The only thing that might hurt is taking your blood.

Can you quit?
You do not have to be in the study and you can stop at any time. No one will be mad at you if you do not want to do this, or if you want to stop part way through. You should tell your parents or your doctor if want to stop.

Who will know?
No one except your parents, your doctor and the research team will know you are in this study unless you want to tell them. Any information we write down about you will be locked up.

Your signature
It will show us that you would like to be in this study. Your mom or dad will be asked to sign another form. This will tell us they are okay with you being in the study.

Do you have more questions?
You can ask your parent or guardian about anything you do not understand. You can also talk to Dr Diana Mager (Ph: 780-492-7687), Dr Andrea Haqq (Ph: 780-248-5488) or Dr Jason Yap (Ph: 780-248-5420). If you have any problems or concerns about any part of this study please call the Human Research Ethics Board (Ph: 780-492-2615). This office has no connection with the study researchers.

I agree to take part in the study: ☐ YES ☐ NO

Signature of research participant:_____Date: _____

Signature of witness:_____Date: _____

Signature of investigator:_____ Date:_____

UNIVERSITY OF ALBERTA

Information Form & Consent for teenagers with NAFLD and parents of children/teenagers with NAFLD

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome

Principal Investigator:	Diana Mager PhD RD	Telephone: 780-492-7687
Co-Investigator:	Andrea Haqq MD, FRCPC	Telephone: 780-248-5488
	Jason Yap, MD, FRACP	Telephone: 780-248-5420
Research Coordinator:	Krista MacDonald	Telephone: 780-298-8442

This information and consent form is for the study participant. When parents/guardians are consenting on behalf of a minor child, "you" should be read as "your child" who is the study participant

Why am I being asked to take part in this study?

You are being asked to take part in this study because you have a fatty liver. We know that low vitamin D levels are common in Alberta and that it is important that you eat enough vitamin D to make your bones and muscles healthy. We would like to study how your muscles work and how the vitamin D you eat affects your liver, bones and body composition. We hope that results from this study will tell us how much vitamin D you need to eat and how this affects your bone health and body composition. In total we would like to recruit 45 children/adolescents for this study.

What will I be asked to do?

This study has one study day. We will ask you to come in to the Clinical Research Unit (CRU) at the University of Alberta. The visit will be for blood work, body measurements and muscle tests (~1 hour).

Study Procedures

Tests in the Clinical Research Unit (CRU) at the University of Alberta

We will ask you to come to the University of Alberta for one study day. The visit will take about 1 hour to complete. These tests are extra to normal clinical care your doctor will ask for. We will pay you back the money for parking your car.

1. Anthropometric Measurements

We will measure your weight and height and take some other body measurements at the beginning of the study. We will measure around your waist, hip and arm with a tape measure.

We will also take measurements of your skin from the back of your arm, calf, behind the back and on the side of the waist with a measuring tool called a caliper. Your knee and elbow diameter will also be measured with a small caliper. Calipers look like tongs. It will look like a little pinch but it does not hurt. You should be fasted for these measurements (nothing to eat from midnight before the test). These measurements will take about 15 minutes to complete.

2. Blood Work

Your doctor will order your regular blood work at the Stollery Children's Hospital. This is normal regular patient care. We will not poke you for an extra blood test. We will take an extra half of a teaspoon of blood when you are having your regular blood work done. We will use this extra blood work to measure markers of inflammation, bone health. You need to be fasted for this blood work.

3. Food Intake

We will ask you to fill out a food record for three days (including one weekend and two weekdays). You will be provided with instructions on how to do this during your visit. These food records will take about 20-30 minutes to review with you in your visit to the CRU. This is extra to regular clinical care.

4. Physical Activity

We will ask you to answer some questions from a list of physical activity questions called the Habitual Estimation Activity Scale (HAES). This list of questions will take about 15 minutes to review with you in your visit to the CRU. You do not have to be fasted for this. This questionnaire is extra to regular clinical care.

5. Functional Capacity Measurements

We would like to find out how strong your muscles are and how they work. We will ask you to squeeze a "hand grip" with your hand and we will ask you to walk as far and as fast as you can for 6 minutes. These tests are like squeezing a sponge ball for a few seconds and like taking a very short walk like you do when you go shopping. These measurements will take about 10 minutes to complete.

6. Medical Records

We would also like to look at your medical records. We would like to collect information about the types of medication you are taking, the lab work and results of medical tests (such as abdominal ultrasounds and Dual-X-ray absorptiometry (DXA) scans). We need this information to understand about your liver and how well it is working and about your body composition.

What are the risks and discomforts?

You may experience mild discomfort during the skin fold measurements and/or when your blood is taken. We will try to reduce the pain associated with blood work by only taking blood when your doctors order your regular clinical blood work. If you or the researcher is worried about your safety at **ANY** point during the test, the test will be stopped.

What are the benefits to me? There are no direct benefits to you. However, we will be able to tell you if you are meeting all of your vitamin D needs from your diet. The information we learn from the study may be able to help other children with fatty liver in the future.

Do I have to take part in the study? Being in this study is your choice. If you decide to be in the study, you can change your mind and stop at any time. Stopping the study will **NOT** affect

the care or treatment that you are entitled to. If at any time you wish to leave the study, please let the researcher know and we will not collect any more information about you. The researcher will need to keep the information we have already collected. However if you wish us to remove your information completely, all you have to do is let a member of the researcher team know this.

What happens if I am injured because of this research? If you become ill or injured as a result of being in this study, you will receive necessary medical treatment, at no additional cost to you. By signing this consent form you are not releasing the investigator(s) and/or institution(s) from their legal and professional responsibilities.

Will my information be kept private? If you like, we will share the results about your diet with your doctor. We will not share any other information in your study record with anyone and will keep it private. Sometimes, by law, we may have to release your information. However we will make every legal effort to make sure that your health information is kept private. Any research data collected about you during the study will not identify you by name, only by initals and a coded number. Your name will not be shared with anyone outside the research team and your name will not be in any reports published from this research.

The study doctors/researcher will need to look at your personal health records held within the Liver Clinic at the Stollery Children's Hospital, and/or kept by other health care providers that he/she may have seen in the past (i.e your family doctor). Any personal health information that we get from these records will only be what is needed for the study.

During research studies it is important that the data we get is accurate. For this reason your health information, including your name, may be looked at by members of the Health Research Ethics Board (HREB) or auditors at the University of Alberta.

By signing the consent form you give permission for the study doctor/researchers to collect, use and share information from your medical records as described above. After the study is done, we will still securely store your health data that was collected as part of the study. At the Universty of Alberta, study information is required to be kept for 5 years.

What if I have questions?

You can ask the doctor or nurse about anything you don't understand. You can also talk to Dr. Diana Mager, Dr. Andrea Haqq or Dr. Jason Yap. If you have any problems or concerns about any part of this study please call the Research Ethics Office at 780-492-2615. This office has no connection with the study researchers.

Principal Investigator:	Diana Mager PhD RD	Telephone: 780-492-7687
Co-Investigator:	Andrea Haqq MD, FRCPC	Telephone: 780-248-5488
	Jason Yap, MD, FRACP	Telephone: 780-248-5420
Research Coordinator:	Krista MacDonald	Telephone: 780-298-8442

UNIVERSITY OF ALBERTA

PARENT CONSENT FORM

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome.

Principal Investigator(s): Dr Diana Mager PhD RD **Phone Number:** 780-492-7687
Co-Investigator (s): Dr. Andrea Haqq MD, FRCPC **Phone Number:** 780-248-5488
Dr. Jason Yap MD, FRACP **Phone Number:** 780-248-5420

	Yes	No
1. Do you understand that your child has been asked to participate in a research study?	☐	☐
2. Have you read and received a copy of the attached Information Sheet?	☐	☐
3. Do you understand the benefits and risks involved for your child in taking part in this research study?	☐	☐
4. Have you had an opportunity to ask questions and discuss this study?	☐	☐
5. Do you understand that you are free to withdraw your child from the study at any time, without having to give a reason and without affecting your child's future medical care?	☐	☐
6. Do you understand who will have access to your child's records, including personally identifiable health information?	☐	☐
7. Do you want the investigator(s) to inform your child's family doctor or pediatrician that your child is participating in this research study?	☐	☐

Doctor's name: _____

Who explained this study to you? _____

Child's Name _____

I agree for my child to take part in this study: ☐ YES ☐ NO

Signature of Parent or Guardian _____ Date & Time _____

(Printed Name) _____

Signature of Parent or Guardian _____ Date & Time _____

(Printed Name) _____

Signature of Witness _____ Date & Time _____

Signature of Investigator or Designee _____ Date & Time _____

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH SUBJECT

UNIVERSITY OF ALBERTA

Assent Form for Children with NAFLD

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome.

Principal Investigator: Diana Mager PhD RD Telephone: 780-492-7687

Co-Investigator: Andrea Haqq MD, FRCPC Telephone: 780-248-5488
Jason Yap, MD, FRACP Telephone: 780-248-5420

Research Coordinator: Krista MacDonald Telephone: 780-298-8442

It is important that you eat enough vitamin D to make your bones and muscles healthy. You get vitamin D from the sun and from eating things like milk and fish. We would like to study how your muscles work and how the vitamin D you eat affects your liver and body composition.

What will you have to do?

If you and your parents say that it is okay to take part in this study we will ask you to:

1. Let us do some measurements on your arm, leg, stomach and back
2. When you see your doctor and they take your blood, is it okay to take a bit more?
3. Squeeze a "hand grip" with your hand (It is like squeezing a sponge)
4. Walk as fast and as far as you can for 6 minutes
5. Write down what you had to eat for three days (Your parents can help)
6. Answer some questions about how much you move during the day (Your parents can help)

Will it help?

We know that some children do not get enough vitamin D. We want to find out how much vitamin D you eat and have in your body so we can find out if children should have more.

Will it hurt?
The only thing that might hurt is taking your blood. We will take blood for our study at the same time you have to give blood to your doctor so you do not have to do it again.

Can you quit?
You do not have to be in the study and you can stop at any time. No one will be mad at you if you do not want to do this, or if you want to stop part way through. You should tell your parents or your doctor if want to stop.

Who will know?
No one except your parents, your doctor and the research team will know you are in this study unless you want to tell them. Any information we have about you will be locked up.

Your signature
It will show us that you would like to be in this study. Your mom or dad will be asked to sign another form. This will tell us they are okay with you being in the study.

Do you have more questions?
You can ask your parent or guardian about anything you do not understand. You can also talk to Dr Diana Mager (Ph: 780-492-7687), Dr Andrea Haqq (Ph: 780-248-5488) or Dr Jason Yap (Ph: 780-248-5420). If you have any problems or concerns about any part of this study please call the Human Research Ethics Board (Ph: 780-492-2615). This office has no connection with the study researchers.

I agree to take part in the study: ☐ YES ☐ NO

Signature of research participant:_____Date: _____

Signature of witness:_____Date: _____

Signature of investigator:_____ Date:_____

UNIVERSITY OF ALBERTA

Information Form & Consent for teenagers with PWS and parents of children/teenagers with PWS

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome

Principal Investigator:	Diana Mager PhD RD	Telephone: 780-492-7687
Co-Investigator:	Andrea Haqq MD, FRCPC	Telephone: 780-248-5488
	Jason Yap, MD, FRACP	Telephone: 780-248-5420
Research Coordinator:	Krista MacDonald	Telephone: 780-298-8442

This information and consent form is for the study participant. When parents/guardians are consenting on behalf of a minor child, "you" should be read as "your child" who is the study participant

Why am I being asked to take part in this study?

You are being asked to take part in this study because you have Prader-Willi syndrome (PWS). We know that low vitamin D levels are common in Alberta and that it is important that you eat enough vitamin D to make your bones and muscles healthy. We would like to study how your muscles work and how the vitamin D you eat affects your bones and body composition. We hope that results from this study will tell us how much vitamin D you need to eat and how this affects your bone health and body composition. In total we would like to recruit 45 children/adolescents for this study.

What will I be asked to do?

This study has one study day. We will ask you to come in to the Clinical Research Unit (CRU) at the University of Alberta. The visit will be for blood work, body measurements and muscle tests (~1 hour).

Study Procedures

Tests in the Clinical Research Unit (CRU) at the University of Alberta

We will ask you to come to the University of Alberta for one study day. The visit will take about 1 hour to complete. These tests are extra to normal clinical care your doctor will ask for. We will pay you back the money for parking your car.

1. Anthropometric Measurements

We will measure your weight and height and take some other body measurements at the beginning of the study. We will measure around your waist, hip and arm with a tape measure. We will also take measurements of your skin from the back of your arm, calf, behind the back and on the side of the waist with a measuring tool called a caliper. Your knee and elbow diameter will also be measured with a small caliper. Calipers look like tongs. It will look like a little pinch but it does not hurt. You should be fasted for these measurements (nothing to eat from midnight before the test). These measurements will take about 15 minutes to complete.

2. Blood Work

Your doctor will order your regular blood work at the Stollery Children's Hospital. This is normal regular patient care. We will not poke you for an extra blood test. We will take an extra half of a teaspoon of blood when you are having your regular blood work done. We will use this extra blood work to measure markers of inflammation, vitamin D and bone health. You need to be fasted for this blood work.

3. Food Intake

We will ask you to fill out a food record for three days (including one weekend and two weekdays). You will be provided with instructions on how to do this during your visit. These food records will take about 20-30 minutes to review with you in your visit to the CRU. This is extra to regular clinical care.

4. Physical Activity

We will ask you to answer some questions from a list of physical activity questions called the Habitual Estimation Activity Scale (HAES). This list of questions will take about 15 minutes to review with you in your visit to the CRU. You do not have to be fasted for this. This questionnaire is extra to regular clinical care.

5. Functional Muscle Measurements

We would like to find out how strong your muscles are and how they work. We will ask you to squeeze a "hand grip" with your hand and we will ask you to walk as far and as fast as you can for 6 minutes. These tests are like squeezing a sponge ball for a few seconds and like taking a very short walk like you do when you go shopping. These measurements will take about 10 minutes to complete.

6. Medical Records

We would also like to look at your medical records. We would like to collect information about the types of medication you are taking, the lab work and results of medical tests (such as abdominal ultrasounds and Dual-X-ray absorptiometry (DXA) scans). We need this information to understand everything about your PWS including your body composition.

What are the risks and discomforts?

You may experience mild discomfort during the skin fold measurements and/or when your blood is taken. We will try to reduce the pain associated with blood work by only taking blood when your doctors order your regular clinical blood work. If you or the researcher is worried about your safety at **ANY** point during the test, the test will be stopped.

What are the benefits to me? There are no direct benefits to you. However, we will be able to tell you if you are meeting all of your vitamin D needs from your diet. The information we learn from the study may be able to help other children with PWS in the future.

Do I have to take part in the study? Being in this study is your choice. If you decide to be in the study, you can change your mind and stop at any time. Stopping the study will **NOT** affect the care or treatment that you are entitled to. If at any time you wish to leave the study, please let the researcher know and we will not collect any more information about you. The

researcher will need to keep the information we have already collected. However if you wish us to remove your information completely, all you have to do is let a member of the researcher team know this.

What happens if I am injured because of this research? If you become ill or injured as a result of being in this study, you will receive necessary medical treatment, at no additional cost to you. By signing this consent form you are not releasing the investigator(s) and/or institution(s) from their legal and professional responsibilities.

Will my information be kept private? If you like, we will share the results about your diet with your doctor. We will not share any other information in your study record with anyone and will keep it private. Sometimes, by law, we may have to release your information. However we will make every legal effort to make sure that your health information is kept private. Any research data collected about you during the study will not identify you by name, only by initals and a coded number. Your name will not be shared with anyone outside the research team and your name will not be in any reports published from this research.

The study doctors/researcher will need to look at your personal health records held within the Endocrine Clinic at the Stollery Children's Hospital, and/or kept by other health care providers that he/she may have seen in the past (i.e your family doctor). Any personal health information that we get from these records will only be what is needed for the study.

During research studies it is important that the data we get is accurate. For this reason your health information, including your name, may be looked at by members of the Health Research Ethics Board (HREB) or auditors at the University of Alberta.

By signing the consent form you give permission for the study doctor/researchers to collect, use and share information from your medical records as described above. After the study is done, we will still securely store your health data that was collected as part of the study. At the Universty of Alberta, study information is required to be kept for 5 years.

What if I have questions?

You can ask the doctor or nurse about anything you don't understand. You can also talk to Dr. Diana Mager, Dr. Andrea Haqq or Dr. Jason Yap. If you have any problems or concerns about any part of this study please call the Research Ethics Office at 780-492-2615. This office has no connection with the study researchers.

Principal Investigator:	Diana Mager PhD RD	Telephone: 780-492-7687
Co-Investigator:	Andrea Haqq MD, FRCPC	Telephone: 780-248-5488
	Jason Yap, MD, FRACP	Telephone: 780-248-5420
Research Coordinator:	Krista MacDonald	Telephone: 780-298-8442

UNIVERSITY OF ALBERTA

PARENT CONSENT FORM

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome.

Principal Investigator(s): Dr Diana Mager PhD RD **Phone Number:** 780-492-7687
Co-Investigator (s): Dr. Andrea Haqq MD, FRCPC **Phone Number:** 780-248-5488
Dr. Jason Yap MD, FRACP **Phone Number:** 780-248-5420

	Yes	No
1. Do you understand that your child has been asked to participate in a research study?	☐	☐
2. Have you read and received a copy of the attached Information Sheet?	☐	☐
3. Do you understand the benefits and risks involved for your child in taking part in this research study?	☐	☐
4. Have you had an opportunity to ask questions and discuss this study?	☐	☐
5. Do you understand that you are free to withdraw your child from the study at any time, without having to give a reason and without affecting your child's future medical care?	☐	☐
6. Do you understand who will have access to your child's records, including personally identifiable health information?	☐	☐
7. Do you want the investigator(s) to inform your child's family doctor or pediatrician that your child is participating in this research study?	☐	☐

Doctor's name: _____

Who explained this study to you? _____

Child's Name _____

I agree for my child to take part in this study: ☐ YES ☐ NO

Signature of Parent or Guardian _____ Date & Time _____

(Printed Name) _____

Signature of Parent or Guardian _____ Date & Time _____

(Printed Name) _____

Signature of Witness _____ Date & Time _____

Signature of Investigator or Designee _____ Date & Time _____

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH SUBJECT

UNIVERSITY OF ALBERTA

Assent Form for Children with PWS

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome.

Principal Investigator: Diana Mager PhD RD Telephone: 780-492-7687

Co-Investigator: Andrea Haqq MD, FRCPC Telephone: 780-248-5488
Jason Yap, MD, FRACP Telephone: 780-248-5420

Research Coordinator: Krista MacDonald Telephone: 780-298-8442

It is important that you eat enough vitamin D to make your bones and muscles healthy. You get vitamin D from the sun and from eating things like milk and fish. We would like to study how your muscles work and how the vitamin D you eat affects your body composition.

What will you have to do?

If you and your parents say that it is okay to take part in this study we will ask you to:

1. Let us do some measurements on your arm, leg, stomach and back
2. When you see your doctor and they take your blood, is it okay to take a bit more?
3. Squeeze a "hand grip" with your hand (It is like squeezing a sponge)
4. Walk as fast and as far as you can for 6 minutes
5. Write down what you had to eat for three days (Your parents can help)
6. Answer some questions about how much you move during the day (Your parents can help)

Will it help?

We know that some children do not get enough vitamin D. We want to find out how much vitamin D you eat and have in your body so we can find out if children should have more.

Will it hurt?

The only thing that might hurt is taking your blood. We will take blood for our study at the same time you have to give blood to your doctor so you do not have to do it again.

Can you quit?

You do not have to be in the study and you can stop at any time. No one will be mad at you if you do not want to do this, or if you want to stop part way through. You should tell your parents or your doctor if want to stop.

Who will know?

No one except your parents, your doctor and the research team will know you are in this study unless you want to tell them. Any information we have about you will be locked up.

Your signature

It will show us that you would like to be in this study. Your mom or dad will be asked to sign another form. This will tell us they are okay with you being in the study.

Do you have more questions?

You can ask your parent or guardian about anything you do not understand. You can also talk to Dr Diana Mager (Ph: 780-492-7687), Dr Andrea Haqq (Ph: 780-248-5488) or Dr Jason Yap (Ph: 780-248-5420). If you have any problems or concerns about any part of this study please call the Human Research Ethics Board (Ph: 780-492-2615). This office has no connection with the study researchers.

I agree to take part in the study: ☐ YES ☐ NO

Signature of research participant:_____Date: _____

Signature of witness:_____Date: _____

Signature of investigator:_____ Date:_____

A1. Form K (Chart Notes)

Version Date: 01 October 2015

Vitamin D status influences markers of insulin resistance, liver function, cardiometabolic disease risk and body composition in obese children with Nonalcoholic fatty liver disease and Prader-Willi Syndrome

Patient ID:_______

CHART NOTES

DATE	COMMENT

Subject Number: __ __ __

Visit Date: __ __/__ __ __/__ __ __ __(day-month-year)

Informed Consent

Date ICF signed: __ __/__ __ __/__ __ __ __ (day-month-year)

Time ICF Signed: _____

ICF Version: _____

Assent

Date Assent signed: __ __/__ __ __/__ __ __ __ (day-month-year)

Assent Version: _____

Did the subject/parent/guardian have adequate time to review ICF? ☐ Yes ☐ No

Were all of the subject's/parent's/guardian's questions answered? ☐ Yes ☐ No ☐ N/A

Did subject/parent/guardian receive a signed copy of ICF and assent (if applicable)? ☐ Yes ☐ No

Document the consent process in the patient's chart

Inclusion Criteria

Date Inclusion/Exclusion Reviewed: __ __/__ __ __/__ __ __ __

All Yes/No Questions must be answered YES in order for the subject to be enrolled in the study.

1. Written informed consent has been provided by the subject/parent or legal guardian.	☐ Yes ☐ No
2. Patient aged 8 to 18 years	☐ Yes ☐ No
3. Patients clinically diagnosed with (check only one) ☐ Prader-Willi Syndrome (PWS) OR ☐ Nonalcoholic fatty liver disease (NAFLD) OR ☐ Healthy control	☐ Yes ☐ No

Exclusion Criteria

All Yes/No questions must be answered NO in order for the subject to be enrolled in the study.

1. All patients with a history of a known primary liver disease associated with steatohepatitis (Wilson disease, various metabolic disorders, viral hepatitis)	☐ Yes ☐ No
2. All patients with a known primary diagnosis of Type 2 Diabetes or those on insulin	☐ Yes ☐ No
3. Patients on medications known to cause hepatic steatosis or interfere with vitamin D metabolism (e.g., corticosteroids, statins, Orlistat etc)	☐ Yes ☐ No
4. Patients with a history of a comorbid conditions known to affect vitamin D metabolism including other liver disorders or GI disorders such as IBD or CD.	☐ Yes ☐ No

***If the child is a Healthy Control, Dr. Diana Mager must sign as the investigator**

***If the child has been clinically diagnosed with NAFLD, Dr. Jason Yap must sign as the investigator**

***If the child has been clinically diagnosed with PWS, Dr. Andrea Haqq must sign as the investigator**

Investigator Signature: _____ Date: _____

Research Coordinator Signature: _____ Date: _____

Questionnaires and Assessments

*Mark if completed and attach report from respective assessment.

☐ Phlebotomy

Who preformed Blood Draw:_____

Time of Blood Draw:_____

Is date different than study date? NO / YES Date: __ __/__ __ __/__ __ __ __(day-month-year)

- ☐ Clinical
- ☐ Vitamin D
- ☐ Study

☐ Anthropometric Measurements

- ☐ Height and Weight
- ☐ Circumferences
- ☐ Skinfolds
- ☐ Bone Breadths

☐ Hand grip

☐ 6 min walk Test

☐ HAES Questionnaire

☐ Three day food intake record

☐ Instructions were provided on how to complete 3-day food record

Comments:_____

_____

_____

_____

- ☐ 1 Day Day of the Week ___ Date: __ __/__ __ __/__ __ __ __(day-month-yr)
- ☐ 2 Day Day of the Week ___ Date: __ __/__ __ __/__ __ __ __(day-month-yr)
- ☐ 3 Day Day of the Week ___ Date: __ __/__ __ __/__ __ __ __(day-month-yr)

Visit Conducted by:

_____	_____	_____
Printed Name	Signature	Date

A1. Form M (Data Collection Sheet)

Version 3,
June 12, 2015

Data Collection Sheet

NAFLD / PWS / Healthy Control

Patient ID: _____ **Gender:** Male / Female

Anthropometric Variables:

Date of Collection: _____**Height**: _____(cm) **Weight**: _____ (kg) **BMI:**_____(kg/m^2)

Date of Collection	**Circumferences**	**Measurement 1**	**Measurement 2**	**Measurement 3**
	Waist (cm)			
	Hip (cm)			
	Mid-arm (cm)			
	Mid-arm flexed (cm)			
	Calf (cm)			
Date of Collection	**Skinfolds**	**Measurement 1**	**Measurement 2**	**Measurement 3**
	Subscapular (mm)			
	Iliac Crest (mm)			
	Supraspinal (mm)			
	Abdominal (mm)			
	Bicep (mm)			
	Tricep (mm)			
	Medial Calf (mm)			
Date of Collection	**Bone Breadths**	**Measurement 1**	**Measurement 2**	**Measurement 3**
	Humerus (cm)			
	Femur (cm)			

Functional Capacity:

Hand Grip:

Date of Collection: _____

Hand Position _____

Start with dominant hand (circle dominant)

RIGHT: Measurement 1:_____(kg) Measurement 2:_____(kg) Measurement 3:_____(kg)

LEFT: Measurement 1:_____(kg) Measurement 2:_____(kg) Measurement 3:_____(kg)

6 Minute Walk Test:

Date of Collection: _____

Pre-6min walk test

Borg scale: _____

BP: _____(mmHg) HR: _____ (b/min) SPO2_____

Distance walked: _____(m)

Post-6min walk test

Borg scale: _____

BP: _____(mmHg) HR: _____ (b/min) SPO2_____

Laboratory Variables:

Date of Lab work	Variable	Level
	25(OH)D (nmol/L)	
	AST (U/L)	
	ALT (U/L)	
	ALP (U/L)	
	GGT (U/L)	
	Albumin (g/L)	
	Glucose (mmol/L)	
	Insulin (mmol/L)	
	Triglyceride (mmol/L)	
	Total Cholesterol (mmol/L)	
	HDL-Cholesterol (mmol/L)	
	LDL-Cholesterol (mmol/L)	
	Creatinine (µmol/L)	
	Ferritin (µg/L)	
	TSH (mU/L)	
	Urate (µmol/L)	
	CRP (mg/L)	
	Anti-nuclear antibody	

A1. Form N (HAES)

UNIVERSITY OF ALBERTA

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome

Principal Investigator:	Diana Mager PhD RD	Telephone: 780-492-7687
Co-Investigator:	Andrea Haqq MD, FRCPC	Telephone: 780-248-5488
	Jason Yap, MD, FRACP	Telephone: 780-248-5420
Research Coordinator:	Krista MacDonald	Telephone: 780-298-8442

THE HAES (HABITUAL ACTIVITY ESTIMATION SCALE)

This questionnaire will ask you questions about your daily activities. Please read all of the instructions carefully and answer each question as truthfully as you can.

Subject ID: _____
Date: _____

INSTRUCTIONS (please read!)

Please recall the activities of *one typical weekday* (choose from Tuesday, Wednesday or Thursday) and *one typical Saturday* within the past 2 weeks. For each given time period, please estimate the percentage of time that you spent in each of 4 different activity levels. For each of the time periods, the total time spent in all activity levels must add up to 100%.

The different activity levels are described below:

ACTIVITY LEVEL DESCRIPTIONS

These descriptions give you examples of activities that are typical of each activity level. You should refer back to these descriptions as often as you need when completing your estimates.

a) **inactive** – *lying down*, sleeping, resting, napping

b) **somewhat inactive** – *sitting*, reading, watching television, playing video games, time in front of the computer, playing games or activities which are mostly done sitting down

c) **somewhat active** – *walking*, shopping, light household chores

d) **very active** – *running*, jumping, skipping, bicycling, skating, swimming, games that require lots of movement and make you breathe/sweat hard

Following is a sample of a completed time period:
SAMPLE

From when you finished breakfast until when you started lunch, please estimate the percentage of time that you spent in each of the following activity levels:

a) inactive	5% (i.e., having a nap)
b) somewhat inactive	60% (i.e., watching TV)
c) somewhat active	25% (i.e., shopping)
d) very active	10% (i.e., riding a bicycle)
TOTAL	100%

WEEKDAY ACTIVITY

For *one typical weekday in the past 2 weeks*, (choose from one of Tuesday, Wednesday or Thursday), please estimate the percentage of time that you spent in each activity level.

1. After getting out of bed until starting breakfast:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

2. After finishing breakfast until starting lunch:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

3. After finishing lunch until starting supper:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

4. After finishing supper until bedtime:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

For the *typical weekday* that you are referring to, please answer the following questions as accurately as possible in the spaces provided.

5. At what time did you get out of bed in the morning? _____

6. At what time did you start eating breakfast? _____

7. How long did you spend eating breakfast? _____ minutes

8. At what time did you start eating lunch? _____

9. How long did you spend eating lunch? _____ minutes

10. At what time did you start eating supper? _____

11. How long did you spend eating supper? _____ minutes

12. At what time did you go to bed that evening? _____

13. For the *typical weekday* that this questionnaire has asked you about, please rate your <u>overall</u> level of activity (please circle one response only):

a) very inactive
b) inactive
c) somewhat inactive
d) somewhat active
e) active
f) very active

14. Is this "typical" Tuesday, Wednesday or Thursday that you described in this questionnaire (please circle one response only):

a) a lot like most weekdays
b) a little bit like most weekdays
c) a little bit different from most weekdays
d) a lot different from most weekdays

SATURDAY ACTIVITY

For *one typical Saturday in the past 2 weeks*, please estimate the percentage of time that you spent in each activity level.

15. After getting out of bed until starting breakfast:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

16. After finishing breakfast until starting lunch:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

17. After finishing lunch until starting supper:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

18. After finishing supper until bedtime:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

For the *typical Saturday* that you are referring to, please answer the following questions as accurately as possible in the spaces provided.

19. At what time did you get out of bed in the morning? _____

20. At what time did you start eating breakfast? _____

21. How long did you spend eating breakfast? _____ minutes

22. At what time did you start eating lunch? _____

23. How long did you spend eating lunch? _____ minutes

24. At what time did you start eating supper? _____

25. How long did you spend eating supper? _____ minutes

26. At what time did you go to bed that evening? _____

27. For the *typical Saturday* that this questionnaire has asked you about, please rate your <u>overall</u> level of activity (please circle one response only):

a) very inactive
b) inactive
c) somewhat inactive
d) somewhat active
e) active
f) very active

28. Is the "typical" Saturday that you described in this questionnaire (please circle one response only):

a) a lot like most Saturdays
b) a little bit like most Saturdays
c) a little bit different from most Saturdays
d) a lot different from most Saturdays

29. If you have any comments about your activity patterns that you think are important, please mention them on the back of this page. Thank-you.

A1. Form O (3-Day Food Record)

UNIVERSITY OF ALBERTA

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome

Principal Investigator: Diana Mager PhD RD Telephone: 492-7687

Co-Investigator: Andrea Haqq MD, FRCPC Telephone: 248-5488
Jason Yap, MD, FRACP Telephone: 248-5420

Child Food and Drink 3-day food record

How to record what your child eats:

- Write down everything that your child eats and drinks for 3 days. Include at least 1 weekend day (Saturday or Sunday).
- Refer to the following example below to help you complete the *Food and Drink Record*. Write down all the foods and drinks your child consumes. Include the amount eaten, how the food was prepared (ex. Baked, fried, boiled, etc) and any added foods like sugar, cream, margarine, sauces and dressings. Make sure to specify if the grain product was whole wheat or white.
- Make sure to write down the brand names of all the foods your child eats and drinks (including those eaten at school, at lessons, after school and at home).
- Make sure you include the amount of water your child drinks.
- Please check the food labels for brand names and include copies if possible of the food label. Always write down what the brand name of the food item is.
- For home made food; please include a copy of the recipe if possible. We will review how you make the food item in your study visit. Please take note of how many servings your recipe makes. For example if you made lasagna, then how many people ate the lasagna in order to finish it? What does one serving look like? Was it half a dinner plate or 2/3 of a dinner plate?
- Help your child eat as they would normally during the recording period. Remember that this form is not a test, but a tool to help you.
- Please write down any supplements you might take, what is in them and the brand name.
- Bring the *Food and Drink Record* with you to your study visit.

Example:

It is best to measure your food using common household measuring cups and measuring spoons. Here are some ways that you can estimate the amount of food you eat when you cannot measure it:

3 oz meat = deck of cards
1 oz of cheese = size of a thumb
1 cup rice, cereal, pasta = size of a women's fist
Medium size of fruit = size of tennis ball
1 teaspoon peanut butter, sugar = size of a thumb tip

Subject ID: (we will fill this part out) **Date:** May 20, 2015 **Day of Week:** Monday

Time	Food/ Drink and Description	Amount Eaten
7:30	Honey Nut Cheerios (General Mills)	1 cup (250 ml)
	2% Milk	½ cup (125 ml)
10:15	Banana	1 medium size
12:00	Peanut Butter and Jam Sandwich (2 slices white bread)	½ sandwich, 2 Tbsp peanut butter, 2 Tbsp jam
	Yogurt: Mini go strawberry flavored; 100 g	1
	Granola Bar (chocolate covered)	1
	Grapes	10
	Juice Box (fruit punch)	1 (200 ml)
4:00	Taco Chips	1 soup bowl full
	Grated Cheddar Cheese	3 Tbsp (45 ml)
	Salsa	¼ cup (50 ml)
	Orange Juice (from crystals)	1 small glass (approx 4 oz)
6:30	1 Medium Chicken Drumstick (dipped in Shake and Bake)	1
	Noodles in Sauce (Sidekicks is the brand name)	½ cup (125 ml)
	Carrot Sticks and Cucumber Slices	3-5
	Ranch Dip	2 Tsp
	2% Milk	½ cup (125 ml)
8:30	Homemade Blueberry Muffin	1 small
	Water	½ water bottle
	Vitamin D supplement (Jamieson) 1000 IU per tablet	

Three-Day Food and Drink Record: Day 1
Subject ID: _____ Date: _____Day of Week:_____

Time	Food/ Drink and Description	Amount Eaten

Don't forget to include Vitamin/Mineral Supplements!

Three-Day Food and Drink Record: Day 2

Subject ID: _____ Date: _____Day of Week:_____

Time	Food/ Drink and Description	Amount Eaten

Don't forget to include Vitamin/Mineral Supplements!

Three-Day Food and Drink Record: Day 3
Subject ID: _____ Date: _____Day of Week:_____

Time	Food/ Drink and Description	Amount Eaten

Don't forget to include Vitamin/Mineral Supplements!

A1. Form P (Health History Questionnaire)

UNIVERSITY OF ALBERTA

Parent Questionnaire regarding Health History of Healthy Children.

Title of Project: Vitamin D and body composition in children and adolescents with Non alcoholic fatty liver disease and Prader-Willi Syndrome

Principal Investigator:	Diana Mager PhD RD	Telephone: 492-7687
Co-Investigator:	Andrea Haqq MD, FRCPC	Telephone: 248-5488
	Jason Yap, MD, FRACP	Telephone: 248-5420

Question 1:

Has your child had any recent episodes of illness (this includes hospitalizations or colds/flu) over the past 6 months: Yes / No?

If yes, please briefly describe:_____

Question 2:

Has your child been on any prescribed medications over the past six months: Yes / No?

If so, please provide the name:_____

Question 3:

Has your child or any family members ever been diagnosed with Type 2 Diabetes or Liver Disease: Your child: Yes / No? Any family members: Yes / No?

If yes, please briefly describe:_____

Question 4:

Has your child even been diagnosed with a gastrointestinal, liver and/or endocrine disorders: Yes / No?

If so, please provide the name:_____

APPENDIX 2: Additional Methods/Protocols

Table A2.1 The use of Normative Data and Evaluating Sarcopenia in Children.

Article	Population	Sample size	Assessment Method	Evaluation of Lean Mass	Sarcopenia Definition
(Burrows, 2015/2016) [97, 98]	Healthy Chilean adolescents (16-17 yrs)	N=667	DXA	-Fat-free mass index (FFMI) estimated (Wells and Fewtrell) -FFMI values expressed as percentage	-Sarcopenia defined as FFMI values ≤ 25 percentile (adjusted for sex)
(Doulgeraki, 2015) [100]	Male children and adolescents with Duchenne muscular dystrophy (DMD)	N=42	DXA	-Lean mass values from 31 age-matched healthy boys were used for z-score calculations -All absolute values from DXA were converted to z-scores (z-score = actual value – mean value/standard deviation)	-Z-scores were compared between groups -No z-score cut-off for sarcopenia suggested
(Rayar, 2013) [196]	Canadian Children with Acute Lymphoblastic Leukemia	N=91	DXA	-Appendicular lean tissue mass was calculated (sum of the lean soft tissue from arms and legs) -Predicted skeletal muscle mass (SMM) was calculated using ALM and 2 tanner stage specific equations (used ≤16 yrs and ≥ 16 yrs instead of tanner stages) -SMM z-scores was calculated as the difference between measured SMM and predicted SMM using gender specific constants established by Webber, 2012 (see Appendix C)	-Z-scores were examine before and after 6 months of therapy -No z-score cut-off for sarcopenia suggested
(Crabtree, 2004) [99]	Group 1: British healthy controls (5-18 yrs) Group 2: British children with chronic diseases (5-18 yrs)	Group 1 N=646 Group 2 N=43	DXA	-Z-scores were calculated from control data -Regression equations were used to calculated z-scores for lean body mass relative to standing height (Z_{LBMHT})	-Z-score of -2 or less was classified as low lean body mass for height or "primary muscle defect-sarcopenia"

A2. Calculating Skeletal Muscle Mass (SMM) z-scores

Step 1) Calculate appendicular lean tissue mass (ALM)

ALM = Σ(lean tissue in arms + lean tissue in legs)

Step 2) Calculate measured skeletal muscle mass [101, 197, 198]

For children below tanner stage 5 **(used for children < 13 years)**
SMM (kg) = (1.115 x ALM (kg)) – 1.135

For children at tanner stage 5 and beyond **(used for children ≥ 13 years)**
SMM (kg) = (1.19x ALM (kg)) – 1.65

Step 3) Calculate predicted skeletal muscle mass [101]

$$\mathrm{SMM}_{\mathrm{predicted}} = \frac{A}{1 + (B \times e^{-C \times \mathrm{Age}})} + \frac{D}{1 + e^{-E \times (\mathrm{Age} - F)}}$$

Constants A-F are gender specific

	A	*B*	*C*	*D*	*E*	*F*
Female	7.0	6.5	0.55	13.0	0.75	11.5
Male	12.4	11.0	0.45	19.7	0.85	13.7

Step 4) Calculate skeletal muscle mass z-score [101]

$$\mathrm{SMM}\ \ (Z\text{-score}) = \left(\mathrm{SMM}_{\mathrm{measured}} - \mathrm{SMM}_{\mathrm{predicted}}\right) / (G + (H \times \mathrm{age}))$$

Constants G and H are gender specific

	G	*H*
Female	0.10	0.16
Male	0.005	0.325

Table A2.2 Additional Validated Measures of Muscle Strength and Physical Functioning in Children.

Test		Evaluates
Pinch Strength [104]	Pinch Strength -Measured using pinch guages (tip, lateral and palmar)	Hand Function/Strength
Jumping Mechanography[199]	-Measured using ground reaction platform -Multiple two-legged hopping (M2LH) -Multiple one-legged hopping (M1LH) -Single two-legged jump (S2LJ) -Heel-Rise Test (HRT) -Chair-Rise Test (CRT)	 -Maximal ground reaction force -Maximal ground reaction force -Maximal jump height -Endurance -Muscle power/reflective of everyday life
Timed Sit-to-stand test [109]	-Child asked to sit and then stand in set positions -Measure number of repetitions over defined time (10 or 30 seconds)	Lower extremity strength and endurance

A2. Scoping Review Methods

This review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines [200]. MEDLINE (1946 to Present) and Embase (1974 to 2015 March 18) databases were searched using Medical Subject Headings (MeSH) and text words. PubMed and Web of Science (core collection, all years) databases were searched using text words. All searches were run and exported during March 2015. PubMed was monitored to check for new publications. The searches involved using associated terms for NAFLD and vitamin D in combination with either body composition or insulin resistance. Terms used to identify potential articles included: *Non-alcoholic Fatty Liver Disease*, NAFLD, fatty liver, steatosis, Non alcoholic steatohepatitis AND *Vitamin D*, Vitamin D deficiency, cholecalciferol, ergocalciferol, 25-hydroxyvitamin D, 25(OH)D, Vitamin D status, calcitriol, calciferol, $1,25(OH)_2D$ AND either *Body Composition,* obese, overweight, body mass, adipose tissue, adipose, fat mass, body weight, total fat, visceral adipose, subcutaneous adipose, subcutaneous fat, visceral fat, Intra-Abdominal Fat OR *Insulin Resistance*, insulin sensitivity, glucose sensitivity, glucose intolerance, glucose tolerance test, insulin, glucose.

The inclusion criteria were as follows: English-language articles available online, primary research articles, human subjects with a NAFLD diagnosis, studies which measured serum 25(OH)D levels and also evaluated the relationship between 25(OH)D with at least one body composition measurement. Body composition methods included either anthropometric measurements (circumferences or skin fold measurements) or direct assessment using bioelectrical impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA), Magnetic resonance imaging (MRI) or computed tomography (CT). The relationship between insulin resistance/hyperinsulinemia and 25(OH)D was examined as a secondary outcome variable.

Articles were excluded if the primary focus of the study was on participants who had other chronic diseases such as type 2 diabetes mellitus or cardiovascular disease.

Quality of evidence for each study was evaluated using the validated Downs and Black (DB) assessment tool. This validated tool was chosen due to its applicability to intervention (randomized and non-randomized) and observational study types [201]. Scores were categorized based on the cut off points suggested by Silverman et al: excellent (26-28), good (20-25), fair (15-19) and poor (≤14) [202]. A maximum score of 28 is possible for randomized controlled trials and a maximum score of 21 is possible for observational studies. Since observational studies automatically receive lower scores, the following ranges were used for observational studies: excellent (19-21), good (14-18), fair (8-13) and poor (≤7). A previous study has re-categorized quality assessment scoring systems due to differences in study design [203]. The Newcastle-Ottawa scale for observational studies was also used to examine the validity of the adjusted Silverman scores for observational studies [203].

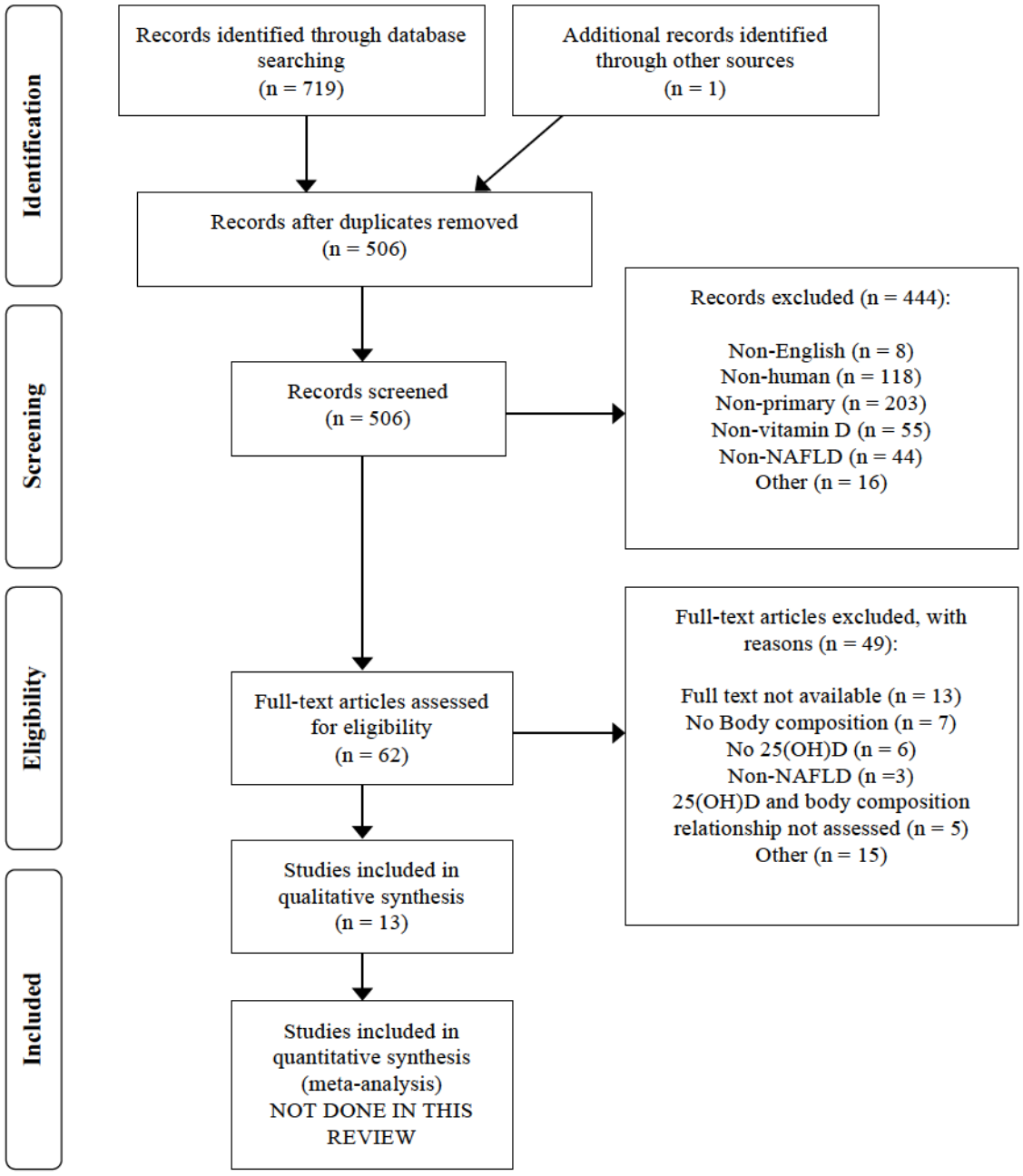

Figure A2.1 *Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flow Diagram of the Systematic Review Process.*

Table A2.3 Quality of Evidence Evaluated Using the Downs and Black Quality Assessment Tool of Articles Included in the Scoping Review.

		Section Totals						
Study	**Study design**	**Reporting**	**External validity**	**Internal validity; bias**	**Internal validity; confounding (selection bias)**	**Power**	**Total (Max 28pts)**	**Quality Category***
Bril, 2015 (USA) [140]	CS	7	2	4	2	0	15	**Fair-Good**
Hourigan, 2015 (USA)[142]	CS	7	2	3	1	0	13	**Poor-Fair**
Lu, 2015 (China) [138]	CS	7.5	0	4	3	0	14.5	**Poor-Good**
Dasarathy, 2014 (USA) [137]	CC	7	2	4	2	0	15	**Fair-Good**
Nobili, 2014 (Italy) [143]	CS	6	2	2	1	0	11	**Poor-Fair**
Rodriguez, 2014 (Spain) [144]	CS	5	2	3	0	0	10	**Poor-Fair**
Yildiz, 2014 (Turkey) [116]	CS	3	2	3	3	0	11	**Poor-Fair**
Bhatt, 2013 (India) [136]	CC	7	2	4	3	0	16	**Fair-Good**
Rhee, 2013 (Korea) [141]	CS	7.5	2	3	3	0	15.5	**Fair-Good**
Barchetta, 2011 (Italy) [135]	CS	7	2	4	3	0	16	**Fair-Good**
Katz, 2010 (USA) [124]	CS	7	2	1	3	0	13	**Poor-Fair**
Targher, 2007 (Italy) [139]	CS	7	2	4	1	0	14	**Poor-Fair**
Sharifi, 2014 (Iran) [147]	RCT	10	1	7	6	1	25	**Good**

Quality of evidence was assessed using the Downs and Black (DB) checklist tool. Scores were categorized based on the cut off points suggested by Silverman et al: excellent (26-28), good (20-25), fair (15-19) and poor (≤14) [202]. The DB checklist is divided into 5 sections: reporting, external validity, internal validity (bias and confounding selection bias) and power [201]. Seven of the questions in the DB checklist are applicable only to randomized studies. As a result the scores were given new cut-offs excluding the 7 questions, which were not applicable to observational studies: excellent (19-21), good (14-18), fair (8-13) and poor (≤7).

***For the observational studies, quality category is expressed as range, category on left uses all questions (max 28 points) and the category on right excludes the 7 questions which were not applicable to observational studies (max 21 points).*

Abbreviations: CS, cross-sectional; CC, case-control; RCT, randomized controlled trial.

Table A2.4 General Study Characteristics of Articles Included in the Scoping Review.

Article	Study Type	Duration of data collection	What study investigated/assessed	Population	NAFLD Sample Size	NAFLD Age (years)	NAFLD BMI (kg/m^2)	Control Sample Size	Control Age (years)	Control BMI (kg/m^2)
Mohamed, 2016 [146]	CS	16 months	Assess vitD status in children with NAFLD	Egyptian children	47	11.1 ± 2.7	BMI percentile 96.9 ± 0.5	23	10.6 ± 3.1	BMI percentile 92.2 ± 9.3
Lorvand, 2016 [148]	Double Blind RCT	12 weeks	Examine effects of vitd supplementation on NAFLD progression (lipids, insulin)	Iranian adults	37*	39.8 ± 11	30.3 ± 3.9	36*	44 ± 10.8	30.3 ± 3.5
Chang, 2015 [145]	CS	2 years	VitD and BMD and to evaluate factors affecting vitD and BMD	Obese children and Adolescents	*Steatosis:* 15 *NASH:* 147	*Steatosis:* 11.0 (8.0-16.1) *NASH:* 11.5 (7.7-18.1)	*Steatosis:* 26.8 (22.2-36.9) *NASH:* 25.6 (19.1-36.3)	32	8.7 (6.6 – 19.3)	23.7 (19.2 – 29.6)
Bril, 2015 (USA) [140]	CS	NR	VitD levels and insulin sensitivity, liver fat accumulation and severity of NASH	Overweight/Obese Adults	185 *Steatosis:* 58 *NASH:* 127	*Steatosis:* 56 ± 1 *NASH:* 54 ± 1	*Steatosis:* 33.3 ± 0.6 *NASH:* 34.6 ± 0.4	23	18 – 70 years	NR (Obese/overweight) Lower BMI than NAFLD
Hourigan, 2015 (USA)[142]	CS	4 years	Relationship between 25(OH)D and biopsy proven NAFLD	Children	102 *Steatosis:* 22 *NASH:* 80	12.9 ± 2.7	BMI-SDS: 2.4 ± 0.5 <2.0 (overweight or less) n= 15 (15%) 2.0 (moderately -severely obese) n=87 (85%)	N/A	N/A	N/A
Lu, 2015 (China) [138]	CS	3 months	Relationship between 25(OH)D and both visceral obesity and NAFLD	Chinese post-menopausal women (visceral obesity: VFA ≥ 80 cm^2)	157	57.3 ± 4.6	*VFA < 80cm^2* 24.1 ± 2.5 *VFA ≥ 80 cm^2* 26.2 ± 2.7	294	57.3 ± 4.6 years	*VFA < 80cm^2* 22.2 ± 2.3 *VFA ≥ 80 cm^2* 24.4 ± 2.3
Dasarathy, 2014 (USA) [137]	CC	NR	Relation between vitD, severity of disease and body composition in NAFLD	Adults	148 *Steatosis:* 67 *NASH:* 81	49.9 ± 12.3 *Steatosis:* 47.9 ±12.3 *NASH:* 51.4 ± 12.2	35.7 ± 7.0 *Steatosis:* 35.2 ± 7.8 *NASH:* 36.1 ± 6.2	39	37.5 ± 10.6	25.5 ± 3.1
Nobili, 2014 (Italy) [143]	CS	3 months	25(OH)D and histological liver damage in NAFLD	Overweight/obese Caucasian children (BMI converted to SDS)	73 *Steatosis:* 24 *NASH:* 49	13 (50th percentile)	BMI: 31.3 BMI-SDS: 2.5 (50th percentile)	Lean: 64 Obese: 21	Lean: 12.8 (10-15) Obese: 11.5 (6-15)	Lean: 20 ± 2.1 Obese: 29.3 ± 2.9
Rodriguez, 2014 (Spain) [144]	CS	4 years	Relationship between 25(OH)D, bone turnover markers, NAFLD and MS	Severely obese adults undergoing bariatric surgery (BMI>40 or BMI>35 with comorbidities)	110 (15% normal biopsy)	44.2 ± 10.2	46.9 ± 6.1	N/A	N/A	N/A
Yildiz, 2014 (Turkey) [116]	CS	9 months	Role of 25(OH)D in children with obesity and hepatosteatosis	Children and Adolescents with obesity (BMI >95th) percentile	58	11.9 ± 2.8	BMI: 30.9 ± 3.9 BMI-SDS: 2.7 ± 0.5	43	11.0 ± 2.8	BMI: 29.3 ± 4.4 BMI-SDS: 2.6 ± 0.6

Table A2.4 General Study Characteristics of Articles Included in the Scoping Review Continued.

Article	Study Type	Duration of data collection	What study investigated/assessed	Population	NAFLD Sample Size	NAFLD Age (years)	NAFLD BMI (kg/m^2)	Control Sample Size	Control Age (years)	Control BMI (kg/m^2)
Bhatt, 2013 (India) [136]	CC	4 years	Association of 25(OH)D and PTH with NAFLD	Adults (Asian Indians residing in North India) Overweight (BMI ≥ 23-24.9 kg/m2)/Obese (BMI ≥ 25kg/m2)	162	38.2 ± 7.0	28.1± 3.2	173	37.1 ± 6.9	26.8 ± 3.2
Rhee, 2013 (Korea) [141]	CS	1 year	25(OH)D with NAFLD to determine if association is independent of obesity and MS	Healthy Korean Men (overweight: 23-25 kg/m2, obese: ≥ 25kg/m2)	2,863	42.3 ± 6.0	26.2 ± 2.6	3,704	41.8 ± 6.6	23.6 ± 2.4
Barchetta, 2011 (Italy) [135]	CS	NR	low 25(OH)D and the presence/degree of NAFLD	Adults	162	52.1 ± 8.2	31.4 ± 5.5	100	49.8 ± 7.7	25.9 ± 5.1
Katz, 2010 (USA) [124]	CS	4 years	If suspected NAFLD is independently associated with HVD	US Adolescents Normal Weight > 5th BMI < 85th Overweight ≥ 85th BMI < 95th Obese BMI ≥95th percentile	1630 (8.39% NAFLD)	12-15 yrs (n=799) 6.82% NAFLD 16-19 yrs (n=831) 9.88% NAFLD	Normal Weight (n=1035; 3.5% NAFLD) Overweight (n=284; 9.0% NAFLD) Obese (n=311; 25.8% NAFLD)	-	-	-
Targher, 2007 (Italy) [139]	CS	5 months	25(OH)D and severity of liver histology in NAFLD	Adults (groups identical for age, sex, BMI)	60 Steatosis: 10 NASH: 50	47 ± 3	26.3± 2	60	48 ± 3 yrs	26.0 ± 2
Sharifi, 2014 (Iran) [147]	RCT Parallel DBPC	4 months	Effects of vitD supplementation on aminotransferases, IR, oxidative stress and inflammation in NAFLD	Adults (50,000 IU cholecalciferol or placebo every 14 days for 4 months)	27*	40.3 ± 8.7	31.3 (28.6, 32.5)	26*	43.9 ± 9.5	29.3 (26.8 – 31.9)

Data expressed as mean ± standard deviation or median (IQR; interquartile range)

**For the RCT trials, the left column refers to the NAFLD group who received the vitamin D treatment and the right column refers to the NAFLD group who received the placebo. Abbreviations: NR, not reported; N/A, not applicable; CS, cross-sectional; CC, case-control; RCT, randomized controlled trial; DBPC, double blind placebo controlled; NAFLD, Nonalcoholic fatty liver disease; NASH, Nonalcoholic steatohepatitis; 25(OH)D, 25-hydroxyvitamin D; HVD, hypovitaminosis D; VitD, vitamin D; VFA, visceral fat area; MS, metabolic syndrome; PTH, parathyroid hormone; IR, insulin resistance; BMI, body mass index; SDS, standard deviation score.*

Table A2.5 Vitamin D Specific Study Characteristics of Articles Included in the Scoping Review.

Article	Average 25(OH)D levels NAFLD group (nmol/l)	Average 25(OH)D levels control group (nmol/L)	25(OH)D reference range used	Prevalence of Hypovitaminosis D	Seasonal Variation considered ?	Assessment of 25(OH)D	Excluded/ identified participants with medications known to influence 25(OH)D status	Excluded/ identified participants taking vitamin D supplements
Mohamed, 2016 [146]	52.1 ± 41.3	104.7 ± 36.2	NR	NR	No	ELISA	Yes	Yes
Lorvand, 2016 [148]	*Baseline** 24.7 ± 9.7 *12 weeks** 67.6 ± 18.0	*Baseline** 25.0 ± 9.5 *12 weeks** 27.5 ± 11.7	Deficient < 37 nmol/l	NS	Yes	ELISA	No	Yes
Chang, 2015 [145]	*Steatosis:* 40.2 (15.7-79.9) *NASH:* 44.9 (18.7-85.1)	44.2 (32.9 – 83.4)	NR	NR	No	UPLC-MS/MS	Yes	No
Bril, 2015 (USA)[140]	56.2 ± 2.0	70.4 ± 5.2	Normal >75 nmol/l Insufficient 50-75 nmol/l Deficient <50 nmol/l	Overall (n=239) *Deficient:* 47% *Insufficient:* 31%	Yes	CLIA	No	No
Hourigan, 2015 (USA)[142]	57.7 ± 22.0	-	Normal ≥75 nmol/l Insufficient 51-74 nmol/l Deficient ≤50 nmol/l	*Deficient:* 43% *Insufficient:* 35%	Yes	CLIA	No	No
Lu, 2015 (China)[138]	*VFA < 80cm²* 27.8 (21.5-34.5) *VFA ≥80 cm²* 27.1 (21.5-33.8)	*VFA < 80cm²* 32.2 (23.7-40.9) *VFA ≥80 cm²* 28.8 (22.0-40.9)	NR	NR	Yes	ECLIA	Yes	No
Dasarathy, 2014 (USA)[137]	*Total:* 52.9 ± 26.0 *Steatosis:* 62.4 ± 28.2 *NASH:* 45.2 ± 21.0	89.1 ± 15.0	Lower limit of 75 nmol/l	*Steatosis:* 70.1% *NASH:* 89.7% *Cirrhosis:* 84.6% *Control:* 0%	Yes	CMM	Yes	No
Nobili, 2014 (Italy)[143]	49.9 (50th percentile)	Lean: 72.4 ± 8.2 Obese: 72.6 ± 20.7	Deficient < 50 nmol/l	NAFLD Deficient: 47%	Yes	HPLC	Yes	Yes
Rodriguez, 2014 (Spain)[144]	58.0 ± 41.5	N/A	Deficient ≤ 50nmol/l	Overall (n=110) Deficient: 60.9%	No	ECLIA	No	No
Yildiz, 2014 (Turkey)[116]	31.4 (23.2 – 45.2)	40.9 (31.0 – 61.9)	Sufficient ≥75nmol/l Insufficient 50-74nmol/l Deficient <50nmol/l	NAFLD: 5.3% Sufficient Control: 10.2% Sufficient	Yes	HPLC	Yes	Yes
Bhatt, 2013 (India)[136]	48.4 ± 21.2	69.4 ± 23.5	Quartile 1= 0-33.9 nmol/l Quartile 2=34.0-58.91 nmol/l Quartile 3=58.93-76.2 nmol/l Quartile 4= >76.2 nmol/l	Quartile 1 n=85 Quartile 2 n=89 Quartile 3 n=78 Quartile 4 n=83	No	RIA	No	No
Rhee, 2013 (Korea)[141]	38.7 ± 9.0	39.7 ± 9.7	Tertile 1 = <33.7 nmol/l Tertile II = 33.7-42.2 nmol/l Tertile III = >42.2 nmol/l	Tertile 1 = 40.0% NAFLD Tertile II = 45% NAFLD Tertile III = 45.9%NAFLD	Yes	ECLIA	No	No

Table A2.5 Vitamin D Specific Study Characteristics of Articles Included in the Scoping Review Continued.

Article	Average 25(OH)D levels NAFLD group (nmol/l)	Average 25(OH)D levels control group (nmol/L)	25(OH)D reference range used	Prevalence of Hypovitaminosis D	Seasonal Variation considered ?	Assessment of 25(OH)D	Excluded/ identified participants with medications known to influence 25(OH)D status	Excluded/ identified participants taking vitamin D supplements
Barchetta, 2011 (Italy)[135]	36.9 ± 23.0	51.2 ± 24.2	Deficient ≤ 50nmol/l	Did not give vitD values for quartiles	Yes	CMM	No	No
Katz, 2010 (USA)[124]	Quartiles I (n=686; 12.31 % NAFLD) II (n=452; 6.91% NAFLD) III (n=297; 6.81% NAFLD) IV (n=195; 7.14% NAFLD)	-	-	Quartiles I (≤47.4 nmol/l) II (47.4<25(OH)D ≤ 62.4 nmol/l) III (62.4<25(OH)D ≤ 77.4 nmol/l) IV (≥77.4 nmol/l)	No	RIA	No	Yes
Targher, 2007 (Italy)[139]	Total: 51.0 ± 22 Steatosis: 59.3 ± 20 NASH: 37.0 ± 23	74.5 ± 15	HVD ≤ 37.5nmol/l	NAFLD: 48.3% HVD Control: 28.3% HVD	Yes	CLIA	Yes	Yes
Sharifi, 2014 (Iran)[147]	Baseline* 28.7 (22.0 – 70.9) 4 months* 74.9 (64.4 – 116.3)	Baseline* 42.1 (29.2 – 61.9) 4 months* 47.9 (36.7 – 66.6)	Sufficient ≥75nmol/l Insufficient ≥50 to <75nmol/l Deficient <50nmol/l	(Deficient, Insufficient, Sufficient) VitD Supplementation Baseline: (70.4%, 18.5%, 11.1%) vs. 4month: (0%, 48%, 52%) Placebo Baseline: (53.8%, 23.1%, 23.1%) vs 4month: (50.0%, 30.8%, 19.2%)	Yes	RIA	Yes	Yes

**For the RCT trials, the left column refers to the NAFLD group who received the vitamin D treatment and the right column is the NAFLD group who received the placebo. 25(OH)D levels are given for baseline and after the 4 month treatment.*

Data expressed as mean ± standard deviation or median (IQR; interquartile range).

Abbreviations: NR, not reported; 25(OH)D, 25-hydroxyvitamin D; NAFLD, Nonalcoholic fatty liver disease; NASH, Nonalcoholic steatohepatitis; VFA, visceral fat area; ELISA, enzyme-linked immunosorbent assay; UPLC-MS/MS, ultra-performance liquid chromatography tandem mass spectrometry; HPLC, high performance liquid chromatography; CLIA, chemiluminescence immunoassay; ECLIA, electrochemiluminescence immunoassay; RIA, radioimmunoassay; CMM, colorimetric methods; HVD, hypovitaminosis D; VitD, vitamin D.

To convert nmol/L serum 25(OH)D to ng/mL, multiply by 0.40. To convert ng/mL serum 25(OH) to nmol/L, multiply ng/mL by 2.496.

Table A2.6 NAFLD, Body Composition and Insulin Resistance Specific Study Characteristics and Methodology and Primary Outcomes of Articles Included in the Scoping Review.

Article	Method of NAFLD diagnosis	Excluded participants with other known causes of steatosis*	All assessment methods of body composition	Measurements of insulin resistance /glycemia /insulinemia	NAFLD mean HOMA-IR value	NAFLD mean insulin value (µU/ml or µIU/ml or mU/L)	Results
Mohamed, 2016 [146]	LU	Yes	BMI	FBG, insulin, HOMA-IR	-	11.7 ± 4.8	No correlation between 25(OH)D and BMI percentile, FBG, insulin or HOMA-IR
Lorvand, 2016 [148]	LU	Yes	BMI, WC, BIA	FPG, insulin, HOMA-IR	*VitaminD at 12 weeks* 3.5 ± 1.3 *Placebo at 12 weeks* 3.3 ± 1.0	*VitaminD at 12 weeks* 15.7 ± 5.5 *Placebo at 12 weeks* 14.8 ± 4.2	-After 12 week intervention, no difference between NAFLD groups for BMI, fat mass, waist circumference, FPG, insulin or HOMA-IR
Chang, 2015 [145]	LU (used for NASH diagnosis to)	Yes	DXA, BMI	FBG, HOMA-IR, HbA1c	*Steatosis:* 5.1 (2.2-9.0) *NASH:* 4.3 (2.2-10.2)	NR	- 25(OH)D did not correlate with BMI, total body fat percentage, extremity fat percentage or trunk fat percentage -25(OH)D was negatively correlated with HOMA-IR in the NASH group
Bril, 2015 (USA) [140]	Biopsy	Yes	DXA, BMI	FPG, FPI, HbA1c, OGTT, euglycemic hyperinsulinemic clamp	*Hepatic IR index:* *Steatosis:* 15 ± 2, *NASH:* 28 ±3, *AT IR index:* *Steatosis:* 5.6 ± 0.8 *NASH:* 8.5 ± 0.7 *Muscle IS:* *Steatosis:* 7.3 ± 0.7, *NASH:* 5.3 ± 0.3	*Steatosis:* 11 ± 1 *NASH:* 18 ± 1	-When NAFLD patients were divided into TBF quartiles there was no sig difference in 25(OH)D between groups -No relationship between 25(OH)D and insulin sensitivity when patients matched for BMI and total adiposity -No difference in plasma vitD in patients with or without NAFLD after adjusting for BMI
Hourigan, 2015 (USA)[142]	Biopsy	Yes	WC	FBG, FINS, HOMA-IR	7.1 ± 4.1	32.2 ± 17.6	-WC, FBG, FINS and HOMA-IR were not significantly different between the three vitamin D groups (sufficient ≥75nmol/l, insufficient 50-74nmol/l deficient ≤50nmol/l)
Lu, 2015 (China) [138]	LU	Yes	MRI, BMI, WC	FPG, HbA1c, FINS, HOMA-IR, OGTT, 2hPG,	*VFA < 80cm²* 2.1 (1.6 – 3.0) *VFA ≥80 cm²* 3.1 (2.3 – 4.3)	*VFA < 80cm²* 8.7 (6.6 – 11.1) *VFA ≥80 cm²* 12.0 (9.0 – 15.6)	-After adjusting for age and BMI, 25(OH)D levels were negatively correlated with VFA and 2hPG -25(OH)D levels lower in NAFLD vs Non-NAFLD regardless of abdominal obesity status
Dasarathy, 2014 (USA) [137]	Biopsy	Yes	WC, BMI, BIA and CT	FBG, FINS, HbA1c, HOMA-IR	*Steatosis:* 5.0 ± 3.9 *NASH:* 9.3 ± 10.4	*Steatosis:* 18.1 ± 11.1 *NASH:* 27.5 ± 21.6	-VitD correlated inversely with body weight, BMI, whole body FM (BIA) and VFA (CT) -NASH and T2DM but not WC independently predicted hypovitaminosis D
Nobili, 2014 (Italy) [143]	Biopsy	Yes	WC, BMI	FSG, FSI, HbA1c, HOMA-IR	4 (50th percentile)	19 (50th percentile)	-No sig difference in BMI, BMI-SDS, WC, FSG, FSI, HOMA-IR or HbA1c between normal 25(OH)D vs low 25(OH)D (<50 nmol/L) -HOMA-IR independently and inversely associated with 25(OH)D

Table A2.6 NAFLD, Body Composition and Insulin Resistance Specific Study Characteristics and Methodology and Primary Outcomes of Articles Included in the Scoping Review Continued.

Article	Method of NAFLD diagnosis	Excluded participants with other known causes of steatosis*	All assessment methods of body composition	Measurements of insulin resistance /glycemia /insulinemia	NAFLD mean HOMA-IR value	NAFLD mean insulin value (µU/ml or µIU/ml or mU/L)	Results
Rodriguez, 2014 (Spain) [144]	Biopsy	Yes	WC, BMI	FSG, FSI, HOMA-IR	6.4 ± 4.1	23.2 ± 12.0	-25(OH)D was not associated with BMI, WC, HOMA-IR or NAFLD -Patients with VDD did not show sig higher HOMA-IR
Yildiz, 2014 (Turkey) [116]	LU	No	WC, WC-SDS, BMI, BMI-SDS	FBG, FINS, HOMA-IR	5.3 ± 4.4	NR	-No correlation between 25(OH)D and WC -25(OH)D levels lower in obese children with hepatosteatosis vs obese children without hepatosteatosis
Bhatt, 2013 (India) [136]	LU	Yes	WC, HC, WHR, MAC, MTC, NC, skinfolds, DXA	FBG, Post-prandial BG, FSI, HOMA-IR	2.5 ± 1.0	11.7 ± 4.0	-In the lowest 25(OH)D quartile, WC, FSI and HOMA-IR sig higher compared to other 25(OH)D quartiles -No correlation between 25(OH)D and fasting insulin or HOMA-IR
Rhee, 2013 (Korea) [141]	LU	Yes	WC, BMI	FBG, FINS, HBA1c, HOMA-IR	1.66 ± 1.0	6.9 ± 3.8	- FINS was weakly negatively correlated with 25(OH)D -25(OH)D levels were still lower in NAFLD patients vs Non-NAFLD after adjusting for BMI and FINS
Barchetta, 2011 (Italy) [135]	LU	No	WC, BMI	FBG, HbA1c, FINS, HOMA-IR	10.4 ± 15.4	NR	-In the lowest 25(OH)D quartile, HOMA-IR and WC sig higher compared to other quartiles -FLI correlated inversely with 25(OH)D independent from sex, age and HOMA-IR - NAFLD and low 25(OH)D association independent from BMI
Katz, 2010 (USA) [124]	ALT ≥ 30U/L	Yes	WC, BMI	N/A	-	-	-25(OH)D was not independently associated with suspected NAFLD in the multivariate analyses that included either BMI or WC (not associated when adjusted for obesity)
Targher, 2007 (Italy) [139]	Biopsy	Yes	WC, BMI	FBG, FSI, HbA1c, HOMA-IR, OGTT, 2hG,	Total: 4.1 ± 2.2 Steatosis: 3.1 ± 1.8 NASH: 5.2 ± 2.6	NR	-25(OH)D correlated negatively with BMI, WC and HOMA-IR -25(OH)D levels lower in NAFLD vs controls and difference persisted after adjustment for sex, age, BMI, HOMA-IR, diabetes status and components of MS
Sharifi, 2014 (Iran) [147]	LU	Yes	BIA, WC, HC, WHR	FBG, FINS, HOMA-IR	VitaminD at 4 months 3.6 (2.4 – 4.3) Placebo at 4 months 2.4 (1.9 – 3.9)	VitaminD at 4 months 14.6 (9.9 – 18.0) Placebo at 4 months 11.5 (8.5 – 15.8)	-Vitamin D supplementation has no significant effect on BMI, WC, WHR, body fat percentage, FBS, FINS or HOMA-IR

*Data expressed as mean ± standard deviation or median (IQR; interquartile range) *Excluded participants if they had excessive alcohol intake (adult population), any form of hepatic disease (Hepatitis C, Hepatitis B, autoimmune liver disease, hemochromatosis, Wilson's disease, etc.), or were taking hepatotoxic medications. Abbreviations: NR, not reported; N/A, not applicable; NAFLD, Nonalcoholic fatty liver disease; NASH, Nonalcoholic steatohepatitis; 25(OH)D, 25-hydroxyvitamin D; VitD, vitamin D; VDD, vitamin D deficiency; VFA, visceral fat area; LU, liver ultrasound; ALT, alanine aminotransferase; DXA, Dual-energy X-ray absorptiometry; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; MAC, mid arm circumference; MTC, mid thigh circumference; NC, neck circumference; BIA, bioelectrical impedance analysis; FM, fat mass; CT, computed tomography; MRI, magnetic resonance imaging; SDS, standard deviation score; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HbA1c, glycosylated hemoglobin; OGTT, oral glucose tolerance test; 2hPG, 2 hour postprandial plasma glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment for insulin resistance; FBG, fasting blood glucose; FSG, fasting serum glucose; FSI, fasting serum insulin; BG, blood glucose; 2hG, 2-hour glucose; IR, insulin resistance; IS, insulin sensitivity; TBF, total body fat; T2DM, type 2 diabetes mellitus; sig, significantly; MS, metabolic syndrome.*

A2. Hand Grip Protocol

Hand Grip Protocol

Equipment required

- JAMAR® Hand Dynamometer (use safety strap to minimize chance of dropping)
- Sturdy Chair (no wheels)
- Foot stool/box if child's feet cannot reach floor

Procedures

•Have the patient sit with feet flat on floor with their shoulder adducted and neutrally rotated, elbow flexed at 90º, forearm in neutral position and wrist in neutral position (wrist between 0 and 30 deg dorsiflexion and between 0 and 15 deg ulnar deviation) (make sure arm is out not tucked into their side)

•Set the JAMAR® Hand Dynamometer to the second handle position from the inside (first setting may have to be used depending on size of child's hand, although considered less accurate) (Before moving the handle from one position to another, note that the handle clip is located at the lower (furthest) post from the gauge. If the handle is not replace in the correct position, the readings will not be accurate)

•Rotate the red peak-hold needle counter clockwise to 0.

•Let the child arrange the instrument so that it fits in his/her hand comfortably.

•Lightly hold around the readout dial to prevent inadvertent dropping.

•After the child is positioned properly, have him/her squeeze with their maximum strength. The peak-hold needle will automatically record the highest force the child has exerted.

Script:

"Are you right handed or left handed? This is a handgrip; I want to see how strong your hands are. I am going to ask you to squeeze this 3 times for each hand. When you squeeze the bar will not move (point to bar) *but squeeze as hard as you can* (put in dominant hand first, use wrist strap) *Does this feel comfortable in your hand? Don't squeeze yet* (turn dial to zero) *on the count of three squeeze as hard as you can… 1, 2, 3. Squeeze...harder...harder... and relax."*

•Record the scores of **three** successive trials for each hand (Reset the peak hold needle to zero before recording new readings). Alternate between each hand (Switch hands starting with the dominant - R L R L R L or L R L R L R, so fatigue isn't a limiting factor)

•The average score of the three trials can be compared to the normative data, which is in pounds (lbs) or kilograms (kg).

•From a statistical perspective, scores within two standard deviations of the mean are considered within normal limits.

A2. Six Minute Walk Test (6MWT) Protocol

6 Minute walk test protocol
(Based on the American Thoracic Society (ATS) standardized guidelines for 6MWT)

LOCATION
The 6MWT course is set up just outside the main entrance of the clinical research unit on the second floor of the Li Ka Shing building. Prior to the study visit measure out 15 meters in length with tape. Place tape in a boarder around line so they don't walk to far away from 15 m line.

MEASUREMENTS
Before the start of the 6MWT, have the patient sit in a chair, located near the starting position, for at least 10 minutes before the test starts. While child is resting, put a pylon at 0m, and 15m and two spaced out inbetween on the course). Depending on time of time/how busy it is,you may be someone to help direct people away from the 6MWT course while child is walking.

1. Measure Blood pressure, Heart rate and pulse oximetry right before the 6MWT (after 10 minutes of resting in chair)
2. While blood pressure machine is measuring (takes 30-45 seconds), ask the child to rate overall fatigue using the modified Borg scale. Show child scale and say: *"How out of breath do you feel right now, in terms of exercise tired, If I said one was you're so relaxed lying on the couch eating candy and ten was you are so out of breath you can barely breathe?"*
3. Instruct the patient as follows:

"The purpose of this test is to see how far you can walk in 6 minutes. You will be walking back and forth in this hallway around the cones. When I say 'Go", you will walk for 6 minutes while I monitor how you are doing. I will stand at the end of the track and let you know how much time you have left in "5, 4, 3, 2, and 1 minute reminders. If you need to slow down or stop and rest, you can stop and stand where you are until you can go again. The GOAL is to try to walk AS FAR AS POSSIBLE (to do as many laps) as you can in the 6 minutes, No jogging or running."

You may demonstrate by walking one lap yourself. Walk and pivot around the cone/marker briskly.

"Are you ready to do that? Do you have any questions? "Can you tell me what you are about to do?"

Position the patient at the starting line. **"You may start on the count of 3. 1, 2, 3, GO!"** Do not walk with the patient. As soon as the patient starts to walk, start the timer.

Use an even tone of voice when using the standard phrases of encouragement.

After the first minute, tell the patient the following:
"You're doing well. You have 5 minutes to go."

When the timer shows 4 minutes remaining, tell the patient the following:
"Keep up the good work. You have 4 minutes to go."

When the timer shows 3 minutes remaining, tell the patient the following:
"You're doing well. You are halfway done."

When the timer shows 2 minutes remaining, tell the patient the following:
"Keep up the good work. You have only 2 minutes left."

When the timer shows 1 minutes remaining, tell the patient the following:
"You're doing well. You have only 1 minute to go."

Do not use other words of encouragement (or body language to speed up).

If the patient stops walking during the test and needs a rest, say this: **"You can lean against the wall if you would like; then continue walking whenever you feel able."** Do not stop the timer. If the patient stops before the 6 minutes are up and refuses to continue (or you decide that they should not continue), wheel the chair over for the patient to sit on, discontinue the walk, and note on the worksheet the distance, the time stopped, and the reason for stopping prematurely.

When the timer is 15 seconds from completion, say this:
"In a moment I'm going to tell you to stop. When I do, just stop right where you are and I will come to you."
When the timer rings (or buzzes), say this: **"STOP!"** Walk over to the patient. Consider taking the chair if they look exhausted. Mark the spot where they stopped by placing a piece of tape on the floor.

4. Ask them to go sit back down in the chair. Take a second measurement of blood pressure, heart rate and pulse oximetry. Ask them to rate their overall level of fatigue again using the Borg scale

BORG SCALE

Rating of Perceived Exertion Chart
(Cardiovascular Endurance)

#10 	**I am dead!!!**
#9 	**I am probably going to die!**
#8 	**I can grunt in response to your questions and can only keep this pace for a short time period.**
#7 	**I can still talk but I don't really want to and I am sweating like a pig!**
#6 	**I can still talk but I am slightly breathless and definitely sweating.**
#5 	**I'm just above comfortable, I am sweating more and can talk easily.**
#4 	**I'm sweating a little, but I feel good and I can carry on a conversation comfortably.**
#3 	**I am still comfortable, but I'm breathing a bit harder.**
#2 	**I'm comfortable and I can maintain this pace all day long.**
#1 	**I'm watching TV and eating bon bons.**

APPENDIX 3: Additional Data

A3. Study Blood Work (ELISA Kits)

Study blood was immediately stored at 2-8°C for approximately 30-60 minutes after time of phlebotomy and then centrifuged at 2500 RPM at 4°C for 15 minutes using a CR4-22 Jouan centrifuge (Winchester, VA, USA). Separated serum and plasma were aliquoted into eppendorf tubes and stored at -80°C until testing. Six markers were analyzed using enzyme-linked immunosorbent assays (ELISA). These markers included: serum bone specific alkaline phosphatase (BAP; MicroVu, Quidel, San Diego, CA, USA, 8012(QI)), osteocalcin (OC; MicroVu, Quidel, San Diego, CA, USA, 8002(QI)), N-telopeptide collagen type 1 (NTX; Osteomark, Wampole Laboratories, Princeton, NJ, USE, X9021), retinol binding protein 4 (RBP-4; R&D Systems, Minneapolis, USA, DRB400) and vitamin D binding protein (VDBP; R&D Systems, Minneapolis, USA, DVDBP0) and interleukin 6 (IL-6; R&D Systems, Minneapolis, USA, HS600B).

Table A3.1 Markers of Inflammation, Bone Turnover and Proteins.

Variable (HC, PWS, NAFLD)	Healthy Control	PWS	NAFLD
IL-6 (pg/mL) (13, 7, 7)	0.4 ± 0.3 (0.1 – 0.9)	0.6 ± 0.3 (0.3 – 1.3)	0.6 ± 0.3 (0.3 – 1.0)
BAP (U/L) (13, 5, 3)	136.6 ± 51.0 (29.8 – 234.9)	101.7 ± 41.3 (64.5 – 172.6)	90.1 ± 42.9 (41.4 – 122.1)
OC (ng/mL) (13, 5, 3)	28.8 ± 7.0 (14.5 – 39.5)	28.4 ± 7.2 (20.9 – 36.7)	23.2 ± 14.0 (8.4 – 36.2)
NTX (nM BCE/L) (13, 5, 3)	398.7 ± 110.0 (265.4 – 592.6)	342.5 ± 91.2 (199.3 – 428.1)	322.1 ± 113.2 (243.9 – 451.9)
RBP-4 (ng/mL) (14, 6, 7)	26.2 ± 7.2 (17.5 – 42.4)	34.5 ± 9.2 (23.3 – 48.4)	32.3 ± 5.9 (24.6 – 38.9)
VDBP (ng/mL) (14, 6, 7)	214.9 ± 88.4 (94.8 – 377.2)	328.1 ± 97.2 (152.6 – 443.5)	326.2 ± 96.2 (198.7 – 510.8)

[1]Values are means ± SD (range). Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease; HC; Healthy Control, IL-6, interleukin 6; BAP, bone specific alkaline phosphatase; OC, osteocalcin; NTX, N-telopeptide collagen type 1; RBP-4, retinol binding protein 4; VDBP, vitamin D binding protein.

A3. Dual X-ray Absorptiometry (DXA) Results

Dual X-ray absorptiometry (DXA) is part of routine clinical care for children with PWS and NAFLD. As DXA is not routinely performed in healthy children in Alberta, no data was available for review from DXA in this study. Body composition data based on DXA was collected for obese children with NAFLD and PWS, if these were completed within 1-2 months of the study visit. Whole body composition (total and regional lean mass, fat mass and total mass) and bone mineral density were measured using a Hologic densitometer (4500A or Discovery A) with Apex System 2.4.2, (Waltham, MA, USA).

Appendicular lean tissue mass (ALM) mass was calculated as the sum of the lean soft tissue (LST) from the arms and legs measured by DXA [102]. Predicted skeletal muscle mass (SMM) was calculated using ALM and tanner stage specific equations [101]. Those with tanner stages <5 were estimated to be <13 years and those with tanner stages ≥5 were ≥13 years, as we did not have data on tanner staging. SMM z-scores was calculated as the difference between measured SMM and predicted SMM using gender specific constants [101].

Table A3.2 Bone Mineral Density and Body Composition (DXA).

	PWS (n=8)	**NAFLD (n=4)**
Total BMD (g/cm^2)	0.9 ± 0.2 (0.7 – 1.2)	1.0 ± 0.1 (0.8 – 1.1)
BMD z-score	-0.3 ± 1.0 (-1.5 – 1.4)	0.9 ± 1.4 (-1.0 – 2.0)
Fat mass total (kg)	22.9 ± 11.0 (9.7 – 45.6)	29.6 ± 13.6 (15.4 – 46.5)
Fat mass/ Height^2 (kg/m^2)	11.1 ± 4.2 (7.1 – 20.5)	12.7 ± 3.2 (9.6 – 16.4)
Fat mass/Height^2 z-score	1.2 ± 0.6 (0.2 – 1.8)	1.5 ± 0.2 (1.2 – 1.7)
Android/Gynoid ratio	0.9 ± 0.1 (0.8 – 1.1)	1.1 ± 0.1 (1.0 – 1.1)
Trunk/ Limb fat mass ratio	0.8 ± 0.2 (0.6 – 1.0)	0.9 ± 0.1 (0.8 – 1.1)
Trunk/ Limb fat mass ratio z-score	0.5 ± 1.1 (-1.3 – 1.8)	1.4 ± 0.4 (1.0 – 1.9)
Lean mass total (kg)	27.3 ± 8.8 (12.9 – 41.1)	36.7 ± 11.3 (21.7 – 46.9)
Lean/Height^2 (kg/m^2)	13.2 ± 2.4 (10.5 – 18.5)	16.2 ± 2.2 (13.4 – 18.7)
Lean/Height z-score	-2.0 ± 0.9 (-1.1 – 1.5)	1.1 ± 1.6 (-1.1 – 2.2)
Appendicular Lean/ Height^2 (kg/m^2)	5.3 ± 1.2 (4.1 – 7.9)	7.0 ± 1.0 (5.7 – 8.0)
Appendicular Lean/ Height^2 z-score	-0.7 ± 0.9 (-1.7 – 1.2)	1.4 ± 0.6 (0.9 – 2.0)
SMM (kg)	11.5 ± 4.7 (4.5 – 19.2)	17.1 ± 6.4 (9.0 – 23.7)
SMM z-score	-1.7 ± 0.9 (-3.3 – -0.23)	1.2 ± 0.5 (0.5 – 1.5)

[1]Values are means ± SD (range). Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease; DXA, dual-x ray absorptiometry; BMD, bone mineral density; SMM, skeletal muscle mass.

A3. Healthy Eating Index, Glycemic Index, Glycemic Load, Fructose and Additional Dietary Intake Data

Diets were assessed for nutritional quality using the Healthy Eating Index-C (HEI-C) [204]. The HEI-C is a validated diet quality scoring system which examines the number of servings consumed from each food group as well as total fat, saturated fat, and cholesterol [204]. Diets were scored from a range of 0-100, with 100 points referring to the "optimal level for" diet quality and lower results indicating larger deviations from the recommended intakes [205]. Children's diets were categorized as 'poor' (≤50 HEI-C score), 'needs improvement' (HEI-C score 50-80), or 'good' (HEI-C score >80) [204, 205].

Glycemic index (GI) was determined using the international table of GI values and the University of Sydney online research database [206, 207]. GI and GL were calculated using the following formulas: GI= Σ (carbohydrate content of food item (g) X GI of food item)/ total carbohydrate content of day (g) and GL= Σ (carbohydrate content of food item (g) X GI of food item)/100 [208, 209]. The GI value of food was categorized as GI (<55 for low GI foods, 55-60 for medium GI foods and >60 for high GI foods)[22]. The GL value of food is categorized as GL (<80 for low GL foods, 80-120 for medium GL foods and >120 for high GL foods)[22]. Dietary fructose and sucrose was analyzed using the Canadian nutrient file, USDA (release 27) and food manufacturers web sites [22]. Fructose intake was calculated as: Σ(free fructose + sucrose/2) [22].

Table A3.3 Healthy Eating Index-C, Glycemic Index and Glycemic Load.

	Healthy Control (n=14)[1]	**PWS (n=7)[1]**	**NAFLD (n=6)[1]**	**P-value[2]**
Total HEI Score	66.7 ± 8.9 (54.3 – 79.2)	79.1 ± 8.1 (62.3– 85.5)	68.2 ± 11.4 (56.0 – 88.3)	0.004Ŧ 0.05*
Gl Mix Meal	51.4 ± 4.4 (42.3 – 58.8)	50.3 ± 3.0 (46.3 – 54.9)	52.8 ± 5.1 (46.4 – 59.8)	NS
GL Sum	139.4 ± 41.2 (83.8 – 226.5)	116.6 ± 38.9 (80.3 – 203.3)	115.3 ± 21.6 (85.5 – 132.2)	NS

**between PWS and NAFLD; Ŧbetween PWS and Control; ϕbetween NAFLD and Control.*
[1]Values are means ± SD (range). [2]p-values <0.05 are considered statistically significant. The Healthy Eating Index-C (HEI-C) scoring system was used to assess diet quality. Diets were scored from a range of 0-100, with 100 points referring to the "perfect" diet and lower results indicating larger deviations from the recommended intakes [204, 205]. HEI-C scores ≤50 are considered 'poor', HEI-C scores between 50-80 'needs improvement' and HEI-C scores >80 'good' [204, 205]. Glycemic index (GI) was determined using the international table of GI values and the University of Sydney online research database [206, 207]. A validated, standardized approach was taken to calculate GI mix meal and glycemic load (GL) [208, 209]. The GI value of food is categorized as GI (<55 for low GI foods, 55-60 for medium GI foods and >60 for high GI foods)[22]. The GL value of food is categorized as GL (<80 for low GL foods, 80-120 for medium GL foods and >120 for high GL foods) [22]. Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease.

Table A3.4 Additional Dietary Intake (3-Day Food record).

	Healthy Control (n=16)[1]	PWS (n=8)[1]	NAFLD (n=7)[1]	P-value[2]	DRI
Total Sugar (g)	101.1 ± 27.7 (70.2 – 173.8)	112.2 ± 25.5 (78.1 – 137.4)	88.5 ± 31.6 (34.6 – 123.3)	NS	-
Fructose (g)	42.4 ± 17.2 (22.1 – 79.8)	40.7 ± 13.1 (26.7 – 68.4)	36.7 ± 15.0 (12.5 – 61.7)	NS	-
Fibre (g)	19.0 ± 5.3 (9.6 – 27.7)	22.7 ± 9.8 (13.7 – 45.9)	17.4 ± 5.0 (11.5 – 26.0)	NS	19-38
Cholesterol (mg)	247.8 ± 109.3 (54.5 – 526.6)	177.0 ± 31.4 (122.4 – 222.1)	246.5 ± 126.3 (109.6 – 426.4)	NS	-
% PUFA	4.8 ± 2.4 (1.7 – 11.2)	5.6 ± 1.3 (3.5 – 7.3)	4.1 ± 2.4 (1.5 – 9.0)	0.01*	10%
% MUFA	10.6 ± 2.5 (5.8 – 15.4)	8.5 ± 1.2 (5.9 – 9.8)	8.9 ± 4.7 (3.5 – 17.6)	0.04Ŧ	10%
Vitamin A (RAE) (µg)	677.4 ± 269.6 (219.9 – 1076.1)	829.0 ± 406.6 (377.7 – 1431.7)	498.0 ± 275.0 (198.7 – 954.4)	NS	300-900
Vitamin E (mg)	5.8 ± 4.0 (1.3 – 19.0)	6.4 ± 3.6 (2.7 – 14.1)	10.2 ± 13 (1.8 – 37.8)	NS	6-15

**between PWS and NAFLD; Ŧbetween PWS and Control; ϕbetween NAFLD and Control.*
[1]Values are expressed as mean ± SD (range). [2]p-values <0.05 are considered statistically significant.
Abbreviations: PWS, Prader-Willi Syndrome; NAFLD, Nonalcoholic fatty liver disease; DRI, Dietary Reference Intakes; PUFA, Polyunsaturated Fatty Acid; MUFA, Monounsaturated Fatty Acid.