Changes in body composition and eating behaviors in 6- to 8-year-old children with obesity participating in a family-centered lifestyle intervention: Results from a 1 year randomized controlled trial

Tamara R. Cohen

School of Human Nutrition
McGill University, Montreal

December 2017

A thesis submitted to McGill University in partial fulfillment of the requirements
of the degree of Doctor of Philosophy

This thesis is dedicated to all those have struggled with obesity during childhood and to those who continue to face its unforgiving consequences in adulthood.

This thesis considers the importance of people-first language: it is important to describe children with obesity and not label them by their disease.

ABSTRACT

Background: Childhood obesity is a complex disease that is associated with health consequences. The literature supports the use of family-centered lifestyle interventions in reducing adiposity in children with obesity, however, the evidence that milk products may favorably modulate these changes are unclear. Further, few interventions include assessment of bone health in this pediatric population. This dissertation aimed to test the effects of increased milk and milk products and weight-bearing types of activity on changes in: (1) body composition; (2) bone health; and (3) eating behaviors in children with obesity participating in a 1 year (y) family-centered lifestyle intervention.

Design: Data are from the McGill Youth Lifestyle Intervention with Food and Exercise (MY LIFE) study, a 1 y randomized controlled trial that took place in Montreal, QC. Eligibility included healthy children classified as overweight or obese as per the World Health Organization. Children (n = 78; ages 6–8.5 y) were randomized to either: control (Ctrl: no intervention); standard intervention (StnTx: 2 servings of milk and milk products/ day (d); meet physical activity (PA) guidelines) or modified intervention (ModTx: 4 servings of milk and milk products/ d; meet PA guidelines plus daily weight-bearing types of PA). Study visits occurred every 3 months (mo) for 1 y; interventions were held once a month for 5 mo with a follow-up session at the end of the 8th mo. Interventions were based on Canada's Food Guide to Healthy Eating (CFG) and Canadian PA guidelines and individualized to meet the needs of the family. Ctrl received counseling after 1 y. Fasting blood samples were collected for various outcomes. Anthropometry were measured; body mass index-for-age and sex z-scores (BAZ) were calculated. Dual-energy x-ray absorptiometry scans were performed for: percent body fat (%BF), fat mass (FM), trunk FM, lean mass. Whole body (WB), lumbar spine (LS) and lumbar lateral spine (LLS) were measured for bone mineral content (BMC) and bone mineral density (BMD). Parents completed the Children's Eating Behavior Questionnaire, reported on diet (3-day food diary) and were surveyed on PA and sedentary behaviors of their child. Fatty acids measured in erythrocytes by gas chromatography were used to assess compliance to the milk intervention during the first 6 months.

Results: (Aim 1) Baseline anthropometry did not differ among groups. At 1 y, all groups increased height ($p<0.001$) and lean mass ($p<0.001$), however BAZ was significantly lower in ModTx ($p<0.001$); %BF was also lower in ModTx ($p=0.02$), but not in StnTx ($p=0.99$) or Ctrl

(p=0.99). FM, waist circumference and trunk FM all significantly increased in Ctrl ($p<0.001$). ModTx significantly decreased erythrocyte fatty acids related to milk product intake by 6 mo ($p<0.05$). (Aim 2) Compared to baseline, 1 y measures of BMC in WB, LS, and LLS had significantly increased among all groups ($p<0.001$). However, WB BMD z-scores were significantly lower in Ctrl at 1 y compared to baseline ($p<0.05$), whereas BMD for WB and LLS were significantly increased in StnTx and ModTx ($p<0.001$) but not in Ctrl. Bone biomarkers did not change over time among groups. (Aim 3) At 6-mo StnTx reduced Emotional Overeating and Desire to Drink scores while Food Responsiveness scores were reduced in both StnTx and ModTx ($p<0.05$). At 1-y, scores for Desire to Drink in StnTx and Food Responsiveness in ModTx remained lower ($p<0.05$). Plasma leptin concentrations were lower in ModTx at 6-mo compared to baseline ($p<0.05$).

Conclusion: Participating in a family-centered lifestyle intervention based on Canadian dietary and PA guidelines had positive effects on reducing adiposity while maintaining bone health and favorably changing eating behaviors in children with obesity. This study suggests that the use of national guidelines as a template for intervention platforms are appropriate, but need to be individualized to meet the needs of the family.

RÉSUMÉ

Contexte: L'obésité juvénile est une maladie complexe. Les interventions qui visent à réduire l'adiposité chez l'enfant obèse devraient être centrées sur la famille, la durabilité des réductions de l'adiposité et la santé osseuse. Cette étude avait pour principal objectif d'évaluer les effets de l'augmentation de la consommation de produits laitiers et de la réalisation d'exercices de port de poids dans : (1) la composition corporelle; (2) la santé osseuse et ses biomarqueurs; (3) le comportement alimentaire.

Méthodologie: Les données sont tirées de McGill Youth Lifestyle Intervention with Food and Exercise (MY LIFE), qui s'est déroulé pendant 1 an dans Montréal (QC). Étaient acceptés les enfants en bonne santé considérés comme obèses ou en surpoids. Des enfants (n=78; 6–8,5 ans) ont été affectés à 3 groupes : groupe témoin (Ctrl : pas d'intervention), groupe d'intervention standard (StnTx: 2 portions de produits laitiers/jour et application des Directives canadiennes en matière d'activité) et groupe d'intervention modifiée (ModTx: 4 portions de produits laitiers/jour, application des Directives et exercices quotidiens de port de poids). Les visites de l'étude ont été organisées tous les 3 mois pendant 1 an; les interventions, une fois par mois pendant 5 mois et une séance à la fin du 8^{e} mois. Des prélèvements sanguins ont été effectués pour divers tests. Les paramètres anthropométriques ont été mesurés, et les écarts z de l'indice de masse corporelle en fonction de l'âge et du sexe (BAZ) ont été calculés. Des analyses par absorptiométrie à rayons X biphotonique ont été effectuées pour établir le pourcentage de masse grasse (%BF), la masse adipeuse (FM), la masse adipeuse du tronc. Le contenu minéral osseux (BMC) et la densité minérale osseuse (BMD) du corps entier (WB), de la colonne lombale (LS) et de la colonne lombale latérale (LLS) ont été mesurés. Les parents ont rempli un questionnaire sur le comportement alimentaire de leur enfant, ont rédigé son journal alimentaire et répondu à des questions sur ses habitudes. Les acides gras, mesurés par chromatographie gazeuse, ont servi à évaluer la conformité aux interventions au cours des 6 premiers mois.

Résultats: (*1er objectif*) L'anthropométrie n'était pas différente au départ entre les groupes. Après 12 mois, la taille ($p<0,001$) a augmenté dans tous les groupes. Cependant, le BAZ a diminué de dans le groupe ModTx ($p<0,001$) ainsi le %BF dans ModTx ($p=0,02$), mais pas dans les groupes StnTx ($p=0,99$) et Ctrl ($p=0,99$). La FM, la circonférence de la taille et la masse adipeuse du tronc ont tous présenté une hausse significative dans le groupe Ctrl ($p<0,001$). Les taux d'acides gras érythrocytaires liés au produits laitiers avaient diminué dans ModTx au 6 mois

(p<0,05). (*2e objectif*) Les mesures du BMC du WB, LS et LLS avaient augmenté dans tous les groupes à 12 mois (p<0,001). Cependant, les écarts z de la BMD du WB était nettement plus bas dans le groupe Ctrl à 12 mois (p=0,01), tandis que la BMD du WB et de la LS avaient augmenté de manière significative dans les groupes StnTx et ModTx (p<0,05). Les biomarqueurs osseux n'ont pas changé. (*3e objectif*) Dans StnTx, à 6 mois les scores pour «Emotional Overeating» et «Desire to Drink» pendant que les scores pour «Food Responsiveness» étaient plus bas dans ModTx. A 1 an, «Desire to Drink» et «Food Responsiveness» étaient plus bas dans StnTx et ModTx. Les concentrations de leptine étaient plus bas dans ModTx à 6 mois.

Conclusion: La participation à une intervention sur le mode de vie centrée sur la famille et utilisant le Guide et les Directives a eu des effets positifs sur la réduction de l'adiposité et le maintien de la santé osseuse chez des enfants obèses. Des changements positifs se sont également opérés dans le comportement alimentaire. Cette étude donne à penser que dans cette population, il est approprié d'utiliser des lignes directrices nationales pour structurer les interventions, mais en les individualisant pour répondre aux besoins des familles.

Statement of Support

This work would not have been possible without support from the following agencies: Dairy Research Cluster Initiative: Dairy Farmers of Canada, Agriculture and Agri-Food Canada, and the Canadian Dairy Commission (operational grant), and the Canadian Foundation for Innovation (infrastructure funding). The Canadian Heart and Stroke Foundation and Kellogg's Canada donated small tokens that were offered to the children in the study.

The Mary Emily Clinical Nutrition Research Unit of McGill University provided the facilities to conduct the clinical trial, in part from Canada Foundation for Innovation funding.

The PhD Candidate was financially supported by the Frederick Banting and Charles Best Canada Graduate Doctoral Award (Canadian Institutes of Health Research) (2012- 2015) and the Walter M. Steward Postgraduate Scholarship in Agriculture (2012).

Preface and Advancement of Scholarly Knowledge

This dissertation is based on the *McGill Youth Lifestyle Intervention with Food and Exercise (MY LIFE) Study*, a study that enrolled healthy children classified as overweight or obese from greater Montreal, Quebec, Canada. It aims to examine the changes in children among and between groups from baseline to 6 months (mo) (end of intervention phase) and at 12-mo (end of study) on various outcomes: (1) body composition; (2) bone health; and (3) eating behaviors. Despite some important limitations, this study supports the use of Canadian diet and activity guidelines for children with obesity in reducing adiposity while maintaining bone health.

The study's originality and contribution to the scientific literature are discussed in detail in the thesis conclusions. The MY LIFE Study was comprehensive: the works presented in this dissertation include novel biochemical assessments to test compliance to the intervention; assessment of bone sites not currently reported on in children with obesity; and include subjective and objective assessments of satiety. The study design described in this thesis was based on established theoretical models of health behavior. However, above all, this study resulted in an extraordinary retention rate of 94% at 1 year. The findings of this thesis support results from previous trials, and offer a realistic intervention platform that resulted in sustainable changes in adiposity after 1 year.

This dissertation is presented in a manuscript-based thesis for submission to peer-reviewed journals. The first manuscript, a study-protocol paper, provides a general overview of the study design and objectives. This paper highlights the intervention platform used in this study, specifically outlining the educational components and details the differences between groups. The first aim of the dissertation was to examine the intervention effects on body composition, specifically adiposity changes, throughout the course of the 1 year. The data presented in this manuscript are unique as they include assessment of fatty acids to test participant's compliance to the intervention, specifically to assess compliance to consuming 4 servings of milk and milk products per day in children randomized to the modified intervention, compared to standard treatment (2 servings of milk and milk products per day) and control (no treatment). To our knowledge, this was the first study that included this type of biochemical analyses in children with obesity who participated in a trial with a treatment arm based on increasing daily servings of milk and milk products. The second aim was to assess changes in bone health biomarkers and reports on changes in different bone sites analyzed by dual-energy x-

ray absorptiometry. To our knowledge, this is the first report that captures changes in bone sites characterized as weight-bearing and non-weight bearing in children with obesity, and include analyses of bone biomarkers in children participating in a 1 year lifestyle intervention. Finally, the third aim was to describe the changes in eating behaviors and includes a biochemical assessment of satiety, leptin. To date, it is unknown if changes in eating behaviors scores are related to changes in a biomarker of satiety, and if children randomized to increase milk and milk product and weight-bearing types of activities resulted in favorable changes in eating behaviors and leptin concentrations after 1 year.

Together, these collections of manuscripts will contribute to literature related to childhood obesity, bone health and eating behaviors in this pediatric population.

Published research articles in peer-reviewed journals

- **Cohen TR**, Hazell TJ, Vanstone CA, Plourde H, Rodd CJ, Weiler HA. A family-centered lifestyle intervention to improve body composition and bone mass in overweight and obese children 6 through 8 years: a randomized controlled trial study protocol. BMC Public Health. 2013 Apr 25; 13:383. (Chapter 3)
- **Cohen TR**, Hazell TJ, Vanstone CA, Rodd C, Weiler HA. A family-centered lifestyle intervention for obese six- to eight-year-old children: Results from a one-year randomized controlled trial conducted in Montreal, Canada. Can J Public Health. 2016 Dec 27; 107(4-5). (Chapter 4)
- **Cohen TR**, Hazell TJ, Vanstone CA, Rodd C, Weiler HA. Bone health is maintained while fat mass is reduced in pre-pubertal children with obesity participating in a 1-year family-centered lifestyle intervention. Calcif Tissue Int. 2017 Dec;101(6):612-622. (Chapter 5)
- Hazell TJ, Ellery CV, **Cohen TR**, Vanstone CA, Rodd CJ, Weiler HA. Assessment of pedometer accuracy in capturing habitual types of physical activities in overweight and obese children. Pediatr Res. 2016 Nov; 80(5): 686-692.
- Kasvis P, **Cohen TR**, Loiselle SÈ, Kim N, Hazell TJ, Vanstone CA, Rodd C, Plourde H, Weiler HA. Foot-to-foot bioelectrical impedance accurately tracks direction of adiposity change in overweight and obese 7- to 13-year-old children. Nutr Res. 2015 Mar; 35(3): 206-13.

Research articles currently submitted or under review in peer-reviewed journals

- **Cohen TR**, Hazell TJ, Vanstone CA, Rodd C, Weiler HA. Changes in eating behavior and plasma leptin in children with obesity participating in a family-centered lifestyle intervention (Chapter 6: *Under peer-review*)

Abstracts at professional meetings

- **Cohen TR**, Hazell TJ, Jean-Philippe, Vanstone CA, Moore M, Plourde H, Rodd C, Weiler HA. Dual-energy x-ray absorptiometry is more informative than body mass index in assessing adiposity in inactive obese children. CSEP October 2011.
- **Cohen TR**, Hazell TJ, Vanstone CA, Moore M, Plourde H, Rodd C, Weiler HA. Body composition of 6 to 8.5-year-old overweight and obese children: 12-week follow-up from an eating and exercise behavior family-centered lifestyle intervention in Quebec (Canada). FASEB J April 2012.
- **Cohen TR**, Hazell TJ, Jean-Philippe S, Pham T, Vanstone CA, Rodd C, Weiler HA. The effects of dairy intake among preschool aged girls and boys on their weight status FASEB J April 2013.

Oral conference presentations

- **Cohen TR**, Hazell TJ, Vanstone CA, Rodd C, Weiler HA. Changes in lean mass and bone parameters in obese children participating in a family-centered lifestyle intervention: results from a 1-year RCT. Experimental Biology, April 2017.
- **Cohen TR**, Hazell TJ, Vanstone CA, Rodd C, Weiler HA. Children's Eating Behavior Questionnaire: associations with body composition and lifestyle behaviors in overweight and obese prepubescent children. 6th Conference on Recent Advances in the Prevention and Treatment of Childhood and Adolescent Obesity, October 2016.

Contribution of Authors

Manuscript 1: Dr. H Weiler is the primary investigator of the McGill Youth Lifestyle Intervention with Food and Exercise (MY LIFE) study and conceptualized it in partnership with Dr. C Rodd and Dr. H Plourde. The candidate, T Cohen, under the guidance of Dr. H Weiler and Dr. H Plourde, created the intervention platform described in Manuscript 1. The candidate created the study forms under the guidance of Mrs. C Vanstone; Dr. H Weiler approved them for use. The candidate and Dr. H Weiler presented the study protocol to various school boards in Montreal, Quebec, to seek ethics approval for recruitment in public schools. C Vanstone and Dr. H Weiler have maintained ethics approval for subsequent use of the study data.

For Manuscript 1, the candidate was the primary author; guided by the study protocol written by Dr. H Weiler, wrote the first draft of the manuscript and edited all subsequent drafts. Dr. T Hazell, Mrs. C Vanstone, Dr. H Pourde, Dr. C Rodd and Dr. H Weiler provided feedback and edited the manuscript prior to submission. This study was funded by the Dairy Research Cluster Initiative, (Dairy Farmers of Canada, Agriculture and Agri-Food Canada, and the Canadian Dairy Commission) (operational grant), and the Canadian Foundation for Innovation (infrastructure funding).

<u>Author study involvement for Manuscripts 2, 3 and 4:</u>

- The candidate, T Cohen, actively participated in participant recruitment. The candidate assisted in study visits, performed anthropometric assessments, was trained on both dual-energy x-ray absorptiometry (DXA) and blood sample procurement, conducted baseline teachings to families, and conducted all family-centered lifestyle interventions. Additionally, the candidate participated in data entry and auditing. Finally, the candidate conducted all the gas chromatography analysis to assess fatty acid composition under the supervision of Mrs. Sherry Agellon.
- Dr. T Hazell was involved with data collection, specifically DXA assessments, blood sample procurement and data entry.
- Mrs. C Vanstone actively participated in recruitment, collected the blood, when necessary performed DXA assessments and anthropometry.
- Dr. C Rodd oversaw laboratory results sent to the Montreal Children’s Hospital (Montreal, Quebec).

- Dr. H Weiler aided the candidate with the intervention preparation and assisted in overseeing the measurements and interventions. Dr. H Weiler approved all study forms.

Manuscript 2: The candidate was the primary author, wrote the first draft of the manuscript, conducted all statistical analyses and edited all subsequent drafts. Dr. T Hazell, Mrs. C Vanstone, Dr. C Rodd aided with revisions to the manuscript. Dr. H Weiler made contributions to the manuscript, aided with data interpretation and statistical analysis. All authors participated in the response to revisions before final approval to publish.

Manuscript 3: The candidate was the primary author, wrote the first draft of the manuscript, conducted all statistical analyses and edited all subsequent drafts. Dr. T Hazell, Dr. C Rodd and Dr. H Weiler provided extensive revisions to the manuscript, including comments to statistical analyses. Mrs. C Vanstone edited the final draft of the manuscript before submission to peer-review. All authors approved the final version of the manuscript.

Manuscript 4: The candidate was the primary author, wrote the first draft of the manuscript, conducted all statistical analyses and edited all subsequent drafts. Dr. T Hazell, Dr. C Rodd and Mrs. C Vanstone provided comments to the draft manuscripts before submission to peer-review. Dr. H Weiler provided comments concerning statistical analyses and interpretation in addition to approving the final draft before submission to peer-review.

Before submission to peer-review, the Dairy Farmers of Canada had the opportunity to review all manuscripts.

Acknowledgements

This work was made possible by numerous individuals who not only supported me, but also believed in the study itself.

First and foremost, my deepest gratitude to my supervisor, Dr. Hope Weiler, who met with me on a sunny day and asked if I would like to be a part of the MY LIFE Study; a journey that has lasted 7 years. I have learned a great deal working under your guidance and appreciate your continuous support and most of all understanding. To the co-applicants of the MY LIFE Study: Dr. Celia Rodd and Dr. Hugues Plourde; thank you for your support and guidance throughout the years. I want to also acknowledge my PhD committee members, Dr. Stan Kubow and Dr. Louise Thibault, for your encouragement and advice throughout this journey; thank you Dr. Stephanie Chevalier for assistance with the final submission process.

My appreciation to the funding agency, Canadian Institutes of Health Research, who believed in my research and supported me throughout the PhD, including two maternity leaves. I am grateful to Lise Grant and all the departmental staff for their advice, encouragement and assistance throughout the degree.

My sincere gratitude to those individuals who worked directly with me: Dr. Tom Hazell, my genuine appreciation for your contributions to this study but most of all, for our friendship; Catherine Vanstone, thank you as well for your work, but most importantly for your reassurance and wise words. To Sarah-Eve Loiselle and Popi Kasvis: together working side-by-side, motivating each other and most of all, being terrific friends. I will forever be thankful for the support you have both given and continue to offer me. Thank you to Sherry Agellon for your work on the biochemical analyses and for making my short experience in the laboratory a pleasure. To the other members of the MY LIFE Study, as well as numerous volunteers, summer students and dietetic students, thank you for making the study the success it has been.

To all the families of the MY LIFE study: thank you for trusting me and for helping me grow as a dietitian and researcher. Without your commitment to the study, none of this would have been possible.

To my friends: Sonia, Negar, Sina, Neil, Jason and Isabelle, I appreciated our time together.

Finally, my deepest indebtedness goes to my family: my parents who have always believed in me and most importantly, have given me the gift of understanding the importance of

a healthy lifestyle. You have taught me from a young age that exercise and a healthy diet are imperative, a lesson that needs to be shown through example. To my sister, Jessica, I am forever grateful for all you do. To my late feline Charlene, who started this degree by my side and whose fluffiness aided me when in need. To my children, Henry and Reuben, who teach me constantly about life. Most of all, to my dear husband, Emmanuel (Eman), who accepts me for me: your unconditional support, love and encouragement have helped me in fulfilling this degree.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Δ	Delta
µg	Microgram
µSv	Microsievert
%tile	Percentile
%BF	Percentage body fat
1,25(OH)$_2$D	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
3DFD	3-day food diary
aBMD	Areal bone mineral density
AgRP	Agouti-related peptide
AFM	Android fat mass
ANOVA	Analysis of variance
AR	Attrition rate
AT	Aerobic training
BAP	Bone specific alkaline phosphatase
BAZ	Body mass index for-age and sex z-score
BIA	Bioelectrical impedance analysis
BL	Baseline
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
Ca	Calcium
CART	Cocaine-and amphetamine-regulated transcript
CCHS	Canadian Health Measures Survey
CCK	Cholecystokinin
CDC	Centers for Disease Control and Prevention
CEBQ	Child Eating Behavior Questionnaire
CFG	Canada's Food Guide
CHMS	Canadian Health Measures Survey
CHQ	Child Health Questionnaire
CI	Confidence interval
CIQ	Child Intention Questionnaire
CLA	Conjugated linoleic acid
CT	Computed tomography
Ctrl	Control

CTX-1	C-telopeptide of type 1 collagen
CV	Coefficient of variation
d	Day
DXA	Dual-energy x-ray absorptiometry
FFM	Fat-free mass
FFMI	Fat-free mass index
FHQ	Family Health Questionnaire
FM	Fat mass
FMI	Fat mass index
FU	Follow-up
g	Gram (s)
GLP-1	Glucagon-like peptide-1
GnRH	Gonadotropin-releasing hormone
h	Hour (s)
HAZ	Height-for-age z-score
IOTF	International Obesity Task Force
IU	International units
kcal	Kilocalorie
kg	Kilograms
L	Liter (s)
LLS	Lateral lumbar spine
LM	Lean mass
LS	Lumbar spine (1-4)
m	Meters
MC(3/4)R	Melanocortin- (3/4) receptor
MF	Milk fat
mg	Milligram
min	Minute (s)
mL	Milliliter (s)
mPAQ-C	Modified Physical Activity Questionnaire for Children
mo	Months
ModTx	Modified treatment
MY LIFE	McGill Youth Lifestyle Intervention for Food and Exercise
NAFLD	Non-alcoholic fatty liver disease
NHANES	National Health and Nutrition Examination Survey
NPY	Neuropeptide Y
OB	Obese
OC	Osteocalcin

OR	Odds ratio
OW/OB	Overweight/ obese
PA	Physical activity
PAQ-C	Physical Activity Questionnaire for Children
POMC	Pro-opiomelanocortin
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
PYY	Peptide YY
RBC	Red blood cell
RCT	Randomized controlled trial
RR	Risk ratio
RT	Resistant training
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SDS	Standard deviation score
StnTx	Standard treatment
TBFM	Total body fat mass
TTM	Transtheoretical model
TXT	Treatment
TV	Television
UCP2	Uncoupling protein 2
UD	Ultra-distal
VAT	Visceral adipose tissue
vBMD	Volumetric bone mineral density
WAZ	Weight-for-age z-score
WB	Whole body
WC	Waist circumference
WHO	World Health Organization
wk	Week (s)
y	Year (s)

CHAPTER 1

Background and Thesis Rationale

1.1 Background

Childhood obesity is a complex medical condition. Globally, it is estimated that over 41 million children under the age of 5 year (y) are overweight or obese [1], with a rising prevalence in low-income to middle-income countries [2]. In Canada, childhood obesity is a public health concern and a priority, and is currently identified as one of many mandates issued by the Public Health Agency of Canada (e.g., Program 1.2: Health promotion and disease prevention; 2017-2018). Results from the Canadian Health Measures Survey (cycle 3.0; 2012- 2013) classified 1 in 3 children 5-17 y of age as overweight or obese [3]. Most troubling, a 2016 systematic review predicted that children and adolescents with obesity are 5 times more likely to be obese in adulthood and that 80% of adolescents with obesity will become adults with obesity [4]. Without any doubt, this problem should be prevented and treated in its infancy.

The causes of childhood obesity are multifaceted. To pinpoint the exact source is nearly impossible as obesity stems from different etiologies (i.e., biological and behavioral) that vary among individuals. Environmental factors (i.e., intrauterine, and post-natal environments), genetics and ecological factors (i.e., family, school and community) all simultaneously affect a child's weight status [5]. Further, certain ethnicity and sociodemographic factors have been identified as risk factors for developing obesity during childhood [6].

The medical and psychological consequences associated with childhood obesity are potentially devastating [7] (**Figure 1.1**). Health consequences can start young; cardiovascular disease risk factors associated with metabolic syndrome have been seen in 5 y old children with obesity [8]. Further, childhood obesity increases the risk of morbidity in adulthood [9]: these children are at an increased odds of developing adult diabetes (odds ratio (OR)=1.70; 95% confidence interval (CI) [1.30, 2.22]), coronary heart disease (OR=1.20; 95% CI [1.10, 1.31]) and certain cancers [9]. From a psychological and emotional standpoint, they are often isolated, stereotyped as lazy, socially inept [7] and are more likely to be bullied [10]; thereby develop low self-image [11] and experience feelings of sadness and depression [12].

Conceptual models or frameworks illustrate the complexity of obesity. The *Ecological Model of Predictors of Childhood Overweight* (**Figure 1.2**) [5] is an example of a model that identifies the different intricacies that may impact a child's weight status. It also considers the modifiable risk factors that should form the basis of treatment programs, specifically dietary intake, physical activity (PA) and sedentary behaviors. Most importantly, these models highlight

the influence that parents and community have on a child's weight status. Therefore, it is not surprising that the literature supports the use of family-centered lifestyle interventions as a treatment option for children [13]. This method of treatment, the family-centered approach, was used in the study design of this dissertation and will be discussed in more detail in the subsequent chapters.

The model proposed in **Figure 1.2** is comprehensive; the study design presented in this thesis was based on many factors proposed in this model, and extends to explore in greater depth a "*child's characteristic*", specifically their eating behaviors. Eating behaviors are important to consider as they have a direct impact on weight [14]; children with obesity often respond differently to satiety cues, rate of meal consumption and sensitivity to environmental cues (i.e., emotional stress) [15-20].

In general, studies based on the family-centered lifestyle approach often exclusively consider changes in body mass index as a primary outcome and include different dietary and physical activity goals [13]. Recently, there has been an increased interest on the impact of milk and milk product consumption on the weight status in children. The findings in these studies are nonetheless mixed: one meta-analysis found that children who consume higher intakes of milk and milk products were 38% less likely to be classified as overweight or obese (OR=0.62; 95% CI [0.49, 0.80]) [21] whereas another meta-analyses that focused specifically on children in developed countries, suggested milk and milk products have a neutral effect on weight status during childhood [22].

Currently, Canadian recommendations for milk and milk products are 2 servings per day for children 4-8 y [23]. The role of milk and milk products in fostering healthy bone development in children has been established. In children with obesity, doubling the daily recommended servings of milk and milk products may be beneficial, as this pediatric population may be subject to potential bone microarchitectural alterations [24]. Specifically, research suggests that children with obesity generally have larger bones, greater bone mineral content (BMC) and bone mineral density (BMD) compared to normal weight peers [25]. However, children with obesity are at greater risk of developing musculoskeletal pain (risk ratio (RR)=1.26; 95% CI [1.09, 1.45]) and compared to normal weight children, are more likely to fracture (RR=2.41; 95% CI [1.42, 4.10]) [26]. Further, at the bone microarchitectural level, cross-sectional studies comparing children by body mass index, classified as normal versus

obese, have shown that fat mass is negatively associated with both cortical and trabecular volumetric BMD [27] and that alterations in cortical density and thickness exist [28]. This dissertation will explore the musculoskeletal health of children with obesity, an outcome that is often not included in interventions aimed at this pediatric population [29].

To date, there exist only a few studies that have examined the mechanisms as to how milk products are related to adiposity, and suggest that calcium and protein are key regulators in this process [21, 30]. Similarly, milk proteins have been shown to increase satiety and decrease hunger [31], therefore may play a role in favorably changing eating behaviors. It is not known if increasing the milk and milk product recommendation from 2 to 4 servings per day will have any added benefit on changes in adiposity, bone health or favorably changing eating behaviors in children with obesity who participate in a 1 year family-centered lifestyle intervention.

The effects of PA on adiposity and bone health are well established [32, 33], however, weight-bearing types of activities have an added benefit on improving BMC in pre-pubertal children [34]. Currently, Canadian recommendations for children 5-11 y are to engage in daily moderate-to-vigorous activities 60 minutes/day; weight-bearing types of activities are suggested 3 times/week (wk) [35]. Given the potentially altered bone microarchitecture in children with obesity, a study that examines the effects of increasing the recommended frequency of participating in weight-bearing types of activities from 3 times/wk to daily, is also of interest.

1.2 Thesis Rationale

The use of family-centered lifestyle interventions to treat childhood obesity is well established. However, these interventions should not be restricted to solely examining changes in adiposity, but also consider the impact of bone health and eating behaviors in children who participate in these programs. Given the importance of milk and milk products and weight-bearing types of activities on overall health, a study that examines the effects of increasing these two health recommendations above current guidelines is warranted, and could be of interest to those who work in the field of childhood obesity.

Study Objectives and Hypotheses

This study aimed to examine the effects of a 1 y family-centered lifestyle intervention on changes in: (1) adiposity; (2) bone health; and (3) eating behaviors in children with obesity. Specific objectives and hypotheses for each of these themes are described as follows:

Aim 1: Adiposity:* (*Manuscript 2)

To test the effects of a 1 y family-centered lifestyle intervention using Canada's dietary and physical activity guidelines to reduce body mass index-for-age and sex z-scores (BAZ) in overweight and obese children; and if increasing milk and milk products and weight-bearing types of activities above current recommendations will result in greater reductions in BAZ.

Hypothesis: Children randomized to increase milk and milk products and focus on daily weight-bearing PA will have a lower BAZ at 12 months compared to children randomized to meet current Canadian dietary and PA recommendations, and the control group.

Aim 2: Bone Health:* (*Manuscript 3)

To determine if children with obesity who participated in a 1 y family-centered lifestyle intervention resulted in favorable changes in bone outcomes and bone biomarkers; and if increasing milk and milk products and weight-bearing types of activities above current recommendations result in additional benefits on bone health.

Hypothesis: Children randomized to increase milk and milk products and engage in daily weight-bearing types of activities will increase BMC and BMD, and favorably change bone biomarkers at 12 months compared to children randomized to meet current Canadian dietary and PA recommendations, and the control group.

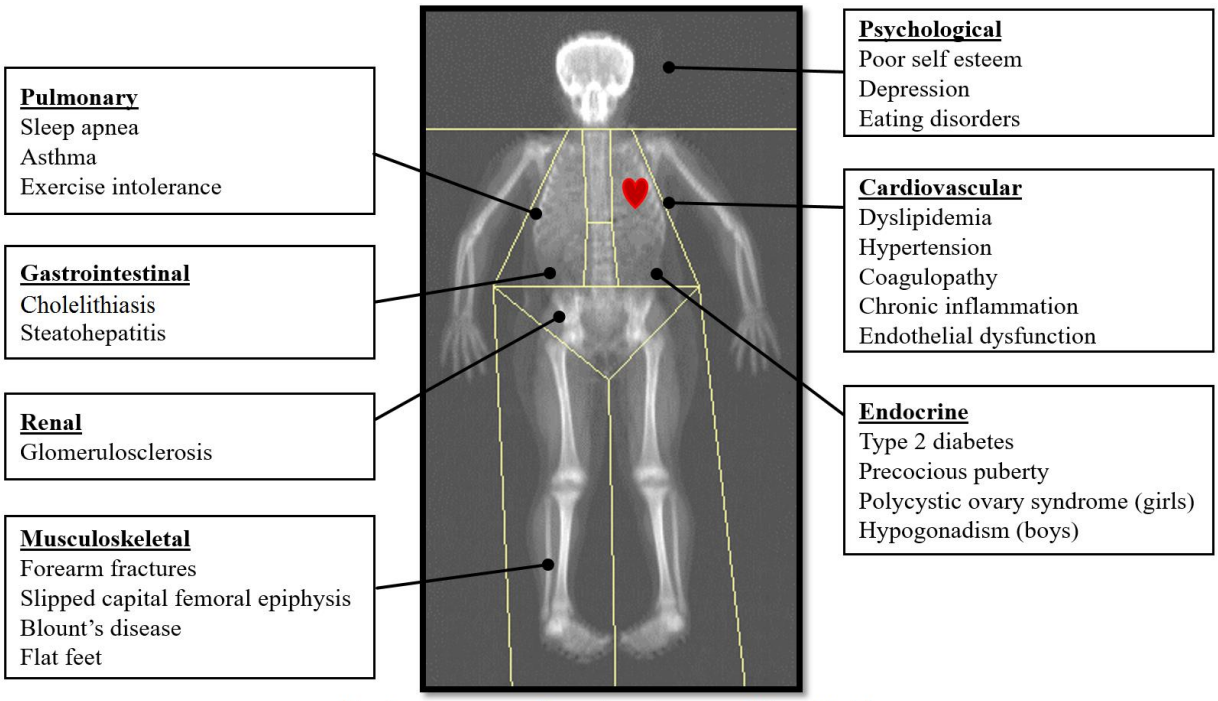
Aim 3: <u>Eating Behaviors</u>*: (*Manuscript 4)

To determine if children with obesity who participated in a 1 y family-centered lifestyle intervention aimed at reducing adiposity favorably change eating behaviors and plasma leptin concentrations; and if increasing milk and milk products and weight-bearing types of activities above current recommendations will result in favorable changes in eating behaviors and plasma leptin concentrations.

<u>Hypothesis:</u> Children randomized to increase milk and milk products and engage in daily weight-bearing types of activities will significantly decrease Food Approach type eating behaviors and will decrease plasma leptin concentrations at 12 months compared to children randomized to meet current Canadian dietary and PA recommendations, and the control group.

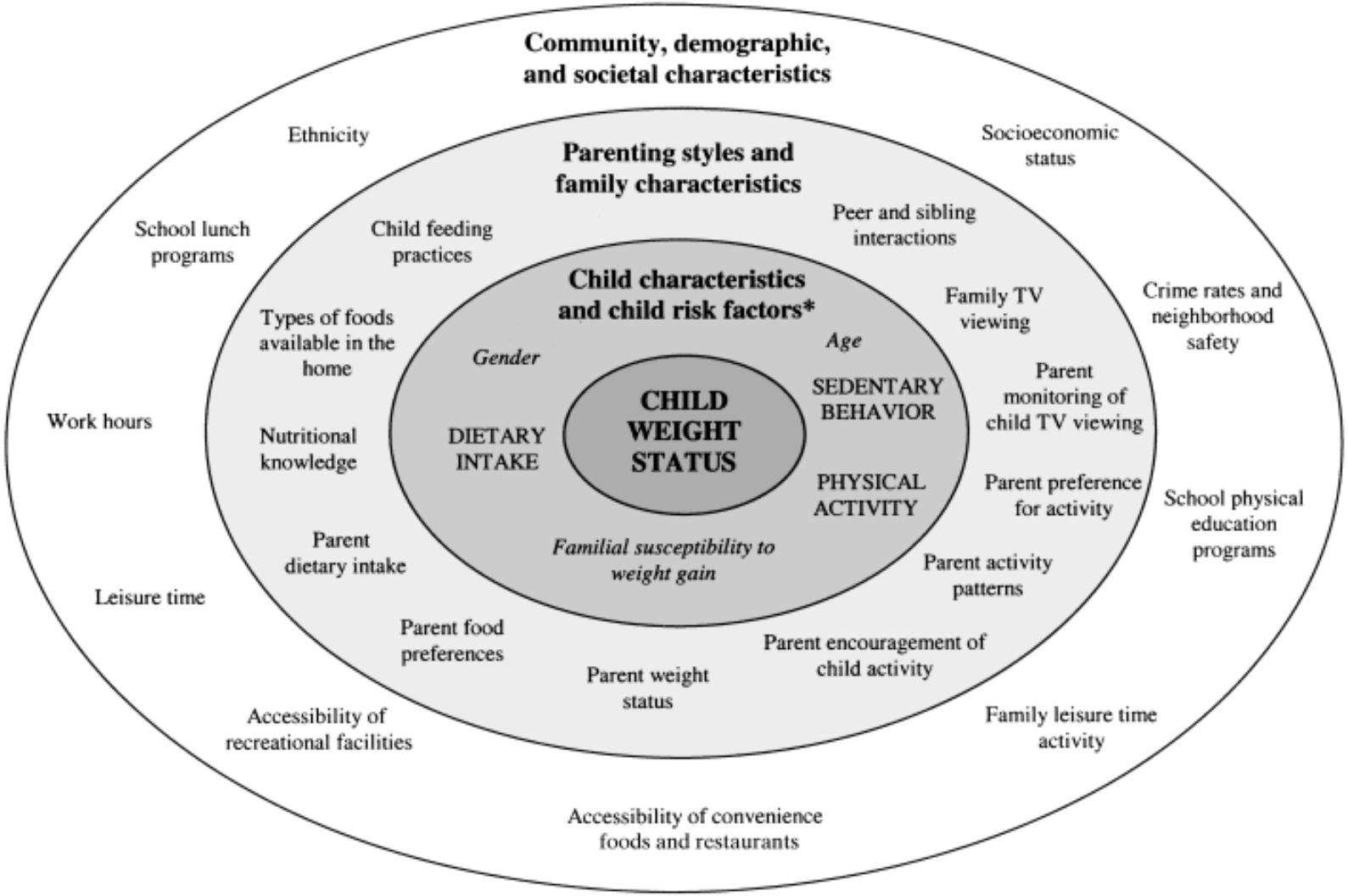
Before addressing these objectives, the following comprehensive review aims to discuss the consequences of childhood obesity, focusing on the musculoskeletal health of overweight and obese children. It will also explore the many causes of childhood obesity, focusing on pathological and environmental contributors; and finally, examine the impact of family-centered lifestyle interventions in treating childhood obesity. This review will also highlight the role of milk and milk products and weight-bearing types of activities as they relate to changes in adiposity, bone health and eating behaviors. Together, this review will further justify the rationale of the study, the study protocol and thus subsequent statistical analyses of the presented work.

Figure 1.1 Health consequences of childhood obesity [7]



Dual-energy x-ray absorptiometry scan of 8-y old girl
Image with permission

Figure 1.2 **Ecological model of predictors of childhood overweight** [5]
*Author permission to reprint: **Appendix 1**.



CHAPTER 2

Literature Review

2.1 Childhood Obesity in Canada

Over the past 25 years (y), the prevalence of childhood obesity has risen in Canada [36]. Data from the Canadian Community Health Survey (CCHS) (cycle 2.2; 2004) estimated that 18% of children 6-11 y were obese [37]. More recently, data from the Canadian Health Measures Survey (CHMS) (cycle 3.0; 2012-2013) suggested that 31%, or 1 in 3 children between the ages of 5 to 17 y, are overweight or obese [3]. Childhood obesity is therefore a public health concern in this country.

2.2 Measuring Growth and Obesity in Children

2.2.1 Defining Obesity

A child is defined as obese by measuring weight (kilograms, kg) and height (meters, m) to calculate a body mass index (BMI) (weight divided by height-squared, kg/m^2). Classification of a child's BMI can be described using different definitions (**Table 2.1**). Growth charts from the World Health Organization (WHO) [38], Centers for Disease Control (CDC) [39] and International Obesity Task Force (IOTF) [40] are examples of classification systems used to define a child's weight status in terms of a percentile (%tile) or z-score; they are age and sex-specific. BMI for-age and sex z-scores (BAZ) are often used in research settings to allow for comparisons of weight change when dealing with children of different ages. BAZ are defined as the BMI transformed into the number of standard deviations (SD) above or below the population mean for age and sex [41].

BMI fluctuates throughout life by age and sex. BMI curves decline after age 1 y, then continue to gradually decrease until a minimum 4-6 y; a gradual increase is seen after 4-6 y of age. This increase in BMI continues throughout the remainder of childhood into adolescence, when children experience a major growth spurt; weight continuously increases for the remainder of adolescence into adulthood [42]. The rebound, or the increase in BMI after its lowest point, is referred to as the "adiposity rebound", a normal occurrence in all children. However, should a child reach this rebound at an early age (before 4-6 y), he/she are at a greater risk of developing obesity in adulthood [43].

In Canada, national surveys and Health Canada define childhood obesity using the WHO BMI classification system [38], whereby a child exceeding 85%tile or 95%tile on a sex-specific

growth curve are considered overweight or obese, respectively (**Table 2.1**). The data presented in this dissertation will follow suit.

Table 2.1 Defining obesity during childhood

Organization	Reference Population	Specifics	Definition of Childhood Obesity
World Health Organization *(WHO)*[38]	National Health and Nutrition Examination Survey I collected from 1971–1974	5-19 y; sex-specific	**Overweight:** BMI >1 SD above the WHO growth standard median; or >85%tile **Obese:** BMI >2 SD above the WHO growth standard median; or >95%tile
U.S. Centers for Disease Control and Prevention *(CDC)*[39]	Five national health examination surveys from the United States (1963-1994) plus five supplement sources	2-19 y; sex-specific	**Overweight:** BMI >85%tile and <95%tile **Obese:** BMI >95%tile
International Obesity Task Force *(IOTF)*[40]	Pooled sample from the United Kingdom, United States, the Netherlands, Singapore, Hong Kong and Brazil using *n*=97,876 males and *n*=94,851 females	2-18 y; sex-specific	BMI cut-off in children corresponds to adult definitions: **Overweight:** BMI ≥25 and <30 **Obese:** BMI ≥30

2.2.2 Measuring Body Composition

Both fat mass and lean mass have important implications in the overall health of children. There are numerous techniques to assess body composition that are suitable and appropriate for use in children with obesity, all presenting with strengths and limitations. A brief review of those described in this dissertation are summarized as follows:

- *Body mass index (BMI)*

Body mass index is a global index of nutritional status that categorizes a person's weight status. However, BMI is not always related to body composition *per se*. Despite BMI correlating with fat mass, it is unable to differentiate between fat mass and lean mass [44]. When measuring weight to calculate a BMI, the entire body mass is considered (i.e., skeletal (bone and muscle) mass, organ mass, adipose tissue mass) and may fluctuate depending on hydration status [45]. Therefore, BMI has a low sensitivity and may underestimate obesity prevalence (false negative) [45]. For these reasons, BMI is best used in population surveillance studies and as a screening tool on an individual basis in conjunction with other assessment methods [42].

- *Waist circumference (WC)*

Obtaining a child's waist circumference can be done to assess abdominal adiposity and potential metabolic and cardiovascular disease risk [41]; WC is measured with a standard measuring tape to the nearest 0.1 cm at the umbilicus. In children, WC positively correlates with visceral adipose tissue (VAT) [46]. In Canada, reference data from the 1981 Canada Fitness Survey has been used to create WC references (%tile) for youth 11-18 y; WC increases with age in both boys and girls, however, boys typically have higher WC at every age and %tile compared to girls [47]. More recently, WC curves have been derived using data from the CHMS (cycle 1.0: 2007-2009; cycle 2.0: 2009-2011) for children 6-19 y whereby WC cutoffs are defined for boys (94%tile) and girls (86%tile) [48].

In addition to WC, the use of waist-to-height ratio can be used to measure a child's risk of developing central adiposity [49]; there is no reference data for waist-to-height measures, however, children's waist circumferences should be kept below half their height [50]. In general, WC is a quick and simple measure of abdominal fat, but is limited in its inability to measure visceral adipose tissue.

- *Bioelectrical impedance analysis (BIA)*

BIA is an instrument that uses a small electrical current to measure different body components; it can be hand-to-foot or foot-to-foot. BIA is a practical and portable method of assessing body fat and lean mass in clinical or community settings. However, it is less accurate compared to other laboratory techniques, such as dual-energy x-ray absorptiometry. Body fat percentile charts have been created using BIA technology for children and youth [46]. Despite its low accuracy, BIA is of value to use during interventions with children with obesity as it may provide an indication of the direction of change in body fat, or lean mass, however, its use in quantifying these changes is cautioned [51].

- *Dual-energy x-ray absorptiometry (DXA)*

DXA is considered a quick and acceptable method of assessing body composition (i.e., fat mass, lean mass) and bone (i.e., mineral content (BMC), mineral density (BMD) and area) in a 2-dimensional manner [51]. DXA uses two different energy levels, specifically ionizing radiation in form of x-rays that are expressed in sivert (Sv); microsiverts (μSv) are one millionth of a sivert [52]. The radiation from a DXA scan for an effective whole body (WB) scan is approximately 4.8 μSv in a 10-y old child, much less than the average radiation exposure due to all natural sources (~2,400 μSv/y) [52].

DXA is based on two energy peaks: one peak (lower energy) is absorbed by soft tissue while the other by bone (higher energy x-ray); what is not absorbed is detected by the instrument and is converted to tissue composition using an algorithm [53]. The use of DXA in children is supported by the International Society for Clinical Densitometry; z-scores and %tiles for children are based on reference data from National Health and Nutrition Examination Survey (NHANES; 1999–2004) [54]. DXA is considered accurate, however the machines are relative inaccessibility, expensive, and although minimal, the technology is based on radiation exposure. Further, accuracy may be limited if positioning is not consistent and uniform, similarly if artifacts are not accounted for before assessment. Further considerations with the use of DXA in children with obesity are discussed in the final chapter of this thesis.

- *Peripheral quantitative computed tomography (pQCT)*

pQCT is used to assess bone in a 3-dimensional manner to quantify BMD as mg/cm^3 (volumetric BMD [vBMD]). Unlike DXA, pQCT can measure cortical and trabecular bone compartments separately, in addition to providing information about bone strength; pQCT measures are often limited to measures of the radius and tibia [55]. Standard outcome measurements include vBMD and cortical and trabecular dimensions whereas derived measures include cortical thickness, muscle and fat indices and bone strength indices. Depending on the model and device, radiation exposure is minimal (i.e., the Stratec 2000 XCT (<1.0 μSv) or the XtremeCT (3.0 μSv)) [55]. pQCT limitations are that it is an expensive, not easily accessible and requires a trained technician to operate.

In general, it is important that a child's weight status be routinely monitored. As previously mentioned, there are different classification systems available to define a child's BMI and different methods that can be used to track changes in body composition over time, all with their own strengths and limitations. Epidemiological studies suggest that certain children are at greater risk of developing obesity, thereby monitoring children's weight status as they grow in these at-risk populations becomes paramount. A brief overview of the etiology of obesity and the potential risk factors in developing childhood obesity is therefore necessary.

2.3 Etiology, Determinants and Risk Factors

The causes of childhood obesity are complex. Many factors, including pathology and environment, simultaneously effect an individual's make-up that may contribute to developing obesity [56]. Pathological factors include genetics and hormones whereas environmental factors include lifestyle behaviors, which itself can be influenced by other circumstances (i.e., family characteristics, socio-demographics, family income, place of residence) [57]. In addition, certain risk factors have been identified, placing some children at greater risk of developing obesity over others. The etiology of obesity is comprehensive; general concepts will be discussed here as they pertain to the dissertation.

2.3.1 Pathological Factors

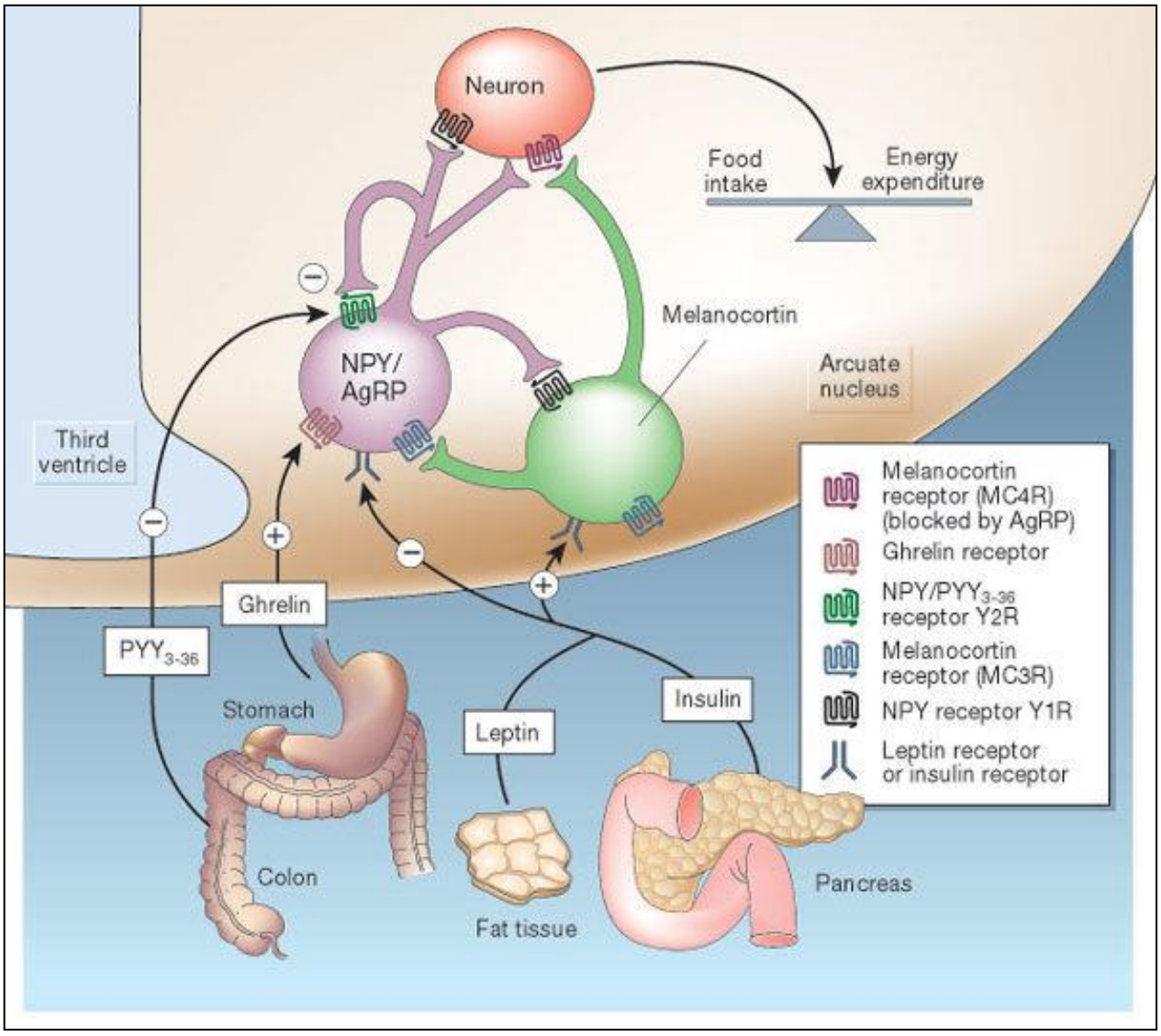
The genetic contribution to obesity is well established. It is estimated that 60-80% of the variance in human body weight can be accounted for by genetic factors [58]. Common obesity is polygenic and involves gene-to-gene and gene-environment interactions [58]. As stated by Bray (2004): "*(for obesity)* genetic background loads the gun, but the environment pulls the trigger" [59]. Currently, research has identified over 300 genetic loci that are involved in human body weight regulation [42]. Significant progress is being made in the research area of epigenetics, which will eventually provide greater insight to individual risks of developing obesity [60].

2.3.2 Energy Regulation

Energy balance can be described in simple terms of energy intake, energy spent and energy storage [61]. However, when describing this type of equation in relation to children, energy intakes should also account for the additional energy costs associated with growth; a negative energy balance in children may have a direct effect on development and growth which should be avoided.

The central nervous system influences energy balance and body weight by its effects on: (1) behavior, including dietary intake and physical activity (PA); (2) the autonomic nervous system which regulates energy expenditure and metabolism; and (3) the neuroendocrine system [62]. Energy balance is induced or suppressed through physiological signals. Specifically, the hypothalamus and brain stem, gastrointestinal system, pancreas and adipose tissue are all involved in eating regulation through different peripheral peptides (**Figure 2.1**) [63]. These systems are complex and are outside the scope of this dissertation: the following will provide a simplified review of these processes with a primary focus on the role of leptin.

Figure 2.1 **Hormones that control eating** [63]
(Nature Publishing Group license agreement with T. Cohen: #4104320392954).



Legend: Leptin and insulin decrease appetite by inhibiting neurons that produce the molecules neuropeptide Y (NPY) and agouti-related peptide (AgRP), while stimulating melanocortin-producing neurons in the arcuate-nucleus region of the hypothalamus, near the third ventricle of the brain. NPY and AgRP stimulate eating, and melanocortins inhibit eating, via other neurons. Activation of NPY/AgRP-expressing neurons inhibits melanocortin-producing neurons. The gastric hormone ghrelin stimulates appetite by activating the NPY/AgRP-expressing neurons.

2.3.2.1 Short-Term Energy Regulation

Hunger

Gut-brain interactions initiate eating, but learned behaviors and habits also play important roles in energy intake [64]. When there is a need for nutrients, signals from receptors in the gut and circulatory systems initiate eating [65]. However, when there is a mismatch between actual energy needs and pleasure from eating, the homeostasis of energy balance is disrupted [64].

The hypothalamus, specifically the arcuate nucleus, acts as the control center for hunger, and allows passage of certain peripheral peptides and proteins through the blood brain barrier to interact directly with its neurons [66]. These peptides include neuropeptide Y (NPY) and agouti-related peptide (AgRP), which promote energy intake. In the hypothalamus, NPY is the most abundant peptide that stimulates feeding through the NPY G-protein coupled receptor (Y1 and Y5); it is regulated by peripheral peptides, such as ghrelin, to stimulate appetite. At the same time, the rise in NPY decreases the actions of Pro-opiomelanocortin (POMC), a hormone also found in the arcuate nucleus responsible for inhibiting feeding. Similarly, AgRP, also produced by neurons in the medial arcuate nucleus, acts by blocking receptors (melanocortin-3 (MC3R) and melanocortin-4 (MC4R) receptors) in the hypothalamus to stimulate feedings [66].

In addition to NPY and AgRP, circulating peripheral peptides also play a role in appetite stimulation. For instance, when energy is needed, ghrelin, a fast-acting hormone, is released from the stomach, travels to the hypothalamic arcuate nucleus to signal the release of NPY/AgRP, stimulating appetite [67]. Ghrelin is made up of 28 amino acids with a fatty chain modification (octanoyl group) on the third amino acid [68]; it is produced by the endocrine cells of the gastric mucosa of the fundus; smaller amounts are found in the small intestine, pituitary gland, hypothalamus, pancreas, lungs, immune cells, placenta, ovary, testis and kidneys [66]. Ghrelin levels increase before eating and fall after eating, hence its primary role in stimulating appetite [66]. In children with obesity, ghrelin concentrations are often lower [67], thereby are negatively correlated with BMI [69].

Satiation and Satiety

Satiation is the process that leads to termination of a meal *after* it has started [70]. It is characterized by the absence of hunger and the feeling of fullness (distension in the stomach) and is directly related to meal size [70]. There are many factors involved in satiation, including

increasing concentrations of peripheral peptides cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) and decreasing concentrations of ghrelin, all which act on different areas of the hypothalamus [70].

Satiety is the process of stopping to eat, and is characterized as a decline in hunger and an increased feeling of fullness [70]. Satiety is also regulated by the hypothalamus, predominantly by producing anorexigenic peptides such as POMC and cocaine- and amphetamine-regulated transcript (CART). Peripheral peptides also play a role in satiety; they originate from different locations and acts on different sites of the brain (**Figure 2.1**). These include glucose, peptide YY, pancreatic peptide, incretins (i.e., oxyntomodulin), glucose-dependent insulinotropic polypeptide, insulin, and adiponectin [66]. In addition, short-term regulation of food intake can be mediated by the leptin secreted from gastric mucosa [71]. Specifically, leptin is secreted by endocrine and exocrine cells from the gastric mucosa, from which it is directed towards blood circulation or gastric secretions to act on different targets to control feeding [71].

Role of Nutrients on Satiation and Satiety: A Case for Milk and Milk Products

Satiety can be regulated by the consumption of food or beverages, however the total energy content, or macronutrient content of the food or beverage, result in different intensities or duration of satiety [72]. In general, foods with higher protein content, compared to carbohydrates and fats, are more satiating and have greater effects on delaying the onset of hunger [73].

Milk products, including cheese and yogurt, are nutritious foods; fluid milk supplies an average of 32 grams (g) of protein per liter [74]. On average by weight, bovine milk is composed of 87% water, 4% to 5% lactose, 3% protein, 3% to 4% fat, 0.8% minerals, and 0.1% vitamins [75]. The proteins found in milk are considered high-quality and are predominantly made up of whey protein (soluble; ~20% protein fraction) and casein (insoluble; ~80% protein fraction) [74]. In Canada, milk and milk product recommendations are 2 servings/day (d) for children (2-8 y) and 3 servings/d for adolescents (9-18 y) (**Table 2.2**) [23]. Milk products contain important macronutrients and micronutrients that support children's growth and development [74].

Compared to other beverages (i.e., juice), milk products have been shown to reduce food intake and appetite in children [76, 77]. In a three-way crossover randomized controlled trial (RCT) of 34 obese boys (10-12 y), children consumed a fixed breakfast with low-fat milk, apple

juice or water for 2 consecutive days, and were asked to rate appetite using a visual analogue scale 1 h post-breakfast [77]. Overall, boys reported higher satiety scores after drinking milk compared to water and apple juice ($p<0.05$). However, these results are limited as the test beverages were not offered blinded, therefore any prior knowledge related to milk or apple juice could have affected their responses. Further, this was a relatively small study that did not include female participants.

Findings from another small study (n=32; 9-14 y) found differences in milk product types on appetite scores in normal (n=16) and overweight (n=16) children [76]. This study compared the effects of water to isocaloric (130 kilocalorie (kcal)/ 250 milliliter (mL)) beverages of 2% milk fat (MF) milk, 1%MF chocolate milk, 1.5%MF yogurt drink and fruit punch consumed 60 minutes before and during a pizza meal on satiety and food intake. Appetite was rated by questionnaire using a visual analogue scale; food intake was assessed by weighing food. Results showed that the chocolate milk and yogurt drink significantly reduced mean pizza intake by 14% ($p<0.001$) and 10% ($p=0.01$), respectively, compared to water but not compared to milk or fruit punch; whereas fruit punch increased energy intake (12%, $p=0.03$) compared to water. Overall, both pre- and post-meal subjective appetite scores were affected by treatment, with a greater increase in appetite scores reported after milk. However, when differentiating between weight-status, the effects of milk on satiety were found only in the normal weight children ($p<0.02$), suggesting that other factors, such as eating behaviors, may play a role in energy intake in the overweight children.

Despite these interesting findings, this study is limited by its study design whereby the satiating effects of cheese from the pizza (pepperoni or 3-cheese deep dish pizza) were not considered as they may independently effect energy intake. Nonetheless, this study suggests that the type of milk product consumed during the meal may induce a different response in appetite [76]. Specifically, yogurt and chocolate milk, but not milk, reduced pizza intake compared to water. Although these beverages have comparable quantities of protein (i.e., milk (8 g/250 mL); chocolate milk (7.8 g/250 mL); yogurt (5.7 g/250 mL)), both chocolate milk and yogurt contained sucrose which in addition to milk proteins, may collectively have a greater satiating effect [76].

Although these studies have their respective limitations, they suggest that certain components of milk products, most notably milk proteins, affect appetite regulation. Milk

proteins are made up of two major types: casein and whey, both of which are considered complete proteins containing all essential amino acids, however, differ in their rate of amino acid appearance in circulation [31]. Whey proteins have been found to induce a fast, high, and transient increase in plasma amino acids whereas casein coagulates in the stomach thereby delaying gastric emptying resulting in a slow increase in plasma amino acids [78].

In addition, milk proteins may affect glycemic control and stimulate the secretion of insulin [31]. Other studies propose that the satiating effects of milk products are due to bioactive compounds particularly found in whey protein (i.e., glycomacropeptide) that may potentially affect CCK thereby playing a role in satiety [31]. Finally, lactose may increase satiety by stimulating gut peptides such as CCK and GLP-1 [79]. Therefore, the unique composition of milk products, as described, may play a role in short-term energy regulation.

2.3.2.2 Long-Term Energy Regulation

In general, obesity can be characterized by an increase in white adipose tissue, a specialized loose connective tissue heavily loaded with adipocytes [80]. Adipose tissue is a well-organized vascular system that is connected to blood vessels and nerves that regulate the body's metabolism. Pre-adipocytes, the precursor cells to adipocytes, are found in the stromal vascular fraction and when obesity is present, macrophages are often infiltrated in adipose tissue [80].

In humans, adipose tissue depots are found beneath the skin (subcutaneous fat), surrounding organs (visceral fat), in bone marrow (yellow bone marrow), and in skeletal muscle (intermuscular). The expansion of adipose tissue is due to the combined hypertrophy of pre-existing adipocytes and de novo adipocyte differentiation [80]. In addition to acting as an energy reservoir, or having a passive role of insulating the body, adipocytes have endocrine functions, as they govern the release of many different proteins or hormones that affect energy balance, such as leptin [80].

Leptin

Leptin, also known as the OB protein, is a 16- kDa polypeptide derived from adipose tissue responsible for body weight regulation; in other words, leptin induces weight loss by suppressing energy intake and stimulating energy expenditure [67]. In addition to adipose tissue, this hormone is also synthesized in the stomach, heart, mammary epithelium, ovarian follicles,

placenta, bone, cartilage and the brain [66, 67]. The *ob* gene is expressed by all sites of adipose tissue, but predominantly in subcutaneous adipose tissue [66].

Leptin acts as a regulatory signal to the central nervous system in relation to stored adipose tissue [80]. Leptin receptors are located on many different sites of the brain including the blood brain barrier; they are important for the transport of leptin into the arcuate nucleus through receptor-mediated endocytosis. Once bound to the receptor, it stimulates a complex signaling cascade. In brief, NYP and AgRP are inhibited while simultaneously, POMC and CART are stimulated, ultimately decreasing appetite and increasing energy expenditure [66, 67]. Pathogenic mutations of the leptin-receptor gene are rare, estimated to be found in <3% of individuals with severe early onset obesity [81].

Leptin secretions are driven by circadian patterns; basal levels are found between 08:00 and 12:00 hours (h), then rise progressively to peak between 24:00 h and 04:00 h and steadily decline by 12:00 h [82]. The expression of leptin may be mediated by diet composition. However, studies that have explored the impact of adjusting macronutrient compositions of diets on changes in leptin concentrations yield different findings. For instance, the effects of a high-fat versus high-carbohydrate diet on leptin concentrations were examined in adults over a 1 y period (n=191; 60-70 y) [83]. Specifically, this study tested the effects of two types of Mediterranean diets on changes in plasma leptin, among other biochemical outcomes. Participants were randomized to 1 of 3 groups: (1) virgin olive oil (participants were provided with 1-liter of oil per week but were advised to consume <4 tablespoon/d); (2) 30 g/d of mixed nuts (15 g walnuts, 7.5 g hazelnuts, 7.5 g almonds); (3) the control group, who were advised to follow a low-fat diet. By 12 months, both Mediterranean groups had significantly reduced body weight (kg) compared to baseline. Specifically, the change from baseline to 12 months in body weight was -0.81±2.2 kg ($p<0.05$) in the olive oil diet group, -0.71±2.41 kg ($p<0.05$) in the nut diet group and -0.29±2.71 kg ($p=0.35$) in control. However, these changes from baseline to 12 months in body weight were not were not significantly different among groups ($p=0.45$). Changes in waist circumferences were also examined: women from all three groups significantly reduced waist circumferences from baseline to 12 months, whereas only men from the olive oil diet group significantly reduced waist circumference at 12 months. Despite these general changes in body composition, plasma leptin concentrations did not significantly change among groups from baseline to 12 months.

Conversely, results from another study suggest that changes in body composition affect leptin concentrations. A 12-week (wk) RCT in overweight men (n=31; 40-70 y) tested the effects of whole eggs (640 milligram (mg) cholesterol/d) versus egg substitute (0 mg cholesterol/d) following a carbohydrate-reduced diet (10-15% of total energy from carbohydrate) on plasma leptin concentrations [84]. By 12-wk, both groups significantly reduced both body weight (i.e., egg group lost 6.7 kg; placebo lost 5.9 kg, both compared to baseline; $p<0.001$) and significantly reduced plasma leptin concentrations (i.e., egg group decreased leptin by 9.7±5.0 microgram (µg)/liter (L); placebo decreased leptin by 3.6±5.4 µg/L, both compared to baseline; $p<0.01$). Changes in plasma leptin from baseline to 12-wk were positively correlated with reductions in body weight ($p<0.01$).

Similar results were seen in a trial that tested the effects of increasing protein (15% versus 30% of total energy from protein) while maintaining the percentage of carbohydrate on changes in plasma leptin in 19 adults [85]. Specifically, participants were placed on a weight-maintenance diet (~1997±2 kcal/d; 15% protein, 35% fat, and 50% carbohydrate of total energy) for 2-wk, followed a diet with similar total energy but different percentage protein (~2001±5 kcal/d; 30% protein, 20% fat, and 50% carbohydrate of total energy) for 2-wk, then an *ad libitum* diet (~2000±3 kcal/d; 30% protein, 20% fat, and 50% carbohydrate of total energy) for 12-wk. By 12-wk, participants lost an average 4.9±0.5 kg; plasma leptin concentrations significantly decreased by end of study ($p<0.05$).

In general, these studies suggest that the changes in leptin concentrations may be mediated by changes in adiposity, and not directly related to diet composition. Few studies report on the effects of changing diet composition on leptin concentrations in children. The effects of low-carbohydrate (50% carbohydrate, 30% fat and 20% protein of total energy) and low-fat (60% carbohydrate, 25% fat and 15% protein of total energy) on serum leptin and eating behaviors were assessed in overweight and obese children (n=120; 6-12 y; mean BMI: 28.1±3.6 kg/m^2) over a 2-mo period in Iran [86]. By end of trial, both groups significantly reduced BMI ($p<0.001$) and serum leptin concentrations ($p<0.001$); however, the change in serum leptin did not significantly differ between groups at 2-month (mo) ($p=0.71$), suggesting that the decreases in BMI and not the different macronutrient distributions of the diets, mediated the changes in leptin concentrations. However, a limitation of this trial was the study's assessment of dietary

intake, whereby compliance to dietary protocol was assessed by questionnaire and not by other subjective assessments such as food diaries.

Nonetheless, irrespective of the macronutrient composition of diet, when used as a biomarker for satiety, a large body of literature reports that leptin concentrations are more suggestive of long-term appetite and not short-term appetite control (i.e., between or immediately after energy intake) [67]. Most importantly, as shown with the studies cited above, leptin concentrations are positively associated with adiposity. In children with obesity, leptin concentrations are often elevated compared to normal weight peers and decrease with reductions in adiposity [87].

Despite leptin's well documented role in energy balance, few trials have included this biomarker as it relates to eating behaviors. Leptin concentration are typically reduced in patients with anorexia nervosa due to reduced body fat stores [88], however, they are also lower in normal weight individual with bulimia nervosa [89], suggesting other factors other than body fat may modulate leptin concentrations. Binge eating disorder is not typically observed during childhood, however, loss of control of eating is often reported in youth [90]. In a cross-sectional study of lean and obese children (n=506, 7-18 y), serum leptin concentrations were higher among children with more frequently reported loss of control eating episodes ($p<0.001$), even after adjusting for adiposity and other covariates [91].

Eating behaviors are typically assessed by questionnaire. To date, the relationship between leptin concentrations and eating behaviors assessed by questionnaire in children with obesity has been reported in only one trial. This Iranian study involved overweight and obese children (n=120), who participated in a 2-mo intervention that tested the effects of a low-fat versus low-carbohydrate diet on changes in body composition, eating behaviors and leptin [86]. Eating behaviors were assessed using the Children's Eating Behavior Questionnaire [92]. After 2-mo, both diets resulted in lower Food Responsiveness ($p<0.001$) and Enjoyment of Food ($p<0.001$) scores while Emotional Overeating scores decreased only in the low-carbohydrate group ($p<0.05$). As mentioned, to date this is the only trial that reports on changes in eating behaviors using a questionnaire and includes an analysis of leptin. However, this study did not test for associations between changes in eating behavior scores and leptin, results that could contribute to our understanding of the role of leptin in long-term energy regulation as it relates to eating behaviors in children with obesity.

2.3.3 Environmental Factors

2.3.3.1 Intrauterine and Postnatal Influences

The "developmental origins of health and disease" hypothesis suggests that a stimulus to an organism during a critical period of development can influence gene expression via epigenetic modifications [93]. Specifically related to childhood obesity, being born small or large for gestational age may increase the risk of developing obesity during childhood [94]. Further, irrespective of birth size, children are at an increased risk of developing obesity if they are born to mothers who smoke during pregnancy [95] or who are themselves obese [94].

Postnatal factors related to childhood obesity include breastfeeding, which has been associated with a reduced risk of developing childhood obesity [94]. However, its effects may not be protective as childhood progresses [96]. Conversely, the associations with early adiposity rebound and childhood obesity are established [43].

2.3.3.2 Sociodemographic Factors

In general, cross-sectional studies have shown certain ethnic groups are at greater risk of developing obesity (i.e., Aboriginal, Hispanic and South Asian) [97], and that children born in low- and middle- income countries with greater food security are now at greater risk of overnutrition [98]. Conversely, in high-income countries, children living in the lowest socioeconomic classes have higher rates of obesity compared to those in affluent families [99].

In Canada, CHMS data (cycle 3.0; 2012-2013) suggest childhood obesity rates differ by age, sex and geographic location [3]. Specifically, the prevalence of overweight and obesity among children is higher in 12-17 y old adolescents (36.8%; 95% confidence interval (CI) [30.0, 43.5]) compared to 6-11 y old children (25.8%; 95% CI [20.5, 31.1]). Sex differences also exist, whereby boys have a higher prevalence of overweight and obesity (33.5%; 95% CI [28.7, 37.9]) compared to girls (28.9%; 95% CI [21.8, 35.9]). Geographic variations also exist: the highest rates of childhood obesity are seen in the Atlantic Provinces, where the prevalence among children 12-17 y old youth is between 45.4-65.4%; both Quebec and Alberta have the lowest prevalence rates of 0.1-26.8% [3]. Finally, ethnic and cultural differences exist among childhood obesity rates and are highest among Canadian Aboriginal children. Data from the 2006 Aboriginal Peoples Survey estimates that 18.5% of Métis boys and 14.4% of girls are classified as obese [100].

In addition to the conventional sociodemographic and economic factors, the concept of a child's "built environment" has emerged as an important determinant of childhood obesity [101]. Specifically, access to parklands or open space, street connectivity, housing density and recreational facilities have been suggested to increase PA and therefore lower rates of obesity [102]. Conversely, living close to convenient stores and fast food restaurants are associated with higher rates of obesity [101]. However, when classifying location of residence as urban or rural, a recent meta-analysis of children (2-19 y; pooled 10 studies) from the United States found that rural children have a 26% increased odds ratio of being classified as obesity compared to urban children (odds ratio (OR)= 1.26; 95% CI [1.21, 1.32]) [103]. Therefore, despite living in an environment that provides a greater opportunity for active transport or more open space for playtime, there are other environmental features, such as diet quality and time engaged in sedentary behaviors, that may have a greater impact on weight status during childhood.

2.3.3.3 Lifestyle Behaviors

Physical Activity and Sedentary Behaviors

Both dietary intake and physical activity play a role in developing obesity during childhood. Simply put, children who spend less time engaging in moderate-to-vigorous activity are at a higher risk of becoming obese [104]. In Canada, children 5-11 y are recommended to engage in daily PA for 60 minutes (min) at a moderate-to-vigorous intensity while weight-bearing types of activities are encouraged 3-times/wk [35]. Sedentary behavior (i.e., television (TV) viewing, computer screen time) should be restricted to <2 h/d [105]. However, Canadian children 5-11 y old are not meeting these guidelines: CHMS (cycle 3.0; 2012-2013) results estimate that only 9% and 24% are meeting PA and sedentary behavior guidelines, respectively [3].

The literature supports the notion that exercise reduces BMI in children [33, 106]. A meta-analysis examining these effects reported that overweight and obese children (2-18 y) who participated in exercise (mean: 43 min, 4-times/wk for 16-wks) significantly reduced BMI by ~3% (mean change (Δ) BMI: -0.06; 95% CI [-0.09, -0.03]) [33]. Another meta-analysis aimed to assess which types of activities resulted in greater reductions in BMI, found that the combination of aerobic and resistance training activities yield greater reductions in BMI (mean ΔBMI: -0.11;

95% CI [-0.19, -0.03]) versus resistance training alone (mean ΔBMI: 0.04; 95% CI [-0.07, 0.15]) [106].

Although children are being encouraged to be active daily, compliance and adherence to programs, particularly those that include a resistance training component, are often poor [107]. It is important that studies consider their target population as the physical and physiological demands may be too overwhelming. Moreover, PA levels among children are recognized as multi-dimensional: demographic, psychologic factors, physical skill, social and cultural influences as well as physical environment may all influence a child's activity level [108]. In a review that aimed to examine predictors of PA among children (4-12 y), positive associations were found between PA and boys (4-12 y), boys who had active parents or parents who supported PA practices [109]; however, there was insufficient data to assess the associations between PA and BMI. These results are transferable to a younger age, whereby in preschool-aged children, BMI does not associate with levels of PA however boys were found to be more active than girls [110]. It is therefore important that studies that include promotion of PA in children understand that sex-differences may exist in terms of correlates of activity, but moreover, that it is important to consider a child's preferences for types of activities so that PA practices are maintained and enjoyed [111].

Sedentary behaviors are considered a separate entity to physical activity. Sedentary behaviors decrease total energy expenditure and are often associated with higher consumption of unhealthy foods [112]. However, research does not suggest that sedentary behaviors *replace* physical activities, *per se*, as they both have different effects on overall health outcomes. This is best demonstrated from results of a large European cross-sectional study (n=1,921; 9-10 y and 15-16 y), that examined the associations between TV viewing (sedentary behavior), PA and metabolic-risk factors [113]. This study found a positive association between TV viewing and adiposity (p=0.02); adiposity was mediating the positive associations between metabolic-risk and TV viewing. However, PA was inversely associated with metabolic-risk indicators, independent of obesity. As mentioned, PA and sedentary behaviors should be considered as separate entities in statistical analyses, as shown in the results of this study, as they mediate different associations to metabolic-risk factors in children [113].

The research examining the effects of interventions aimed at reducing screen time on changes in BMI are mixed: some report no effect on BMI when screen time is reduced [114],

whereas others suggest reduction in screen time (~4.63 h/wk) significantly reduced BMI by -0.15 kg/m^2 [115]. In most studies, the message to decrease sedentary behaviors are made in conjunction with other lifestyle behavioral changes, therefore it is difficult to draw conclusions as to whether sedentary behaviors influence weight changes in children alone [116].

Dietary Intakes

Positive energy balance contributes to the deposition of excess adipose tissue; it has been estimated that as little as 100 kcal/d above recommended energy needs contribute to obesity during childhood [117]. When assessing dietary risk factors that contribute to childhood obesity, an individual's overall food consumption, eating patterns, socio-economic status and culture should all be considered [118].

National dietary data (CCHS cycle 2.2; 2004) suggest that Canadian children are consuming diets within acceptable ranges for macronutrients [37], however total energy intakes may exceed recommendations. Further, when categorizing foods by Canada's Food Guide to Healthy Eating (CFG), children's diets are not balanced [119]. Specifically, only 41% of children (2-17 y) are meeting the recommended daily servings for fruit and vegetables; whereas one-third of children aged 4-9 y are not meeting the minimum daily servings of milk and milk products. Further, generally children are consuming too few or too many grain products and consumed a high quantity of "other" foods, whereby "other foods" (i.e., high-fat and/or high-salt foods such as chips or cakes) were providing the second highest percentage of daily energy intake (22%) after grain products (31%) [119]. These findings are troublesome as are the findings from a Canadian study that found that consuming ≥5 servings of grain products per day at 4 y of age, specifically in boys, significantly increase the odds of being overweight at 6 y of age (adjusted OR=3.20; 95% CI [1.72, 5.97]) [120].

Similarly, dietary intakes that are characterized as energy-dense, high-fat, and low-fiber are associated with obesity during childhood [121]. A longitudinal study from the United Kingdom (n=6,772) examined the relationships between dietary pattern and body composition in 7-15 y old children [122]. Dietary intake was assessed by 3-day food diary at ages 7, 10 and 13 y. At each age, dietary intakes from food diaries were categorized by energy density (percentage of total energy from fat and fiber) and assessed for patterns (i.e., energy-dense, high-fat, low-fiber) which were identified using a reduced rank regression; each food diary was assigned a

dietary pattern score, whereby depending on the degree to which their reported dietary intakes reflected the dietary pattern, a z-score was calculated. Fat mass to calculate fat mass index (FMI) was measured by DXA at follow-up visits when children were 11, 13 and 15 y. For each standard deviation increase in the dietary pattern z-score, the odds of being in the highest FMI increased by 13% (95% CI [1, 27%]), suggesting that improving dietary intakes may reduce the risk of childhood obesity. Nonetheless, these results should be interpreted with caution as the age of participants at the time of DXA and dietary assessments were not the same.

Additionally, research suggests that children who do not consume adequate fruit and vegetable intakes are at an increased risk of developing obesity. In Canada, data analyzed from the CCHS (cycle 2.2; 2004) suggests that children who consumed fruit and vegetables ≥5 times/wk were less likely to be overweight or obese [123]. In a review that examined determinants of fruit and vegetable consumption among 6-12 y children, it was found that fruit and vegetable availability, accessibility, taste preference and parental intakes were most consistently and positively related to fruit and vegetable consumption in children [124]. It is important that childhood obesity treatment programs be aware of these barriers to healthy eating to help children achieve balanced dietary intakes.

Meal distribution is also associated with body composition in children. Data from NHANES (1999-2006) was used to examine the relationship between breakfast skipping and body composition among children (n=4,320; 9-13 y) and adolescents (n=5,339; 14-18 y) [125]. In this study, breakfast consumption was self-reported; dietary intakes were assessed using 24-h dietary recalls. Results showed that 20% of children and 31.5% of adolescents were breakfast skippers; breakfast skippers had higher BAZ ($p<0.05$) and higher waist circumferences ($p<0.05$) compared to children and adolescents who ate ready-to-eat cereals or other breakfast foods.

Dietary familial or cultural factors also affect weight status; a large Canadian cross-sectional study investigated the associations between risk factors for childhood obesity and lifestyle behaviors among children [126]. Grade 5 children (n=4,298 10-11 y) had their height and weight measured; parents were surveyed on different sociodemographic and lifestyle behaviors. The risk of being overweight increased among children who ate in front of the TV (>5 times/wk; OR=1.44; 95% CI [1.18, 1.74]) or did not bring lunches to school (OR=1.39; 95% CI [1.16, 1.67]). These results suggest that healthy habits need to extend beyond the home environment, as they have potential to impact a child's weight status.

The effect of snacking between meals on weight status in children has been studied. A review that examined the impact of snacking on weight status using 19 cross-sectional studies and 5 longitudinal studies in children found that snacking either: (1) had no associations with weight status; (2) children who consumed more snacks were less likely to be obese; and (3) the percentage of energy consumed per snack may have a greater impact on weight status [127]. A Canadian study analyzed the data from the CCHS (cycle 2.2; 2004) for snacking patterns (i.e., dietary intakes between the hours of 3:00 pm to 6:00 pm, Monday through Friday) in 4-18 y old children (n=9,131) and found that snacks were consumed by 63% of children, representing 13% of total daily energy intakes [128]. Although fruits were cited as the most frequently consumed food group, majority of snacks were classified as energy-dense (i.e., sugar-sweetened beverages, sweets and cookies).

Snacking while engaging in sedentary behaviors has also been shown to be associated with adiposity. Canadian data from a population-based birth cohort of preschool children (Quebec, Canada; n=1,549; mean age: 4.5 y) was used to assess the relationship between food consumption during TV viewing and BMI [129]. Dietary intakes were reported by 24-h recall while questionnaires were used to calculate TV time; height and weight were measured. One-third of children ate snacks in front of the TV; children who snacked while watching television had a higher mean BMI (15.9 kg/m^2) compared to those who did not snack while watching TV (15.5 kg/m^2) ($p<0.05$). Despite this large and representative sample, these results were based on parent reports of dietary intakes and children's TV viewing practices.

The evidence on consumption of sweetened-beverages and weight status is clear. Research suggests a positive relationship between sugar-sweetened beverages and obesity development [130]. In a 2-y follow-up study of school-aged children (n=548; mean age 11.7 y), after adjusting for anthropometric, demographic, dietary, and lifestyle variables, each additional daily serving of sugar-sweetened beverage increased BMI by 0.24 kg/m^2 (95% CI [0.10, 0.39]; p=0.03) and increased the risk of obesity (OR) by 1.6 (95% CI [1.14, 2.24]; p=0.02). At baseline, the consumption of sugar-sweetened beverages was independently associated with change in BMI (mean ΔBMI of 0.18 kg/m^2 for each daily serving; 95% CI [0.09, 0.27]; p=0.02). However, this study was limited in statistical power, as at the 2-y follow-up, of the 548 children analyzed, only 37 were classified as obese.

Among Canadian children, one study using CCHS data (cycle 2.2; 2004) found that the odds of being overweight or obese were greatest among boys (6-11 y) who drank >2 cups (553±29 g/d) of soft drinks per day compared to boys who were moderate drinkers (i.e., 23±4 g) (OR=2.3; 95% CI [1.2, 4.1]) [131]. However, data from the United States (NHANES 1999-2002; n=1,572; 2-5 y) found that weight status of children does not associate with beverage consumption (i.e., milk, 100% fruit juice, fruit drink, soda) [132]. This study found total beverage consumption was positively associated with increased total energy intake, but not with BMI. Generally, it can be stated that the relationship between an increased risk of obesity and consumption of sugar-sweetened beverages may be attributed to the failure to reduce solid food consumption needed to compensate for the energy from the beverage [118]. In addition, it is worthy to note that these beverages typically contain high-fructose corn syrup that may contribute to weight gain through different mechanisms, such as lipogenesis, insulin secretion or leptin production [133].

Finally, eating outside of the home environment may be related to childhood obesity. Fast food restaurants are often palatable, cheap, easy to access and fun for children, however these may have adverse effects on diet quality [118]. Under free-living conditions, a study of 26 overweight and 24 lean adolescents (age 13-17 y) showed that energy intake increased by 409 kcal/d in overweight subjects on days eating out at fast-food restaurants; no effects were seen in lean adolescents [134]. However, this study was limited by its small sample size, and relied on self-reported energy intakes. Similar results were seen from secondary analyses of a large international study (n=17 countries; n=72,900 children) that examined whether reported fast food consumption was associated with BMI [135]. Parents reported on fast-food consumption, which was a reflection of consumption over the past year; they also reported the height and weight of their child. Despite data being self-reported, this study showed that children who frequently or very frequently consumed fast-food had BMI that were 0.15 kg/m^2 and 0.23 kg/m^2, respectively, higher than those in the infrequent group ($p<0.001$).

Special Focus: Milk Products and Body Composition in Children

In addition to their effects on satiety, the effects of milk products on adiposity have been explored. Research has proposed numerous mechanisms as to how milk products may play a role

in the regulation of energy metabolism or body weight; these postulated theories will be discussed in brief at the end of this dissertation.

In general, epidemiological studies suggest that fluid milk is the most consumed or favored milk product among children [21]. However, globally, milk product consumption among children is decreasing. Consequently, the overall proportion of children meeting their respective national recommendation tends to decrease with age [136]. Factors related to this decreasing trend can be attributed to parents who infrequently consume milk products themselves and/ or the replacement of milk with sugar-sweetened beverages [137]. Skipping breakfast, a common occurrence in adolescence, is also related to decreased milk consumption [138].

The literature that reports on the associations between dairy consumption and the risk of developing obesity during childhood is mixed [21, 22, 136]. A systematic review based on 23 studies (15 cross-sectional, 5 prospective cohort studies, 1 case control and 2 RCT) examined the association between adiposity and milk product consumption in children, and concluded there were no associations between milk intake and adiposity [22]. A major limitation to this review was its method of statistical analysis whereby all studies were pooled and were not differentiated by assessment of body composition as some used BMI while others used DXA to explore adiposity outcomes.

Conversely, a meta-analysis that analyzed only prospective cohort studies with a minimum of a 3-y follow-up on: (1) the risk of being overweight/ obesity; (2) changes in body fat; or (3) BMI gains in children and adolescents, found that milk product intakes were positively associated with body composition [21]. Specifically, studies were categorized as “low” and “high” milk product consumers and their risk of developing obesity were assessed. Results showed that “high” milk product consumers were 38% less likely to be obese (OR=0.62; 95% CI [0.49, 0.80]). When authors standardized the number of servings per day among studies to estimate a dose-response relationship, the risk of developing obesity was 13% lower with each (one) serving/d increment of milk product intake (OR=0.87; 95% CI [0.76, 0.98]).

The same meta-analysis also examined the associations between milk product consumption and BMI gains and found that with each 1 serving/d increment of milk product, BMI increased only by 0.02 (95% CI [0.01, 0.03]) kg/m^2 during follow-up [21]. Additionally, using two prospective cohort studies, although not significant, there was suggestion of an inverse

relationship between milk product consumption and percentage body fat (%BF) analyzed by DXA, whereby %BF was reduced by 0.65% (β=0.65; 95% CI [-1.35, 0.06]; p=0.07) [21].

As mentioned, the effects of milk products on mediating positive effects on body composition in children is controversial: some studies suggest there are benefits to consuming higher daily servings of milk products per day while other studies have found they have no added benefit on a child's weight status. Generally, milk product intakes are low among children, an eating behavior which may impact not only body composition but also a child's bone health. Eating behaviors are the result of many different factors and are not easily changeable; a discussion concerning the etiology and influences of eating behaviors in children warrants a brief discussion.

2.3.3.4 Eating Behaviors

Eating behaviors are complex and are regulated by many different pathways that are independent of energy needs [139]. Genetics, food availability and environment all influence an individual's eating behavior.

Genetic Determinants of Food Preferences

Genetics and environment can influence food preferences. Children tend to prefer sweet and salty snacks over bitter tastes which suggest a possible innate predisposition towards sweet foods and beverages [140]. Children's likes and dislikes and therefore preference towards foods that are high in fat, sugar and energy may also be influenced by the environment, especially environments where these foods are present [141].

Evidence from twin studies also confirms the genetic influence on appetitive traits in children [140]. In a large population-based twin study (n=2,402 pairs), infants eating behavior were assessed to describe appetitive traits [142]. This study found that heritability was high for Slowness in Eating and Satiety Responsiveness scores, which can be described as "Food Avoidance" type eating behaviors. Moderate significance was found for Food Responsiveness and Enjoyment of Food scores, which fall under the "Food Approach" umbrella. These findings suggest that genetics may play a role in appetitive traits. Research in genetics and eating behaviors is ongoing, however, until personalized medicine is a reality in the management of childhood obesity, eating behaviors are addressed in treatment programs through behavioral counselling.

Parental Influences and Family Environment

Parents and the overall family environment can influence a child's early experiences with food and eating [143], as do parenting styles and parenting practices [140]. The literature has identified 4 major parenting styles: authoritative, authoritarian, indulgent or neglectful; whereas parenting practices describe the strategy of control used by the parent, such as pressure to eat certain foods (i.e., fruits and vegetables) while possibly restricting others (i.e., fatty snacks) [140].

Both parenting styles and practices can influence a child's eating behavior and BMI. Although with good intention, children of restrictive parents often display poorer self-regulation and tend to overeat [144]. Similarly, pressuring children to eat certain foods (i.e., vegetables) may disrupt their short term behavioral control over their energy intake, thereby leading to disliking a food item [143]. Further, children's dietary intake is not only influenced by the types of foods present at home but also by the portion sizes available to them [143]. Similar to adults, children will often eat more if given the opportunity [145] and are able to override satiety signals that indicate the feeling of "fullness" depending on the food item [146]. Finally, children may attempt to exercise control over their parents, and change dietary intakes for others whereas resist eating certain foods when asked directly by parents.

It is important that assessments of eating behaviors be included in childhood obesity treatment programs as they are directly related to weight status. Assessing stage of change may aid interventions in understanding where children and parents stand in terms of understanding the need to change health behaviors, or for example, their readiness to try new foods. It is clear that there is a complex interplay between children and their parents regarding eating [147], and it is important to address these issues before children develop health consequences that may carry on throughout life.

2.4 Consequences of Childhood Obesity

Obesity is a pro-inflammatory state that can have immediate and long-term health consequences, which may persist into adulthood [4]. Children with obesity are at risk of developing similar health consequences commonly seen in adult-onset obesity [7]. The following section will provide a brief overview of related comorbidities, grouped as physical and

psychosocial, and offer an extended review of one specific consequence: the musculoskeletal health of children with obesity.

2.4.1 Physical Comorbidities

Children with obesity are more likely to develop cardiovascular disease risk factors [148], including hypertension [149], dyslipidemia [150] and impaired glucose tolerance [151] (**Figure 1.1**) compared to normal weight peers. However, development of these cardiovascular risk factors in youth, such as insulin resistance, may differ depending on ethnicity, sex, age and location of adipose tissue (i.e., total, visceral or subcutaneous adipose tissue) [152]. Specifically, cross-sectional data have shown that visceral adipose tissue (VAT) is related to early formation of insulin resistance in adolescents with obesity [153], however VAT volumes differ among ethnicities and sex whereby Caucasian ($p<0.001$) and boys ($p<0.001$) (aged 5-18 y) have been shown to have higher volumes of VAT compared to African Americans and females, respectively [152].

The increasing incidence of pediatric type 2 diabetes parallels the rise in childhood obesity. In Canada, it has been estimated that the incidence rates of type 2 diabetes are 11.3 cases per 100,000 children (<18 y) [154], a similar finding in the US [155]. Children with obesity also show signs of non-alcoholic fatty liver disease (NAFLD) which differs by ethnicity and age [156]. Other comorbidities include sleep apnea, which is estimated to be 4 to 6 times higher among children and adolescents with obesity compared to normal weight peers [157]. Dermatologic conditions, specifically acanthosis nigricans, are also common in children with obesity [158].

Nutrient deficiencies, such as low vitamin D status and low iron status have been reported in this population group [159]. Specifically, serum vitamin D, or 25-hydroxyvitamin D [25(OH)D] has been shown to be lower in obese children compared to normal weight peers [160]. The lower 25(OH)D concentrations may be due to decreased sun exposure from increased sedentary lifestyles, poor diet, or increased vitamin D storage as it sequesters in adipose tissue [161]. Factors related to low iron status include genetic influences, physical inactivity and inadequate dietary intakes, or low grade inflammation that is caused by increased fat mass which may explain in part the greater risk of iron deficiency [162].

2.4.2 Psychosocial Comorbidities

Children with obesity may experience numerous psychosocial and school-related problems. Clinical studies show that this population are more likely to experience more episodes of anxiety, depression, stress and generally have lower self-esteem [11]. They also show higher rates of eating disorder pathology [163], with binge eating disorder reported in 20- 40% of adolescents with obesity [11]. Finally, this population are 4 times more likely to experience poor school performance, including more missed school days, homework incompletion and difficulty with concentration compared to healthy weight controls [164].

2.4.3 Specific Focus: Musculoskeletal Consequences

Only within the past decade have studies explored the impact of excessive fat mass on bone health of children. Although heredity accounts for variations in a person's bone mineral content (BMC) and bone mineral density (BMD), the development of bone is also influenced by modifiable factors including dietary intake, PA and overall body composition [24].

It has been suggested that children with obesity complain of more musculoskeletal pain [26] and through observational studies, appear to have a greater incidence of forearm fractures compared to normal weight children [165]. Studies sought to understand why this may be, and demonstrated that children with obesity were more likely to exhibit abnormalities in gait, thereby making them more susceptible to falls [166]. Additionally, inadequate dietary calcium intakes and unbalanced diets may further exacerbate low bone mass and thereby increase fracture risk [167]. In particular, as mentioned, low circulating 25(OH)D concentrations are common in children with obesity [161].

In adults, being overweight may be protective to bone to a certain extent; the increased loading caused by excessive adipose tissue on weight bearing bones is suggestive to decrease the risk of osteoporosis and fractures [168]. In earlier work, when examining the bone health of children by DXA, children with obesity appeared to have denser bones compared to controls, suggesting that an increased body weight may have a beneficial effect on bone mass [169]. However, when examining bone mass in relation to total body weight, bone mass is often lower in children with obesity, as is bone strength [170], thereby supporting the evidence that the growing skeletal system are unable to adequately adapt to their increasing body weights [171], resulting in increased fractures.

Additionally, and notably to the growth and development in pre-pubertal children, excessive adipose tissue may alter bone development by way of timing of puberty. Specifically, puberty may start earlier as excessive fat mass mediates the release of growth factors that are both beneficial (i.e., insulin like growth factor 1, estrogen) or negative (i.e., pro-inflammatory cytokines, tumor necrosis factor 1, interleukin 6) to bone health [172]. In a study in adolescent girls with obesity, it has been shown that a higher ratio of VAT to subcutaneous adipose tissue (SAT) was significantly related to lower whole body (WB) BMD, suggesting the associations between fat and bone are also mediated by other factors such as adipokines (adiponectin and leptin) among other modulators [173].

In general, overweight children have lower bone mass relative to bone size [165]. However, findings are inconsistent as to whether fat mass exhibits positive or negative effects on bone in this population [165]. These relationships are difficult to decipher as studies use different technologies to assess bone outcomes (i.e., DXA [2- dimensional] versus pQCT [3-dimensional]), statistical approaches (i.e., account for different confounders and covariates) and evaluate different skeletal sites [24].

For instance, positive associations are found between lean mass and BMC, BMD and bone area using DXA [24]. However, the relationship between fat mass and on bone outcomes are not as clear, as findings range from positive [174], negative [175] to non-significant [176], which largely depend on the skeletal site, sex and pubertal status of the child [24]. Similar mixed findings are seen in studies that have assessed lumbar spine [24].

Although cross-sectional data using pQCT have shown that children with obesity have reduced radial cortical diameter [177] and tibia volumetric BMD [27] compared to normal-weight peers, the associations between bone outcomes and fat mass using pQCT are also inconsistent [27, 172], whereby the relationship between areal BMD and fat mass may be both negative [178] or non-significant [170]. In addition, depending on site (i.e., weight-bearing versus non-weight bearing bones), fat mass may mediate different outcomes [24]. These findings are supported by cross-sectional data that found positive (weak) associations between fat mass and tibia strength in both boys (β=0.09, p=0.07) and girls (β=0.17, p=0.03), but no association between fat mass and radial bone strength [178].

It is therefore important that studies differentiate between weight bearing and non-weight bearing sites, particularly in children with obesity. Further, it is important that children are

encouraged to participate in activities that not only promote cardiovascular benefits but also strengthen bones. Specifically, activities that are of high magnitude that impose a dynamic and abnormal strain on the skeleton system are encouraged in addition to general PA guidelines that may not emphasize weight-bearing types of activities [179]. Weight-bearing activities have been shown to be beneficial to bone development [32, 34], whereby it has been estimated that compared to control, exercise interventions may account for a 0.6% to 1.7% annual increase in bone accrual, with greatest effects among pre-pubertal children [32].

In addition to weight bearing activities, the impact of dietary calcium supplementation [180, 181] or milk and milk products [182] on BMC in school-aged children has been studied. It has been suggested that doubling calcium (i.e., 750 mg/d to 1600 mg/d) in normal weight children increases BMD by 18.8% [180] suggesting a possible role for doubling milk products to improve bone health. However, pooled data have not concluded such straightforward findings. For instance, data from a meta-analysis of 23 RCT in healthy children assessed the impact of different dosages of calcium supplementation on WB BMC found no added benefit of calcium supplementation (~2 g/d) on bone outcomes [183]. However, when categorizing control groups as low versus adequate for baseline calcium intakes, BMC increased significantly by 49 g (95% CI [24.0, 76.6]; $p<0.05$).

In adult weight-loss trials, weight-loss induced bone loss is a common concern [184] although the mechanism as to how this occurs remains unknown [29]. To counteract potential losses in BMC, interventions typically involve an exercise component in addition to a dietary intervention, however, scarcely do childhood obesity trials include assessment of bone health to confirm changes in adiposity are not negatively affecting bone health.

The effects of weight loss on bone health have been explored primarily in adolescents as this is a critical time of bone development [185-187]. In a 6-mo trial with obese adolescent girls (n=92; 8-14 y), the effects of weight loss following a lifestyle intervention on bone outcomes (i.e., WB, lumbar spine (L1-L4)) were assessed at 6 and 12-mo [187]. Details concerning the intervention were not disclosed. At both 6 and 12-mo, BMI and %BF decreased, but were not significantly different from baseline. Similarly, both WB and lumbar spine BMC increased at 6 and 12-mo but were not significantly different compared to baseline; WB and lumbar spine BMD remained relatively unchanged.

Similar results are seen in a 12-mo trial, where adolescents (n=40, 15-19 y) with or without NAFLD were guided to follow a restricted diet plus participate in exercise (aerobic + resistance training) group sessions to assess the impact of weight loss on WB bone outcomes [185]. In adolescents without NAFLD, by 12-mo, BMI (mean ΔBMI: -3.5±3.2 kg/m^2; $p<0.05$) and FM (mean ΔFM: -12.0±7.4 kg; $p<0.05$) significantly decreased while WB BMC significantly increased (mean ΔWB BMC: 109.7±189.9 g; $p<0.05$); WB BMD remained unchanged by 12-mo. Similar findings are seen in a 12-mo adolescent study with only girls (n=20; 14-18 y) [186]. This study design used a similar exercise platform (i.e., aerobic + resistant training) but the dietary intervention component was based on education not energy restriction. DXA assessments for WB bone outcomes were performed at baseline and 12-mo. By 12-mo, BMI significantly decreased (mean ΔBMI: –3.2±3.03 kg/m^2) as did total fat mass (FM) (mean ΔFM: –7.3±5.6 kg); WB BMD significantly decreased (mean ΔWB BMD: 0±0.1 kg/cm^2) whereas WB BMC and lean mass did not significantly change. **Table 2.3** includes a more detailed review of these trials.

In general, these studies suggest that interventions in the adolescent obese population do not negatively affect bone health when adiposity is reduced; there is a need to confirm these findings in the pre-pubertal population. The emphasis of a high daily consumption of milk and milk products and participation in daily weight-bearing types of activities may favorably benefit bone health, in addition to body composition in children with obesity. A trial that assesses these two components in children with obesity is certainly warranted.

Bone Biomarkers

In addition to assessing bone status by quantitative bone absorptiometry, direct serum measures of bone markers offer a fast and risk-free method of assessing bone health. Bone markers are molecules that can be classified as either bone formation or bone resorption. Bone formation markers reflect osteoblast function whereas bone resorption markers are the product of bone type 1 collagen degradation [188]. Bone markers are commonly elevated during childhood due to the high skeletal growth velocity and rapid bone turnover [189]. Measuring bone markers, in addition to other assessments of bone health (e.g., vitamin D and parathyroid hormone) may provide insight to skeletal diseases in children or response to treatment. Measurement of bone markers may be especially important for the obese population participating in weight-loss trials,

as increased bone resorption markers and variable changes in bone formation markers have been seen in adult studies [190]. However, there is limited research on the changes in bone markers in children with obesity participating in an intervention [29] and none that report on a trial that assesses the impact of increasing milk and milk products and engaging in daily weight-bearing types of activities above National (Canadian) recommendations on bone health. There are many bone markers that are used in research; their mechanisms of action are complex and are beyond the scope of this thesis. The following provides a simplified and brief overview of the bone biomarkers described in this dissertation:

1. Markers of Bone Formation

- *Bone alkaline phosphatase (BAP)*

Bone alkaline phosphatase is a bone-specific isoform of alkaline phosphatase; it is a glycoprotein found on the surface of osteoblasts and is measured as serum. Serum BAP is not affected by circadian variations and has a long half-life (1-2 days). Measured using immunoassays, BAP is stable when stored frozen and is not affected by repeated freezing-thawing [189]. Serum BAP concentrations peak during infancy and puberty whereby girls peak before boys, therefore age-related changes in BAP positively correlate with growth velocity [189]. In a study examining the effects of a 3-mo lifestyle intervention in children with obesity (n=100 obese; n=70 non-obese children; 5–10 y), BAP concentrations were 20% higher in children with obesity compared to normal-weight control ($p<0.001$) and decreased significantly after a -0.96 change in mean BMI standard-deviation score ($p<0.001$); changes in BAP were significantly correlated with changes in BMI ($r=0.35$; $p<0.001$) [191]. To date, this has been the only published study assessing BAP in children with obesity. Reference values for serum BAP are only available for preterm infants [192] and healthy Caucasian pre-pubertal girls [193].

- *Osteocalcin*

Osteocalcin is a non-collagenous protein found in the bone matrix and is a specific product of osteoblasts [194]. Serum concentrations increase significantly in response to rapid skeletal growth [189]. Unlike BAP, osteocalcin is an unstable molecule that is greatly affected by circadian variations, whereby its highest concentrations are found in the morning [189]. Further, it has a short half-life (~5 min) and may decrease up to 70% if left at room temperature for 6 to 24 hours [195]. Therefore, careful handling of samples used for analyses of osteocalcin is required: samples should be collected on ice, separated within the hour of collection and frozen

immediately. Further, repeated freeze-thawing should be avoided as it may reduce serum osteocalcin values [196]. Reference values for osteocalcin have been established for healthy Caucasian children using radioimmunoassay [197], however, enzyme-linked immunosorbent assay is the more commonly used method of assessment [189]. Compared to normal-weight peers, children with obesity have lower osteocalcin concentrations, however, osteocalcin concentrations have been shown to significantly increase following significant reductions in BAZ (ΔBAZ: -0.72±0.02) following a 1 y lifestyle intervention in children with obesity [198].

2. Marker of Bone Resorption

- *C-terminal telopeptide of type I collagen (CTX-1)*

C-terminal telopeptide of type I collagen assays measure a fragment of the C-terminal telopeptide of type 1 collagen released during resorption from mature bone and into circulation [199]. Urine and serum specimens can be collected; serum is often measured by immunoassay. CTX-1 is a stable molecule, however, it is best analyzed after mixing with ethylene diamine tetra-acetate plasma if left at room temperature for a couple of hours; it is also stable after exposure to numerous freeze-thaws [199]. CTX-1 should be measured fasted as its highest concentrations are in the morning then nadir concentrations occur by mid-day [199]. Reference data are available for children whereby serum CTX-1 concentrations reflect growth patterns of healthy children, with highest concentrations in the first years then typically a second peak occurs when children enter a puberty growth spurt [200]. CTX-1 concentrations have been shown to be the same [201] or increased [202] in children with obesity compared to normal-weight peers. In a 3-mo lifestyle intervention in children with obesity, CTX-1 concentrations remained unchanged despite a significant decline in BMI at 3-mo compared to baseline [191].

3. Other Measured Markers Important to Bone Health

- **Vitamin D**

Vitamin D is a hormone important for calcium homeostasis and bone health. Briefly, vitamin D enters the circulation by ultraviolet B wavelengths from the sun that trigger vitamin D synthesis in the skin by converting 7-dehydrocholesterol to cholecalciferol (vitamin D_3) then enters circulation. Vitamin D_3 is modified by 2 hydroxylation steps to the active form. The first is in the liver, whereby it is converted to 25-hydroxyvitamin D (25[OH]D). The second is in the kidneys, where 25(OH)D is converted to 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) [203]. It is best measured as 25(OH)D using different techniques, such as immunoassays or chromatographic

techniques. Only small amounts of vitamin D are obtained from the diet (e.g., oily fish, milk and milk products, eggs and meat) [203].

The recommended dietary vitamin D intake for children 4-8 y differs depending on the professional society. For instance, children 4-8 y of age are recommended 400-800 international units (IU)/d by the Canadian Paediatric Society [204] whereas the Institute of Medicine advises an estimated average requirement of 400 IU/d, a recommended dietary allowance of 600 IU/D and a tolerable upper intake level of 3000 IU/d [205]. However, these reference ranges may not reflect the needs of children with obesity: compared to normal-weight peers, children with obesity often result with lower concentrations of 25(OH)D [206], however, concentrations increase when BMI declines [207]. Decreased serum concentrations of 25(OH)D in children with obesity may be attributed to decreased sun exposure due to sedentary lifestyles, poor diet with inadequate dietary intakes of vitamin D, or increased vitamin D storage as it may become sequestered in adipose tissue [161].

2.5 Treatment of Childhood Obesity

There are multiple ways to treat obesity, including behavioral, pharmacological, and surgical treatments [208, 209]. Behavioral approaches include modifications to diet-only, PA-only or a combination of diet and PA; they can be individually-focused (i.e., child and interventionist only), directed only to the parents or include the child and family (i.e., family-based). Interventions can be delivered in individualized sessions with an interventionist or in group settings [209]. This dissertation will focus primarily on the family-based, or family-centered lifestyle intervention (interventionist and family) for treating childhood obesity.

In general, the goal of treatment in pre-pubertal children is weight maintenance, where ideally “children outgrow adiposity” to ensure height reaches its growth trajectory. After puberty, a steady-gradual decrease of <0.5 kg per week can be recommended [210]. The message should be improving health outcomes and not on achieving a particular weight, *per se* [56]. In general, lifestyle behavior programs include discussions concerning dietary intake, PA and health [9, 10].

In the past, the general recommendation of achieving a healthy lifestyle to decrease adiposity was not the focus of childhood obesity interventions. Before the 1970s, childhood obesity programs were governed by the weight-reduction model. Evidence later emerged

suggesting that structured lifestyle approaches that combined behavioral strategies were more effective at reducing body weight in children than the former strict model [208]. More importantly, these reductions in weight were more likely to be sustained when they also addressed lifestyle approaches [210]. Therefore, the research shifted and started to propose the family model, as studies that were designed to target children individually were not as successful [211]. This shift in treatment was pivotal; researchers were recognizing that children are greatly influenced by social and familial factors. Children are nurtured by families and most of their behaviors are learned from their parents, therefore parents can act as the agents of change [212]. Moreover, family-centered programs could be used to educate parents to act as healthy role models.

A well cited study by Epstein et al. provided key evidence that showed the effectiveness of this type of intervention in sustaining long term successful reductions in adiposity [211]. This trial examined the effects of a behavioral family-based treatment in 6-12 y old (n=76) obese children and followed them after 5 and 10 years. Families were randomized to 1 of 3 groups: (1) control; (2) child and parent target; or (3) child target. Groups were provided similar diet, exercise, and behavior management training one time per week for 8 weeks; 6 additional meetings distributed over 6-mo were provided. They were followed at 21, 60 and 120 months. Components of the intervention were as follows: (*Diet*) The traffic-light diet: a color-coded, calorie-based food exchange system whereby foods were divided by caloric density per average serving (i.e., green: <20 kcal/serving; yellow: ≤20 kcal/serving above the standard for the group; red: >20 kcal/serving)[213, 214]; (*PA*) Participants were provided information on aerobic exercise and stretching; (*Behavioral*) Contracting, self-monitoring and social reinforcement techniques were used during interventions. The control group was encouraged to attend follow-up sessions. All children were measured for weight and height to calculate BMI. At both 5 y (n=67) and 10 y (n=61) follow-up, significantly less children were classified as overweight in the child and parent target group (-11.2% and -7.5%, respectively ($p<0.05$)) compared to the control (+7.9% and +14.3%, respectively ($p<0.05$)). Further, the control group increased in the percentage of children classified as overweight at both 5 and 10 y follow-up (+2.7% and +4.5%, respectively ($p<0.05$)) [211]. This study highlighted the value of family involvement in interventions aimed at children with obesity. It also illustrated how lifestyle interventions can yield successful reductions in adiposity from childhood through adolescence.

Since the study by Epstein et al., numerous family-centered interventions have been published using study designs that are based on theoretical frameworks, or behavioral [209, 215]. The behavioral components that are taught during these interventions include self-monitoring, goal setting, problem solving, behavioral contracting and relapse prevention techniques [211, 215].

The effectiveness of reducing BAZ in children with obesity who participate in lifestyle interventions have been subject to many review papers [13, 209, 215-218] including a Cochrane review [219]. Given the evidence that early childhood weight status tracks into adolescence and adulthood, interventions using the family-centered, multidisciplinary approach are valid in children as young as 5 y [220]. However, the age appropriateness of interventions should be considered [209, 221]. Younger children (i.e., <12 y) are under the direct care of parents and do not have the same level of freedom to make choices, particularly when purchasing food items at school, in comparison to children >12 y who may have more perceived control over their dietary intakes.

In a meta-analysis that examined trials (n=61) involving overweight children and adolescents (2-18 y) participating in obesity interventions published up to February 2006 found that when dividing by age (i.e., ≤8 y of age versus ≥9 y), greater reductions in BMI in younger children (-0.70; 95% CI [-1.00, -0.40]; $p<0.001$) compared to interventions targeting 9-18 y old children (-0.49; 95% CI [-0.81, -0.18]; $p<0.01$) were seen [221]. Similar results are seen in another meta-analysis that examined the differences in BMI in children and youth participating in treatment programs by age [209]. Specifically, this study reports on trial up to August 2013 and included RCT of primary care behavioral studies and pharmacological interventions in children and youth aged 2-18 y if post-baseline data were provided past 6-months. Analysis of trials (n=31) suggested that children 2-12 y yielded greater reductions in BMI (ΔBMI: -0.54; 95% CI [-0.76, -0.32]; $p<0.001$) compared to 13-18 y (ΔBMI: -0.29; 95% CI [-0.92, -0.25]; $p<0.001$). These findings suggest that interventions designed for children <8 y are appropriate and can result in significant reductions in adiposity.

The effects of interventions that focus solely on diet or PA on reducing BMI in children with obesity have been reviewed and suggest that interventions that focused solely on changing diet ($p=0.27$) or PA (p-value not disclosed) yielded insignificant results [209]. These findings parallel another meta-analysis that examined changes in BMI in children with obesity who

participated in interventions that focused solely on either diet or PA compared to control (i.e., self-help or standard care) [221]. Results showed that changing only diet (ΔBMI: -0.22; 95% CI [-0.21, -0.18,]; p-value not disclosed, but reported not significant), PA-only (ΔBMI: -0.02; 95% CI [-0.21, 0.18]; p=0.86) or focusing on reducing sedentary-behaviors only (ΔBMI: 0.02; 95% CI [-0.35, 0.39]; p=0.91) yielded non-significant results. This study also found that family-centered (ΔBMI: -0.64; 95% CI [-0.88, -0.39]; p<0.001) compared to child-interventionist approach (ΔBMI: -0.17; 95% CI [-0.40, 0.05]; p=0.13) yielded significant reductions in BMI.

In general, successful lifestyle interventions are those with treatment periods no less than 6 months [218, 219] and last a minimum of 1 year [209, 218]. Further, sessions that occur every 1-3 months are effective at reducing BMI in overweight children [218]. In terms of the family-centered approach, the literature has identified four distinct types of intervention designs. Family-based lifestyle interventions can be described as: (1) behavioral; (2) behavioral plus parent education; (3) family therapy; or (4) family therapy plus behaviorally oriented psycho-education [215]. These distinctions were made in a systematic review that found that "family-centered trials" that were based specifically on behavioral changes were more successful at reducing BMI compared to the other three types of family-based platforms [215]. A description of the family-centered trials using behavioral component are summarized in **Table 2.4**.

Despite the minor yet important differences under the family-based intervention "umbrella", children are either responders or non-responders to treatments, irrespective of intervention type. Studies have aimed to examine predictors of success and have shown that younger children are more likely to succeed in interventions aimed at reducing adiposity, as their weight-related habits are less ingrained and easier to change [222]. Higher initial body weight may also predict better outcomes [223], whereas sex may play a role, by which females respond better to treatments [224], although these findings are mixed [225].

Milk Products as Treatment in Intervention Trials

Previously in this review, the role of milk products in modulating adiposity were proposed; it was suggested that the composition of milk products may play a role in regulating satiety. However, research was limited to observational and longitudinal studies. Randomized controlled trials have been conducted to test the effects of different servings of milk products in relation to changes in adiposity outcomes. In adult trials, the effects of milk product consumption

compared to control on changes in body composition are inconsistent [226]; similar inconsistencies are seen among overweight and obese children [22, 227-229]. In a 16-wk trial of overweight children (n=55; 8-10 y), the effects of high versus low milk consumption on changes in body composition were tested [228]. All children were counselled to consume a stop-light diet; the high-milk group were counseled to consume milk daily [skim milk: 3 x 236 mL/d; 1%MF chocolate milk: 1 x 236 mL/d]. Children in the low-milk group were counseled to consume milk on a weekly basis [skim milk: 4 x 236 mL/wk; 1%MF chocolate milk: 5 x 236 mL/wk] plus consume sugar-sweetened beverages [3 x 200 mL/d]. Body composition was assessed by magnetic resonance imaging. Compliance to beverage intakes were assessed by 3-day food diary at 12-wks and 24-h recalls were conducted at each study visit. By the end of trial, compliance to the milk interventions in both groups were described as "excellent". Body composition did not significantly differ among groups, however, both groups were trending towards significant reductions in BMI, suggesting obesity was resolving.

In another 16-wk trial in overweight children (n=98; 8-10 y), significant increases in lean mass ($p=0.04$) and height ($p=0.01$) but not fat mass or percent body fat were found in the milk intervention group compared to control. Specifically, this study tested the effects of consuming 3 servings/d (200 g/serving) of milk in addition to being counselled to not drink sugar-sweetened beverages compared to a normal diet (control) [229]. Families were provided with milk that was home delivered; body composition was assessed by DXA at the study center and compliance to the milk intervention was assessed by food frequency questionnaire, both performed at baseline and at 16-wk. From baseline to 16-wk, milk intakes significantly increased in the intervention group ($p<0.001$), suggesting compliance to treatment.

Trials that have assessed the effects of energy restriction compared to different servings of milk products in children and youth have also resulted in mixed findings. A 6-mo trial in overweight and obese children (n=120; 5-6 y) tested the effects of either a dairy-rich diet or an energy-restriction diet compared to control after 6-mo and at a 3-y follow-up session on changes in body composition assessed by DXA [227]. Both treatment groups participated in 6 monthly family-centered lifestyle interventions that focused on basic diet and PA practices. Dietary intake was assessed 3-day food diary; PA by questionnaire. The dairy rich group was encouraged to consume foods that would total or exceed 800 mg of dietary calcium per day, specifically from milk products; energy restrictive group was restricted to the energy requirement for height (as

described by authors); control was only seen for DXA assessments. By 6-mo, all three groups resulted in significantly decreasing BMI and waist circumference ($p<0.01$), which all increased at the 3-y follow-up. However, the rise in BMI and waist circumference from the dairy-rich group was significantly lower than the other two groups. The dairy-rich group also significantly increased dietary calcium compared to the energy-restriction group and control.

A similar study that used energy restriction to reduce adiposity in overweight and obese adolescents resulted in similar findings [230]. In this short trial (12-wk), adolescents (n=120; 12-18 y) were counselled to follow an energy restricted diet (500 kcal deficit of total energy expenditure) and to consume either 2, 3, or 4 servings of dairy per day. Body composition was assessed using a BIA; compliance to the treatment was assessed using 24-h recalls at baseline and end of study. By 12-wks, all groups significantly decreased BMI, BAZ and %BF with no differences among groups. Calcium intake was significantly greater by 12-wk in the 4 servings/d group versus others.

Overall, these trials are of relatively short duration (12-wk to 6-mo), and most importantly, do not all use treatment regimens that are advisable or sustainable for children with obesity. The use of the family-centered approach to address changes in adiposity in children is established. Further, behavioral techniques used during these interventions are important, as they support changes in lifestyle behaviors that can be sustained.

Compliance to Milk Interventions

The studies mentioned above used different methods to assess compliance to the various milk product interventions: food frequency questionnaires [229], 24-h recalls [230], 3-day food diaries [227] or the combination of 3-day food diaries and 24-h recalls [228] were used to compare baseline to end of study dietary intakes. Biomarkers of milk products, specifically fatty acid concentrations, can be used to assess compliance to milk product intakes; they are advantageous as they are objective and are free of measurement bias possibly associated with dietary recall error or analysis [231]. Most importantly, when assessing children, dietary recalls are limited to parent recalls [232].

In fluid milk, fats are made up as globules that are resistant to pancreatic lipolysis unless exposed to gastric digestion [74]. Triacylglycerol represent ~98% of milk fat fraction, while diacylglycerol (~2%), cholesterol (<0.5%), phospholipids (~1%) and free fatty acids (0.1%)

make up the remaining lipid profile [233]. However, the composition and amount of milk fat may slightly vary depending on different variables, including animal origin, season, and the stage of lactation of the animal [233]. Nonetheless, approximately 70% of the fat fraction are saturated fatty acids with the remaining 30% being unsaturated fatty acids [233]. Plasma phospholipid saturated fatty acids [234] and serum saturated fatty acids [231] are considered valid biomarkers for assessing milk and milk intakes, specifically pentadecanoic acid (15:0) and heptadecanoic (17:0) acid [235]. One study found 15:0 to be moderately correlated with dairy fat intake based on 24-h recalls ($r=0.45$; $p<0.001$) [236]; another study in 4-13 y old children (42% of n=93 classified as overweight and obese) used 15:0 as a biomarker to test compliance of reducing full-fat milk to reduced-fat milk consumption over 24-wks [231]. To date, there are no reports of a trial specifically aimed at children with obesity that assess compliance to a milk intervention using these biomarkers in addition to standard measures of dietary intake.

2.6 Conclusion

It is clear from this review that the topic of childhood obesity is complex. The etiology of childhood obesity is wide-ranging, spanning from pathological non-modifiable factors to lifestyle behaviors that can develop into bad habits, which may be overcome with proper treatment, including family-based interventions. However, the short- and long-term consequences of childhood obesity are potentially devastating and may be a challenge to treat as children grow into adulthood should obesity persist. The literature supports the use of family-centered lifestyle interventions in children <8 y of age; should obesity present at a young age, children should be treated accordingly.

Research suggests that milk and milk products may play a role in modulating healthy body compositions. Childhood obesity interventions that have used milk products as a treatment arm typically assess compliance using standard dietary recall measures and have not considered using objective measures to assess milk fat concentrations. Further, few childhood obesity trials include bone outcomes in their analyses, which may be particularly important given the potential bone alterations in this pediatric population. Similarly, there is limited information on the impact of reductions in adiposity on both weight-bearing and non-weight-bearing bone sites in children with obesity. As mentioned, the role of milk and milk products and weight-bearing types of

activities are known to benefit bone health, however, if both are increased above current guidelines to elicit greater changes in BMC in children with obesity has yet to be tested.

Finally, this review briefly discussed factors that influence energy intake and thereby mediate a child's weight status. The role of leptin in long-term energy regulation was reviewed and it was suggested that leptin concentrations are associated with adiposity. Eating behaviors are also associated with a child's weight status whereby children with obesity are more sensitive to external cues of food, for instance, compared to normal weight peers. Milk and milk products may play a role in regulating appetite, however, it is not known if increasing milk and milk products as well as weight-bearing types of activities will favorably change leptin concentrations or eating behaviors in children with obesity.

Several gaps have been identified in the literature and are important to consider when treating children with obesity. Childhood obesity continues to be a public health concern: well-designed trials that consider the complexity of this disease are needed to offer realistic and sustainable outcomes for this population.

Table 2.2 Canada's Food Guide serving sizes of milk and milk products (weight or volume) [23]

Food Item	Serving Equivalent
Milk, skim, 1%, 2%, whole	250 mL, 1 cup
Milk, chocolate	250 mL, 1 cup
Milk, evaporated, canned	125 mL, ½ cup- undiluted
Milk, goat, enriched	250 mL, 1 cup
Milk, lactose reduced	250 mL, 1 cup
Milk, powdered	25 g, 75 mL, 1⁄3 cup
Buttermilk	250 mL, 1 cup
Cheese, block (i.e., cheddar, Mozzarella, Swiss, feta)	50 g, 1 ½ oz
Cheese, cottage or quark	250 mL, 1 cup
Cheese, goat	50 g, 1 ½ oz
Kefir	175 g, 175 mL, 3⁄4 cup
Paneer	50 g, 1 ½ oz
Pudding/custard (made with milk)	125 mL, ½ cup
Yogurt (plain and flavored)	175 g, 175 mL, 3⁄4 cup
Yogurt drinks	200 mL
Fortified soy beverage*	250 mL, 1 cup

*recommended when not able to consume cow's milk

Table 2.3 Summary of weight loss trials with bone outcomes

Reference; Location	Age (y), n; AR; %	Assessment	Intervention type	Results	
Campos (2012) [185] Brazil	OB 17±1.7 y; n=40 NAFLD n=18 Non-NAFLD n=22; AR 12-mo: 0% *Study length: 1-y*	DXA: WB	(Txt length: 1-y) Hypo caloric diet + diet lessons 1x/wk; 3x/wk supervised AT (30min treadmill); RT 30min	Δ from BL to 1-y: **NAFLD:** ΔFM: -11±7.2 kg* ΔLM: 2.8±5.8 kg ΔBMC: 1.7±2.1 kg* ΔBMD z-score: 0.2±0.5	**Non-NAFLD:** ΔFM: -9±8.3 kg ΔLM: 3.0±3.5 kg ΔBMC: 1.1±1.9 kg* ΔBMD z-score: 0.1±0.3
Campos (2013) [186] Brazil	OB 16±1.5 y; n=20 Female only; AR 12-mo: 40% *Study length: 1-y*	DXA: WB	(Txt length: 1-y) Food diet + diet lesson 1/wk; 3/wk supervised (30min AT); RT 30min	Δ from BL to 1-y: ΔFM: -7.3±5.6 kg* ΔLM: 0.9±3.8 kg ΔBMC: 2.4±3.8 kg ΔBMD: -0.0±0.1 kg/cm^2 *	
Rouke (2003) [223] USA	OB 11.1±1.6 y; n=92 Female only; AR 12-mo: 0% *Study length: 1-y*	DXA: WB LS (L1-4)	(Txt length: 6-mo) No details provided; participated in a weight-loss trial	Δ from BL to 1-y: **WB:** Δ%BF: -0.8±2.8 % ΔBMC: 2.9±1.3 kg ΔBMD: 0.1±0.0 kg/cm^2	**LS 1-4:** ΔBMC: 7.9±5.3 kg ΔBMD: 0.1±0.1 kg/cm^2

Abbreviations: Δ: change; %BF: percent body fat; AR: attrition rate; AT: aerobic training; BL: baseline; BMC: bone mineral content; BMD: bone mineral density; DXA: dual-energy x-ray absorptiometry; FM: fat mass; LS: lumbar spine; min: minute; mo: month; NAFLD: non-alcoholic fatty liver disease; OB: obese; Txt: treatment; RT: resistant training; WB: whole body; wk: week; y: year.
*Statistically significant changes from BL to 12-mo within a group; $p<0.05$.

Table 2.4 Summary of family-centered lifestyle interventions: Behavioral focused

Study;	Study Length; Age (y)	Groups	n; AR (%)	Dose	End of Trial Results
Aragona (1975) [237] USA	12-wk 5-10 y Female only	• Txt 1: Diet, exercise, parent involvement, stimulus control, self-monitoring, behavioral control with reinforcement • Txt 2: Same as above; less reinforcement • Ctrl: F/U visits	15; 20%	Weekly group sessions	• By 12-wks: Txt 1 (11.3lbs) and Txt 2 (9.5lbs) lost significantly more weight than Ctrl (+0.9lbs) ($p<0.05$) • By 31-wk F/U: Txt 1 regained less than other two groups but not statistically different
Epstein (1984) [238] USA	1-y 8-12 y	• Txt 1: Diet and parent involvement • Txt 2: Diet, exercise and parent involvement • Ctrl: Wait list	53; 11%	First 8 sessions: Weekly; 7 sessions spread out over 20 weeks with 3 bi-weekly meetings; remaining 4 meetings at each month until 1-y	• By 6-mo: Percent overweight reductions of both Txt were significantly greater than Ctrl ($p<0.01$) • F/U 6-mo post-Txt: Txt 1 and 2 continued to be lighter than at baseline • No significant difference between two treatment groups at 6 and 12-mo
Janicke (2008) [239] USA	4-mo 8-14 y	• Txt 1: Family-based: separate group sessions for parents and children on PA and diet • Txt 2: Parent only group sessions • Ctrl: Wait list	93; 24%	Weekly 90-min sessions for 8-wk; biweekly for the following 8-wk	• At 4-mo: BAZ was lower in Txt 2 compared to Ctrl ($p<0.05$) • By 10-mo F/U: BAZ reduced in group 1 (ΔBAZ: -0.12±0.05) and Txt 2 (ΔBAZ: -0.09±0.04) compared to Ctrl (ΔBAZ: 0.02±0.17) ($p<0.05$) • No differences in change of BMI at 4-or 10-mo between Txt groups ($p=0.68$)
Jiang (2005) [240] China	2-y 12-14 y	• Txt: Family-based intervention on diet and PA • Ctrl: F/U visits	68; 9%	Monthly home visits and diet modification plan	• At 24-mo F/U: Txt (ΔBMI: -2.6±1.6) compared to Ctrl (ΔBMI: -0.1±1.1) ($p<0.001$)

Table 2.4 Summary of family-centered lifestyle interventions: Behavioral focused *(continued)*

Study;	Study Length; Age (y)	Groups	n; AR (%)	Dose	End of Trial Results
Kirschenbaum (1984) [241] USA	9-wk 9-13 y	• Txt 1: Diet, PA, parent, reinforcement, self-monitoring (family) • Txt 2: Above (child-only) • Ctrl: Wait list	40; 25%	90-min group sessions; weekly	• At 3-mo F/U: Significantly less children classified as overweight (BMI) in Txt 1 and Txt 2 compared to Ctrl ($p<0.01$). • At 1-y F/U: Children in both Txt sustained their initial and similar degree of weight loss ($p=0.04$)
Rodearmel (2006) [242] USA	13-wk 8-12 y	• Txt: Family-based focused on cereal intake (2 servings/d) and steps (additional 2000 steps/d) • Ctrl: F/U visit	105; 23%	3 group sessions (at beginning, middle, end)	• At 14-wk F/U: Txt reduced %BAZ (Δ%BAZ: -0.65) compared to Ctrl (Δ%BAZ: 0.47) ($p=0.03$)
Wheeler (1976) [243] USA	7-mo 2-11 y	• Txt: Parent involvement; reinforcement, stimulus control, self-monitoring • Ctrl: F/U visit	40; 46%	30-min family sessions biweekly; spaced out depending on progress	• By 7-mo: Less children classified as overweight in Txt compared to Ctrl by end of study
White (2004) [244] USA	6-mo 11-15y	• Txt: Diet, PA, parent involvement, self-monitoring • Ctrl: Information only	57; 12%	Weekly information on website	• At 6-mo: Txt significantly reduced BMI (ΔBMI: -0.24±1.38) compared to Ctrl ΔBMI: 0.71 ±1.19) ($p<0.01$)

Abbreviations: Δ: change; AR: attrition rate; BAZ: body mass index-for-age and sex z-score; BMI: body mass index; Ctrl: control; d: day; F/U: follow-up; min: minute; mo: month; PA: physical activity; Txt: treatment; wk: week; y: year.

Table 2.5 Methodological quality of studies included in this dissertation[a] using the Cochrane bias assessment tool

	Bias Criteria[b]						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Campos (2012) [221]	Low	Unclear	NA	NA	Low	Low	Low
Campos (2013) [222]	NA	NA	NA	NA	Unclear	Low	Unclear
Rouke (2003) [223]	NA	NA	NA	NA	Low	Low	Unclear
Aragona (1975) [237]	Unclear	Unclear	NA	NA	Unclear	Low	Unclear
Epstein (1984) [238]	Unclear	Unclear	NA	NA	Unclear	Low	Unclear
Janicke (2008) [239]	Low	Low	NA	NA	Low	Low	Low
Jiang (2005) [240]	High	Unclear	NA	NA	Low	Low	Low
Kirschenbaum (1984) [241]	Low	Unclear	NA	NA	Low	Low	Unclear
Rodearmel (2006) [242]	Low	Unclear	NA	NA	Low	Low	Low
Wheeler (1976) [243]	Unclear	Unclear	NA	NA	Low	Low	Unclear
White (2004) [244]	Unclear	Unclear	NA	NA	Low	Low	Low

[a] Specifically from Table 2.3 and 2.4, only; defined as "low Risk", "high risk"; "unclear risk"; "N/A: not applicable".
[b] Columns: 1: random sequence generation, 2: allocation concealment, 3: blinding of participants and personnel, 4: blinding of outcome assessment, 5: incomplete outcome data, 6: selective reporting, 7: free of source of funding.

Bridge Statement 1

The literature review of this dissertation has outlined the complexities of childhood obesity and has discussed the different factors that may govern a child's weight status. Children with obesity are at greater risk of developing adult obesity, therefore the need to start treatment at a young age is justified and supported by previous work. Further, the use of family-centered lifestyle interventions that include behavioral components have shown greater reductions in adiposity compared to those that are tailored to individuals.

However, this review has highlighted some research gaps and has brought forth conflicting literature. The effects of milk and milk products on bone health are established. However, when examining their role in changing adiposity measures, the literature is not as clear. Further, few trials have considered the impact of reducing adiposity on bone health in children with obesity. In adult weight-loss trials, bone loss can occur as a side-effect of weight loss. Given that children with obesity are at a risk of fractures, the potential of bone loss when fat mass is reduced should be considered. Therefore, the use of more sophisticated bone assessment techniques, such as pQCT, is warranted to assess bone at the microarchitectural level to ensure reductions in adiposity do not negatively affect bone health. Further, assessment by DXA that includes weight and non-weight bearing sites is also needed in this population.

Most of all, interventions designed for children with obesity need to be realistic and reflective of their everyday living for goals to be sustained. Canadian dietary and physical activity guidelines have been established; however, on average, Canadian children and youth are not meeting these recommendations. Currently, there have been no Canadian reports of a family-centered lifestyle intervention based on these current guidelines as a treatment option for children with obesity. Further, given the importance of milk and milk products and weight-bearing types of activities on bone health, and possibly body composition, a trial designed to test these effects should be reported.

The following chapter will describe the study protocol of which this dissertation is based upon, providing a detailed overview of the assessment tools that will be described in subsequent chapters. Specifically, this chapter will detail the intervention platform, describe the various questionnaires that were developed to capture different study outcomes.

CHAPTER 3

Manuscript 1: Study Design and Protocol

Published: *BMC Public Health*, 2013; 13:383
Article reprinted in accordance with BioMed Central's Open Access Charter

A family-centered lifestyle intervention to improve body composition and bone mass in overweight and obese children 6 through 8 years: A randomized controlled trial study protocol

Tamara R. Cohen[1], Tom J. Hazell[2], Catherine A. Vanstone[1], Hugues Plourde[1], Celia J. Rodd[3], Hope A. Weiler[1]

Author's Affiliation:

[1] School of Human Nutrition, McGill University, Montreal, QC
[2] Department of Kinesiology and Physical Education, University of Lethbridge, Lethbridge, AB
[3] Department of Pediatrics, McGill University, Montreal, QC
Montreal Children's Hospital, Montreal, QC

Corresponding Author:

Hope A. Weiler
McGill University, School of Human Nutrition
21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Québec H9X 3V9 Canada
Tel: (514) 398-7905
Fax: (514) 398-7739
E-mail: hope.weiler@mcgill.ca

3.1 ABSTRACT

Background: Childhood obesity gives rise to health complications including impaired musculoskeletal development that associates with increased risk of fractures. Prevention and treatment programs should focus on nutrition education, increasing physical activity (PA), reducing sedentary behaviours, and should monitor bone mass as a component of body composition. To ensure lifestyle changes are sustained in the home environment, programs need to be family-centered. To date, no study has reported on a family-centered lifestyle intervention for obese children that aims to not only ameliorate adiposity, but also support increases in bone and lean muscle mass. Furthermore, it is unknown if programs of such nature can also favorably change eating and activity behaviors. The aim of this study is to determine the effects of a 1 y family-centered lifestyle intervention, focused on both nutrient dense foods including increased intakes of milk and alternatives, plus total and weight-bearing PA, on body composition and bone mass in overweight or obese children.

Methods: The study design is a randomized controlled trial for overweight or obese children (6-8 y). Participants are randomized to control, standard treatment (StnTx) or modified treatment (ModTx). This study is family-centred and includes individualized counselling sessions on nutrition, PA and sedentary behaviors occurring 4 weeks after baseline for 5 months, then at the end of month 8. The control group receives counselling at the end of the study. All groups are measured at baseline and every 3 months for the primary outcome of changes in body mass index z-scores. At each visit blood is drawn and children complete a researcher-administered behavior questionnaire and muscle function testing. Changes from baseline to 12 months in body fat (% and mass), waist circumference, lean body mass, bone (mineral content, mineral density, size and volumetric density), dietary intake, self-reported PA and sedentary behaviour are examined.

Discussion: This family-centered theory-based study permits for biochemical and physiological assessments. This trial will assess the effectiveness of the intervention at changing lifestyle behaviours by decreasing adiposity while enhancing lean and bone mass. If successful, the intervention proposed offers new insights for the management or treatment of childhood obesity.

3.2 BACKGROUND

Childhood obesity is a worldwide problem with immediate and long-term health consequences [245, 246]. Not only are obese children at a greater risk of developing medical complications that include cardiovascular disease, type 2 diabetes, hypertension and lipid disorders [246], they are also at risk for developing low self esteem and often experience psychological distress [247]. Furthermore, it is reported that obese children may also present with orthopaedic and bone-related problems, including lower bone mass for their body weight [202, 248], reduced bone strength [249] and increased risk of bone fractures [249, 250].

Modifiable determinants of childhood obesity include energy intake (diet) and energy expenditure (physical activity (PA) and inactivity); these two components typically form the basis of prevention and treatment programs. Health Canada's strategy to reduce childhood obesity addresses these two components, encouraging children to follow Canada's Food Guide (CFG) [23] and participate in daily moderate to vigorous cardiovascular PA for 60-minutes per day [251]. Despite public health initiatives, statistics show that 1 in 5 Canadian children have energy intakes that exceed their energy expenditure [119]. Although children exceed their energy intakes, 71%, 37%, and 27% of children 4 to 8 y are not meeting the recommended servings of vegetables and fruit, milk and alternatives, and cereal products, respectively, per day [119]. Furthermore, only 9% of boys and 4% of Canadian girls accumulate 60 minutes of moderate-to-vigorous PA on at least 6 days a week, averaging 8.6 hours per day, or 62%, of their waking hours being sedentary [252].

Interventions involving overweight or obese children should address the modifiable behaviors of obesity together, including discussions about both diet and physical activity [253, 254]. They should also be "family-centered" with at least one parent involved in the program [215]. The Transtheoretical Model (TTM) allows for assessment of participant awareness and acceptance of the obesity problem including their desires to change behaviors [255]. Applying TTM in programs can be accomplished through different interviewing techniques and counselling strategies in order to achieve individual goals [256]. Interventions should be sensitive to culture and socioeconomic status, as specific population groups may differ in terms of cultural needs and realistic lifestyle changes [257]. In addition, programs should to be tailored and include strategies such as goal setting [258], and educational sessions conducted by health professionals who deliver short and simple messages [259].

It is important that intervention programs start young, as health consequences can carry throughout adolescences to [245, 246]. Programs targeting childhood obesity have been published using different study designs and interventions, therefore it is difficult to determine if the effects of the interventions favourably change body mass index (BMI) z-scores and adiposity (fat mass) [219, 260]. Musculoskeletal health of obese children has also been examined, showing that despite having higher bone mineral density (BMD) and bone size [202, 248], obese children are at an increased risk of facture [170, 261]. Studies assessing bone geometry and strength propose that the bones are not as "developed" and that the extra weight (fat mass) negatively effects bone [170]. The muscle-system is important for cortical bone health [262] and despite having elevated BMD, the increased weight on the cortical mass may be too high, thereby leading to increased fractures [170, 261]. Furthermore, bone adapts to dynamic forces of muscle contractions, not by static forces of fat mass [263].

Research that has studied bone health in children uses different methodology techniques for assessing bone [171, 261]. Recent studies that have use both dual-energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) allow for assessment of volumetric BMD (vBMD) (g/cm^3) and bone geometry and not exclusively area BMD (aBMD) by DXA [261]. This has allowed for the discovery of negative relation to cortical and trabecular vBMD in obese children [27]. The relationship between bone and obesity also extends to environmental behaviors, including physical activity and nutrition [264]; research suggests that low circulating 25-hydroxyvitamin D (25(OH)D) concentrations [265-267] due to decreased sun exposure from sedentary lifestyles [268], poor diet [265], and increased vitamin D storage as it sequesters in adipose tissue [161, 207]. Studies have attempted to enhance bone mass in children using dairy foods [181, 269, 270] or calcium supplements [271-274]. Doubling of calcium intake (from 750 mg/d to 1600 mg/d) in non-obese children enhanced bone density by 18.8% [180], suggesting a possible role for higher milk (calcium and vitamin D) intakes in improving bone health [275]. In addition to increased calcium intake, adding weight-bearing PA improves bone mass [276] and whole body bone mineral density (WB BMD) [277].

Despite the established role of dietary calcium and milk product intakes on developing healthy bone mass, the contribution of milk and alternatives (i.e., cheese, yogurt, cottage cheese, soy beverages) and/or calcium to the achievement or maintenance of healthy body weight is less clear [230, 278-280]. Reviews that aim to conclude if increased dairy or calcium intakes affect

body weight report mixed evidence [279, 281]. To date no study has evaluated the effects of increased milk and alternatives plus total and weight-bearing PA on body composition and indices of bone health in prepubescent children, using a family-centered lifestyle intervention encompassing both education and behavioral modifying techniques. A study that assesses bone and body composition in addition to biomarkers of bone and obesity in overweight/ obese children who participate in a lifestyle intervention by both DXA and pQCT is warranted [27].

To our knowledge, there is no study that has examined the effects of a family-centered lifestyle intervention that investigated the altered bone structure [28] or modulators of bone health in addition to those that can reduce adiposity [282]. This paper shares a comprehensive approach that will be used in a randomized controlled trial of a 1-y lifestyle intervention in overweight and obese prepubescent children. This study will involve two levels of intervention: one focused on meeting recommended age-specific targets for nutrition and physical activity (standard intervention [StnTx]) and a second focused on increased milk and alternatives consumption and weight-bearing activities (modified intervention [ModTx]) to enhance support for bone mass. It is hypothesized that children receiving the ModTx will have a lower BMI z-score, lower waist circumference and improved weight-for-age z-score and increased bone mass and strength at 12-months compared to children in the StnTx and control groups. Secondary objectives are to examine the effects of the intervention on changes of children's eating behaviour throughout the intervention compared to control.

This study, the McGill Youth Lifestyle Intervention for Food and Exercise (MY LIFE Study), embraces the family-centered approach in a non-institutional setting. The primary focus of the MY LIFE Study is to create realistic nutrition and PA goals, while providing families with the necessary education and behavioral tools necessary to help the child meet their immediate and long-term goals of attaining a healthy lifestyle. The study methods are in accordance with the CONSORT guidelines for reporting randomized trials [283]. Ethical approvals were obtained from McGill University Faculty of Medicine Institutional Review Board and Montreal Public School Boards. The MY LIFE Study is registered online at www.Clinicaltrials.gov (Trial # NCT01290016).

3.3 METHODS

Participants

Eligible participants are healthy children 6-8 y, living in or near Montréal, Québec, who are overweight or obese according to the World Health Organization weight-for-height BMI cut-off criteria [38]. In addition to parental consent, participants provide written informed assent by reading and signing an assent form written at the grade 1 level. Participants must speak either English or French. Only one eligible child per household is included in the study. Exclusion criteria includes: (1) known or suspected serious, chronic illness of childhood, such as cancer, Crohn's disease, nephrotic syndrome, rheumatic conditions, and diabetes, etc., or those with disturbances in bone, vitamin D or mineral ion metabolism including rickets, osteomalacia, liver disease, renal disease, immobilization (complete or partial), current fractures, and disorders of the parathyroid gland; (2) use in the past 3 months, medications known to affect bone and/or mineral ion metabolism including all glucocorticoids, phosphate therapy or vitamin D analogues and any bisphosphonates; (3) severe anemia precluding blood sampling (previously diagnosed); (4) established diabetes mellitus (any type) and (5) hyperlipidemia ascribed to non-dietary causes.

Recruitment strategy

Participants are recruited through public and private elementary schools (kindergarten through grade 3), primary healthcare organizations including physician referrals, word of mouth and local advertisements through newspaper, internet, radio commercials and postal mailings. Recruitment in schools included placing a bilingual study brochure in the children's homework folders. Due to the sensitivity of the topic, all brochures are placed in envelopes addressed "*To the Parents/ Pour les parents*". Interested participants contacted the research team by electronic mail or telephone. Participants are screened for eligibility by a researcher during the initial telephone call. If eligible, the family is scheduled for a baseline assessment within three weeks of the screening call at the research unit.

Outcomes

The primary outcome of this study is change in BMI z-score from baseline to 12 months. Secondary outcome measures include changes in body weight (kg), waist circumference (cm), %

body fat (%), lean body mass (g), BMD (g) and bone mineral content (BMC: g), architecture of bone (radius and tibia) as well as bone strength. Changes in food intake, PA and average time spent engaging in sedentary activity (minutes/day) are also assessed. Other outcomes include changes in eating behaviour and child's perceptions of healthy nutrition and physical activity behaviours.

Randomization and blinding

Children are randomized by a computer-generated list using stratified block randomization of three per block to maintain balance. Two stratification factors are implemented: gender and BMI (overweight and obese). To maintain blinding for non-intervention related measurements, children are randomized to control, StnTx or ModTx groups by the registered dietitian who conducts the interventions. Participants are informed of their group at the end of their baseline visit. All other research staff are blinded.

Study protocol

All assessments take place at the same research unit every 3 months (**Table 3.1**). Appointments are scheduled at the convenience of the family including weekend and evenings.

Baseline

All participants arrive at the research unit fasted for 12-hours. Child assent and parent consent are obtained. Child's weight and height are measured to confirm BMI criteria are met. All children are tested for normal blood glucose using a glucometer; those with blood glucose above ≥6.9 mmol/L are advised to seek subsequent medical assessment and are not enrolled in the study. Those with a normal blood glucose value (<6.9 mmol/L) have a 9-mL blood sample taken. Parents complete a socio-demographic questionnaire and with a researcher, the child answers a series of questions from a study-specific questionnaire (*Child Intention Questionnaire* (CIQ)). All families receive a general nutrition and PA educational session by a registered dietitian. Parents are instructed how to complete a 3-day food diary; they are provided with a pre-paid envelope and asked to mail the diary once completed. The food diary will capture 3 non-consecutive days including one weekend day. At the end of the baseline visit, children choose from a ball, ball and racket or jumping rope, as a part of remuneration for their time with

the dual purpose to encourage physical activity. Parents are told which group they are in and schedule their next visit.

Follow-up visits

Children return to the clinic every 3 months in the 12-h fasted state for blood sampling (**Table 3.1**). Parents complete a questionnaire about child's eating behavior, physical activity level, and sun exposure. During these visits, blood tests, anthropometry, body composition, bone assessments, and muscle function tests are performed. The CIQ is also completed. A dietitian is present at all follow-up visits to ensure parents receive the same nutrition and physical activity advice throughout the study. We believe that families require continuous support and therefore having a dietitian present at all study visits helps reduce participant withdrawal, especially for the control group.

Measurements

1. Biochemistry

Immediately after blood is drawn, plasma samples are spun for 20-minutes. Serum samples are spun after 30 min, and used to measure insulin (0.5 mL) while plasma (0.5 mL) is used for measurement of glucose, liver enzymes (alanine transaminase and aspartate transaminase) and lipids (low-density lipoprotein, high-density lipoprotein , total cholesterol, triglycerides and hormones) using autoanalzyers (Beckman Access and Beckman DxC600 CA, USA) at the Montreal Children's Hospital clinical chemistry laboratory (certified by the provincial quality assurance program, the Laboratoire de santé publique du QC). These samples are sent for analysis to the Montreal Children's Hospital within 4-hours of blood sampling. A pediatrician reviews all biochemical data; abnormal results are addressed on an individual basis to ensure children are healthy. These biochemical outcomes are in line with the 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [284].

In addition to the recommended biochemistry, we will also examine in our research unit the following in the view of bone health outcomes and satiety. Vitamin D status is examined using plasma 25(OH)D concentration, as well as osteocalcin and parathyroid hormone concentrations using an autoanalyzer (Liaison, DiaSorin, Ontario, Canada). Plasma is reserved to measure 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) as needed using LC-MS/MS (Warnex

Bioanalytical Services, Quebec, Canada). Blood samples to analyze satiety markers are prepared by adding inhibitors (20 uL AEBSF and 20 uL DPP-4) to one 2 mL tube. Satiety markers are measured at McGill University using multiplex assays and Luminex technology (EMD Millipore Corporation, Billerica, MA, USA): ghrelin (acylated and nonacylated), GLP-1, neuropeptide YY, adiponectin and leptin. For red blood cell analysis, methanol-H_2O-BHT is added in equivalent volume to red blood cell volume and spun; these samples can be used to assess the fatty acid content of red blood cells. All CV% are <5% and typically 1 to 2 %. HOMA-IR is calculated as the product of the fasting insulin (IU/mL) and plasma glucose (mmol/L)/22.5 [285]; HOMA-IR has been validated for use in children [286].

2. Anthropometry

Anthropometry is measured using standard practices: height is measured to the nearest 0.1 cm using a stadiometer (Seca 214, Hamburg, Germany) and weight is measured to the nearest 0.1 lb using a calibrated balance-beam scale (Detecto, Missouri, USA) in standardized children's clothing (facility's cotton shorts, T-shirt, socks). Weight is converted to kg and BMI is calculated (kg/m^2) and z-scores are derived using the World Health Organization's ANTHRO software (version 3.2.2, January 2011, Switzerland, Europe). Waist circumference is measured to the nearest 0.1 cm at the umbilicus [287]. All values are expressed in absolute units with standard deviation scores using the data from the World Health Organization [288].

3. Health measures

During all visits, a registered nurse measures blood pressure using a standard sphygmomanometer (Trimline PyMaH Corp, Flemington, New Jersey, USA) and manually takes the child's pulse. Pubertal stage is assessed by parent's identification of physical development using the Tanner criteria [289, 290]. Fasting serum concentrations of luteinizing hormone (LH) in boys, and LH and estradiol (E2) in girls is used to confirm Tanner stage.

4. Body and bone assessment

Dual-energy x-ray absorptiometry (DXA): DXA is used in the pediatric population to provide measurements of BMC and areal-BMD (aBMD) [291]. As per the International Society for Clinical Densitometry [291], whole body, lumbar spine vertebrae 1 to 4, total hip and forearm

(non-dominant) BMC and aBMD are measured using a Hologic 4500A clinical densitometer (APEX version 13.2:3, Hologic QDR-4500A Discovery Series, Bedford, MA). For this test, children wear standardized clothing (cotton shorts, T-shirt, socks). Values are compared to the Hologic normative databases and thus expressed as absolute BMC and BMD along with z-scores where applicable [291]. This Hologic model also measures soft tissue composition from the whole body scan, but does not distinguish subcutaneous from visceral fat depots. Information about android and gynoid regions, adipose indices (example: ratio of fat mass: height (kg/m^2)) and lean plus BMC indices (example: [lean + BMC]/ height (kg/m^2)) are also available and have been validated in children [292].

To complement the waist circumference measures, abdominal adiposity will be estimated using sub-region analysis of the full torso. Total body fat (kg and %) and trunk fat (kg) values are available from the DXA software. Although DXA measures of trunk fat mass reflect fatty elements in soft tissue as well as adipose tissue in subcutaneous and visceral depots, DXA-derived measures account for 80% of the variation in intra-abdominal fat as measured by computed tomography (CT) [248] and explain 79% of the variance in insulin sensitivity [293]. Values for total abdominal fat obtained using DXA are not different from CT measures [294]. Phantom scans are conducted daily to maintain quality assurance and the CV% for the phantom BMD and BMC will be calculated over the study period.

Peripheral quantitative computed tomography (pQCT): To measure volumetric BMD (vBMD) as well as bone architecture, pQCT (XCT-2000; Stratec, Pforzheim, Germany) will be used [295]. This technique distinguishes the type of bone (cortical and trabecular) [296] in the non-dominant radius and tibia. Dominance will be established by asking the child which hand they write with; that side determines the dominant leg too. A 30-mm planar scout view is used to locate a standard anatomical site for the reference line at the distal end of the limb being measured. For the radius, length of the non-dominant forearm is measured as the distance between the olecranon and the styloid process forming the basis for the location of the distal and proximal slices. Single axis 2.5-mm slices (voxel size, 0.5 mm) are measured 4% and 66% proximally from the distal end of the radius. For the tibia, length of the non-dominant tibia is measured as the distance from the distance between the palpated lateral condyle and the lateral malleolus. Single slices are measured 4%, 14% 38% and 66% proximally from the distal end of the radius and tibia.

The scans are analyzed using contour mode 2 (45%) and peel mode 1 to assess total (TB) and trabecular bone (Trab) parameters at the 4% site. At the 66% site, cortical bone (Cort) is detected with separation mode 1 and a threshold of 710 mg/cm^3. A similar method will be used for distal tibia. Normative data for adults not pediatrics exist for this model [297] and thus values will be tested for changes over time or as absolute changes. vBMD measurements are performed with a repeated-measures program that allows the starting point to be set according to previous measurements. Phantom scans are conducted daily to maintain quality assurance and the CV% for the phantom vBMD will be calculated over the study period. Data is analyzed using the manufacturer's software package in which the outer contour of bone is defined with a threshold of 280 mg/cm^3.

5. Muscle function

At each research visit, balance and muscle strength are determined using standardized manoeuvres on a force plate (Model 9260AA, Kistler Instrument Corp., NY, USA). This force plate measures ground reaction forces, moments, and centre of pressure during balance testing using a piezoelectric 3-component force sensor in 3 planar directions. Manoeuvres include two-legged jumps, sit-to-stand exercises and a balance test. First, in 5-second increments, children are instructed to stand on the platform, bend their knees slightly and jump as high as they can with their hands freely beside them. They perform 6 repetitions. Secondly, seated with legs at a 90-degree angle and feet firmly placed on the platform, children "hug themselves" and rise out of the chair into a standing position (~4 seconds) and then sit back down in the chair back to the initial seating position for 4 seconds before repeating this exercise for 6 repetitions. To assess balance, children were told to either close or open their eyes for 25 seconds at a time. This was done 3 times using a randomly generated list. All data is analysed using Bioware Software (Kistler Instrument Corp.) and statistically used with DXA, pQCT, PA questionnaire and dietary data to assess for changes in muscle strength throughout the intervention.

6. Demographics and home setting

At baseline only, parents complete a questionnaire that contains questions adapted from the Canadian Community Health Survey (CCHS) [37]. This questionnaire asks for parental age at birth of the index child, education, ethnicity, family income range and current employment.

Neighbourhood environment is assessed by asking parents to report on proximity to outdoor and indoor recreational facilities, supermarkets and neighbourhood safety [298]. These questions are found in the study-created questionnaire titled the "Family Health Questionnaire" (FHQ).

7. Parental perceptions of child obesity, intentions, nutrition knowledge and perceived behavioral control

Parents complete a set of questions about previous dieting and weight history, specifically how they perceive themselves as a child, adolescent and adult prior to birth of the participant [255]. They self-report current weight and height and report physical activity practices using the validated International Physical Activity Questionnaire [299]. Parents are also surveyed on their perceptions of their child's weight using a visual analog scale [300]. Nutrition beliefs and practices regarding healthy snacks and milk are assessed using questions from a validated nutrition survey [301]. Parental intention and perceived behavioral control are addressed by asking parents: "*How committed are you to participating regularly in family physical activity over the next month*?" and "*If you were motivated, how confident are you that you could participate in regular family-based physical activity over the next month*?" Parents answer on a 7-point Likert scale: "extremely uncommitted"; "very uncommitted"; "somewhat uncommitted"; "somewhat committed"; "committed"; "very committed" and "extremely committed" [302]. These questions form part of the FHQ.

8. Daily physical activity

Parents report their child's weekly physical activity by completing a modified Physical Activity Questionnaire–Older Children (PAQ-C) [303]. The PAQ-C is valid for 8 y old children (Canadian) and reflects the past week and captures activity in general, at school (physical education classes, recess, lunch), after school and weekends. This questionnaire does not include time and intensity and was therefore modified using the Canada Fitness Survey for children 7 y of age and older [304]. Modifications included using the duration and intensity format from the fitness survey and metabolic equivalent calculations. We also included "other" questions for PAQ-C to capture screen time including video gaming and surveying non-active or interactive involvement. The PAQ-C is part of our study questionnaire titled the "Child Health Questionnaire" (CHQ).

Validation of mPAQ-C: In view of our modified PAQ-C, 30 children (n=10/group), are asked to complete a 7-day activity diary to serve as a mini-validation of our modified questionnaire. To reduce participate burden the 7-day diary will be voluntary at a convenient time. This validation will include a pedometer (StepCounts Co., Diabeters Inc., Deep River, ON) and accelerometer (ActiGraph GT3X, ActiGraph LLC, Fort Walton Beach, Florida). During a visit, children are shown how to wear both accelerometer and pedometer, how to conduct a 20-step test and how to reset the pedometer. Children are sent home with the pedometer, accelerometer, a pedometer log book, activity diary and a copy of the modified PAQ-C. Children wear the pedometer and accelerometer for 7 days and at night record the pedometer wear time. Each day, child and parent complete the activity diary reflecting the activity done on that day, including the intensity of activity. To validate the questionnaire, at the end of the week, parents complete the modified PAQ-C and mail all material back to the research unit. Data analysis includes comparing pedometer log books to accelerometer readings. Activity diary, log book and accelerometer analysis is used to validate the modified PAQ-C by quantifying activity in terms of intensity (light, moderate or vigorous), type of activity and metabolic equivalents.

9. Dietary intake

Dietary intake is documented using 3-day food diaries. Parents complete the food diaries before each visit at baseline, 3, 6, 9 and 12 mo. These diaries reflect 2 weekdays and 1 weekend day and have been used previously in this age group [305]. Diaries are analyzed using Nutritionist Pro software (Axxya Systems, Stafford, TX), a program that uses the Canadian Nutrient File 2010. Data are analyzed according to CFG food groups, foods considered “extras”, macronutrients (carbohydrate, protein and fat), as well as micronutrients (calcium, vitamin D, sodium, potassium and iron). Differences in dietary intake are analysed throughout the study and considered as possible covariates in all statistical analyses.

10. Exposure to ultraviolet B

To augment other information (outdoor activity, mobilization of fat stores or milk intake) as related to vitamin D status, exposure to sunshine, typical clothing habits, duration and frequency of exposure to sun, travel, and use of sunscreen are assessed using a questionnaire, which is found in the CHQ. To complement this questionnaire, we measure skin pigmentation

using a computerized narrow band reflectometer spectrophotometer (CM-700d/600d, Konica Minolta, Ramsey, NJ, USA). The spectrophotometer provides measures of melanin content of the skin or *Melanin Index* which has been shown to influence circulating 25(OH)D concentrations [306]. Measurements performed on the unexposed skin of the inner upper arm have higher correlation to the *Melanin Index* than measurements performed on the exposed forehead [307] according to standard guidelines [308]. At each visit, skin pigmentation established by measuring pigmentation three times at each site for constitutive pigmentation at the inner upper arm and facultative pigmentation at the forehead, mid-forearm and lower leg using the spectrophotometer. Individual typological angle (ITA^{o}) is calculated with the L* and b* values using the equation from the Commission D'Éclairage [309] and are classified into 6 skin types based on Fitzpatrick descriptions [310, 311].

11. Parental perception of child eating behavior

Parents self-report on their child's eating behaviour by completing the Child Eating Behaviour Questionnaire (CEBQ) [92]. This validated questionnaire for children 4 to 13 requires parents to score 35 questions identifying different eating behaviours as "never", "rarely", "sometimes", "often" or "always" [18, 312, 313]. This 2-page survey identifies 7 eating styles: (1) Food Responsiveness; (2) Enjoyment of Food; (3) Emotional Overeating; (4) Emotional Under Eating; (5) Desire to Drink; (6) Satiety Responsiveness; (7) Slowness in Eating; and (7) Food Fussiness. The CEBQ is completed at each research visit to help identify changes in child's eating styles throughout the year and interventions. Unique to other studies, this questionnaire is used to individualization of the intervention sessions, by guiding the interventionist to discuss and prioritize negative eating behaviours. This questionnaire is part of our CHQ.

12. Child intention and self-perception of nutrition and physical activity

A series of questions asked by a trained researcher to the child was created on the basis of the theory of planned behaviour [314]. Briefly, this theory postulates that human behaviour is guided by behavioral beliefs (e.g., consequences or attributes of the behavior), normative beliefs (e.g., expectations of others) and control beliefs (e.g., presence of factors that hinder or enhance behavior) [315]. These concepts are captured in the Child Intention Questionnaire (CIQ), a questionnaire that is research-administered every visit and asks children about their intentions

and behaviours for engaging in physical activity, self-perception, attitude towards physical activity and nutrition and behavioural control [316, 317]. Children self-identify themselves by identifying a picture that best describes them. The concept of self-perception is identified as it will influence decision making and ultimately the child's actions [318]. Using a gender specific 5-point visual analogue scale, children identify how they feel about certain activities. Intensity of activity is determined using a gender specific Rated Perceived Exertion Scale [319]. With parental permission, this questionnaire is completed without the presence of the parents or family. This type of questionnaire is important to include in a study of this nature, as it identifies concepts that are important in weight interventions, especially when evaluating the family's confidence in achieving a goal or changing a behavior. If interventions do not include such questions, it is likely that goals will not be met and negative feelings towards the program may occur [318].

Intervention

In line with the TTM [255] and the Theory of Planned Behavior [320], this study is based on similar principles of the "Obeldicks Light" framework [321]. Our intervention is based on physical activity, nutrition education and behavioral counselling. Interventions are family-centered and use motivational interviewing techniques, specifically engaging in reflective listening, sharing decision making with the child and families, and setting realistic goals with the child [322]. For example, the interventionist suggests goals to the child and as a group, all present discuss how the goals will be attained. A goal is never set unless the child agrees to the goal and can independently strategize how they can achieve the goal in a realistic manner. All members of the family are encouraged to participate in the interventions particularly those caregivers who have a direct impact on the child's eating and activity (i.e., meal preparation, afterschool care etc.).

Intervention sessions are held at the end of each of the first 5-mo of the study, at the end of 8-mo, concluding with a debriefing session at 12 mo. Families participate in a total of 9 hours of counselling, based on 5 x 1.5 h visits at the end of months 1 through 5. The additional visit at the end of 8 months (1.5 h) is used to determine if new diet and activity intervention strategies are necessary over time as the child grows/matures and due to season. Counselling visits at the end of months 2, 5 and 8 overlap with regular study visits and are conducted after study data is

collected to limit leading and bias (same day if need be to limit travel). **Table 3.2** outlines the overall study design. All interventions have been designed by and will be carried out by a bilingual registered dietitian with experience in physical education.

The overall focus of the intervention is to help families make healthier lifestyle choices in terms of nutrition and physical activity, and to reduce screen time and other (sedentary behaviours). Differences between StnTx and ModTx intervention lie in the CFG for children aged 4-8 y for all food group servings (**Table 3.3**). The modified intervention group is instructed to consume 4 servings of milk and alternatives per day. Although both interventions will be counselled to participate in 60-minutes of activity per day, special emphasis placed on activities that are also weight-bearing for ModTx. Examples of weight-bearing activities include jumping rope, brisk walking, dancing, racket sports (i.e., tennis) and soccer. Notably, these are also aerobic activities.

All intervention sessions are recorded using a standardized tracking sheet for all components, ensuring all sessions follow the same structure with room for individualism and variety. Within each session, the dietitian identifies a key area that requires further discussion, which is either identified by evaluating the CEBQ [92] or through parent concern or a concept that the interventionist has picked-up on during the session. This provides for individualization of sessions to meet family needs and to provide for self-directed change. All sessions are directed at the index child and are performed at the grade 1 reading and learning level.

Components of the interventions

Education: The underlying principles of nutrition education are embedded in both StnTx and ModTx groups. The goal of the education sessions is to improve self-efficacy through empowerment of healthy choices and behaviors. **Table 3.3** details the education topics for each session. In brief, session 1 reviews the food and exercise guides. During session 2, families are taught how to read food labels and identify healthy food choices from labels using a tear-sheet from Health Canada. Session 3 reviews eating out at restaurants or other environments. Session 4 asks children to create a meal plan using food models; during this session children are also taught about different types of hunger, a concept modeled from the Craving Change© series that focuses on three types of hunger (e.g., “tummy hunger”, “mouth and eye hunger” and “heart hunger”) [323]. Sessions 5 and 6 are open to reviewing concepts already discussed, and are

necessary to discuss relapse prevention and reinforce positive lifestyle and behavioral changes. In line with TTM, sessions 5 and 6 are critical components of an intervention program as it ensures children and parents are able to remain in the action phase of change [255]. In addition, these sessions are used to increase self-efficacy of the child by discussing challenges that may arise and how to sustain positive thinking and motivation to stay on track.

Nutrition intervention and nutrition evaluation: According to the randomization, the dietitian counsel's children to consume either 2 servings per day or 4 servings per day of milk and alternatives. Two servings per day is the recommendation for this age group (6-8 y). The child is given a certificate to remind parents of their specific milk and alternative requirements. Parents are also provided with the extended Health Canada list of milk and alternatives to ensure they understand this food group well. At this time, the dietitian works with the parents and child to strategize ways they can meet their recommendation. Ethically, and in line with Canada's Food Guide, children who turn 9 will be instructed to consume 3 or 4 servings of milk and alternative as the recommendations suggest, but 3 per day accepted as meeting the servings.

At every intervention, parents and children complete a 24-h recall. Using the recall, the dietitian reinforces the appropriate quantity of milk and alternatives according to their group. If they are not meeting the recommendations, time is taken to discuss measures to get back on track and to identify barriers to change. Children are evaluated on their understanding of healthy snack choices using a modified traffic light diet evaluation, a form of evaluation often used with children [208]. The original traffic light diet uses a color-coded, calorie based exchange system [211]. We used the same concepts of coloring food choices and ask children to identify "go to foods" (green), "foods that are healthy but when eaten too much become unhealthy (yellow), and "foods that should be avoided on a routine basis, or "sometimes foods" (red). If necessary, the interventionist coaches the child by repeating the description of the food classification, but does not answer for the child. The interventionist records the answers and categorized by food group, or extra-foods (salty, sugary).

Physical activity intervention: Daily physical activity is discussed at every session. Children are asked if they remember the activity recommendations and how they can achieve it. Discussions are individualized per family using the *FITT principle*: frequency of activity, intensity, time and type [324]. Children are instructed to rate the intensity of their activities using a modified Borg Scale [319], which shows a child climbing stairs. Children rate their intensity by

identifying the picture of the child on the stairs, with at the bottom "1" representing low intensity to the top with heavily sweating, red in the face and needing to stop "10" representing high intensity. At every session, children are encouraged to attain a 6-8 scale-rating (feeling sweaty, heart beating fast) for a total of 60-min of activity per day [251]. Using this scale not only allows researchers to understand child's perceived intensity of activity, but also links to child's self-efficacy. Different from the standard intervention, the modified intervention focuses on weight-bearing activities, such as jumping, running, or light strength training activities. These activities are recommended by the Canadian Society of Exercise Physiology and Health Canada and are monitored at every session [251].

Sedentary behaviour intervention: Time spent engaging in screen time is discussed during each intervention. Parents and children are taught that screen-time should be less than 2-h per day [105]; this includes computer gaming, telephone or internet time not related to schooling and television. Children are provided with a series of activities at each intervention session that will help them decrease their screen-time behaviours. They are asked to bring the completed activities to the next session for discussion. Specifically, session 1 asks children to record their total TV time for one-week using a calendar divided by 30-min slots. At the end of each day, if they successfully watch less than 2-h per day, they are asked to color in a star. Session 2 children are encouraged to play a BINGO activity where they choose a different activity to play each day using a similar BINGO template. The time spent performing each activity is set by the child. Session 3 includes all family members as they try to complete a 1-month walking calendar where children color in feet (1 foot=30 min of activity). All family members are encouraged to participate in this activity. Session 4 asks children to play a game based on a walk from their house to a castle, for every 30-min of moderate-intensity activity they may progress in the game by coloring in footstep towards the castle. Sessions 5 and 6 are open to provide children the activity they preferred the most and wish to complete, such as the walking calendar or BINGO. Should the child watch less than 2-hours of screen time prior to the intervention, sedentary activity is still discussed, monitored and positively reinforced.

Overcoming barriers: Relapse prevention: Each visit includes a discussion with the dietitian about "tricky situations"; situations where one would either not follow CFG or have time to engage in PA. These situations may place children in a vulnerable situation where they know they will not be necessarily practicing healthy lifestyle choices, suggesting they may resort

back to old habits of unhealthy lifestyles. Examples of tricky situations include birthdays, holidays, other celebrations, "sleep-over" parties, rainy days and vacations. With the help of the interventionist, realistic strategies and solutions are discussed to help child and family handle the situation to ensure they stay on track either on the day of or after the event

Goal Setting: Goal setting is a valid tool with this age group [318] and is a successful component of treatment programs. Goal setting with children provides insight to the child's attitude and level of understanding of discussions during the intervention. Using a template adapted from Health Canada (Website: http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/educ-comm/toolkit-trousse/plan-3a-eng.php), three SMART goals are set by the child. The goals must consist of at least one nutrition and one physical activity goal. The parents are present and can help suggest goals, but the child decides which goals they wish to pursue. The SMART goals consist of the following: *specific:* "What do I want to do?"; *measurable:* "How much and how often will I do it?"; *attainable:* "How will I do it?"; *realistic:* "Can I do it?" and *time:* "When will I do it/ When will I start?". The child signs the contract with the dietitian and is provided a copy for home. This is the final component of every intervention. Follow-up interventions always start with a review of goals. Children are reminded to be honest and to have open discussion as to why or why not the goals were attained in order to identify why some approaches work and barriers to others. Results from the interventions are recorded as: "yes, no or sometimes". Creating realistic goals that are attainable and set by the participant is in line with clinical recommendations [284].

Control

The control group visits the clinic every three months for the various assessments (**Table 3.1**). Ethically, appropriate care of the control group includes receiving the exact same number of counseling sessions at the end of the study. Specifically, after participating for one year, these families are offered the same number of counselling visits (**Table 3.2**). In terms of food group recommendations, specifically milk and alternatives, children will be instructed to follow the food guide appropriate for their age (i.e., 2-3 servings of milk and alternatives for 6-8 y old and 3 to 4 servings of milk and alternatives for ≥9-y old). Children are also encouraged to engage in 60 minutes of activity per day, including a variety of cardiovascular and strength training exercises. Frequency of these counselling sessions are determined by families.

Sample size calculation

One-hundred and sixteen overweight/ obese children will be recruited to participate in this study. Based on similar studies [320-322], this sample size provides 80% power at a 5% significance level (two-sided) to detect a mean of change of BMI z-score of -0.2 (SD 0.3). Estimating a 10% drop out rate, we aim to recruit 39 children for control, 39 children for StnTx and 39 children for ModTx.

Statistical analysis

All data is analyzed using SAS (Version 9.2, SAS Inc., Cary, NC). Summary statistics are computed for all baseline characteristics to ensure that the randomized treatment groups are not different, and reported as 95% confidence intervals for each group. When baseline imbalances occur between groups despite randomization, these are treated as covariates and adjusted for. Mean absolute and change in BMI z-score, waist circumference, lean, fat and bone mass, and biochemical indices are compared in each dose group under a mixed effects analysis of variance (ANOVA) model (similar to mixed effect regression as used in child obesity interventions [325]), with significant group differences localized by suitable post-hoc testing (e.g. Tukey method), again with adjustment for multiple comparisons to ensure a family-wise error rate of 0.05. Time course is evaluated under a mixed effects ANOVA model (with the addition of time as a random effect). Milk intake at baseline (0, 1 or 2 servings/d) may be used as a covariate. Relationships between outcomes are examined using correlation and regression analyses. For determinants of change in adiposity and bone mass, we explore age, sex, demographics, total energy intake, milk intake total activity or categories of inactive, activity etc., as possible "predictor" variables. During analysis of the data, when a mixed-effects regression model is deemed to give greater latitude in exploring the data we adopt that approach and consider logistic regression.

3.4 DISCUSSION

This is the first family-based lifestyle intervention that targets prepubescent overweight and obese children with the intention to ameliorate both body composition and bone mass. The study focuses on the family, recognizing that both child and parents need to set attainable goals that focus on the child and indirectly require change at the home environment level. If effective,

the tools and methods used in this study can be used in existing weight loss or child obesity treatment centers. A major strength of our study is our study design, that will determine if a program of such nature can support increases in lean mass, ameliorate impaired muscle function and increase bone density (areal and volumetric) as well as changes in adiposity (fat mass) over 1 year.

Our study is unique as it considers frameworks, such as behavioural and control beliefs of parent and self-identity of child [318]. From the child's perspective, our study will be able to track changes in these beliefs and behaviours through research-administered questionnaires. This will help us understand the child's intentions to change their behavior in order to sustain a lifestyle of healthy eating and regular physical activity. To date, our study is the first to develop a series of questions and track over 1 y changes in a child's personal beliefs, self-perceptions and behaviours. Furthermore, we will be able to correlate these behavioral changes with changes in BMI z-scores and changes in body composition from DXA. Unlike other studies, our intervention also includes a bone component, where we will be able to assess changes in dietary intake and physical activity as they relate to bone health, measured by DXA and pQCT.

The methods presented in this study build on previous work and findings in the research area of child education, dietetic practice and nutritional assessments. Few studies have examined the effects of a long intervention of 1 y to reduce adiposity in children. This study is adequately powered to investigate the changes in both bone and body composition as they relate to the study group.

Nevertheless, counselling obese children poses unique challenges and barriers, including lack of overall family involvement, identifying participant motivation, support services, time and reimbursement [326]. Although including all family members in all sessions is important, it is true for some it may not benefit the child, particularly if parental level of support or willingness to change behaviour is not beneficial to the child [327]. A strength of the MY LIFE Study is the focus on the family environment and allows for sessions to be tailored to family needs. Our study offers continuous support to families by having dietitians available at all visits and conducting all follow-up visits at times that meet family needs. It is important that clinicians are capable of identifying behaviors that contribute to the child's weight status and are able to work with the child to modify these behaviors [328]. The MY LIFE Study interventions are designed in a manner to support these needs, by using standard documentation techniques that utilize study

tools (CEBQ and PAQ-C) and still allow for variability of counselling topics. The MY LIFE Study provides an excellent example of how national educational tools, specifically the Canada's Food Guide and Physical Activity Guide, can be used in a clinical setting. We believe that employing a family-centered approach that considers the family environment, developmental stage, as well as food and activity preferences will improve the program's success. A focus on fun yet feasible activities will assist both children and parents in adhering to the program.

The results of the MY LIFE Study will be published in 2014 in relevant organizations and peer-reviewed academic journals. The study protocol presented in this paper is anticipated to help others who research the same area, and ultimately in creating treatment programs to combat childhood obesity.

3.5 ACKNOWLEDGEMENTS

TC is supported by Canadian Institute of Health Research Doctoral Research Award. HW is supported by Canada Research Chair and Canada Foundation for Innovation.

Table 3.1 The MY LIFE study design: Assessment and scheduling of baseline, follow-up visits and intervention sessions

	Baseline	3-mo			6-mo			9-mo			12-mo		
Blood Sampled [a]	●	●			●			●			●		
Anthropometry [b]	●	●			●			●			●		
Body Composition Assessment													
DXA [c]													
Whole-body	●	●			●			●			●		
Bone Assessments													
DXA [d]													
Whole Body	●	●			●			●			●		
Lumbar spine vertebrae 1-4 (AP and AP-Lateral)	●				●						●		
Total hip	●				●						●		
Forearm (1/3 distal radius)	●				●						●		
pQCT [e]													
Radius (%: 4, 66)	●				●						●		
Tibia (%: 4, 14, 38, 66)	●				●						●		
Muscle Function [f]	●	●			●			●			●		
Questionnaires													
Family Health (FHQ)	●												
Child Health (CHQ)	●	●			●			●			●		
Child Intention (CIQ)	●	●			●			●			●		
Baseline Education [g]	●	● [h]			● [h]			● [h]			● [h]		
Interventions													
Lifestyle intervention	**End of Mo**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Intervention groups		●	●	●	●	●			●				
Control group [i]													●

[a] Includes: Glucose, HbA1C, insulin, calcium, CBC-profile, lipid-profile, AST/ ALT, C-reactive protein, Estradiol, LH.
[b] Includes: Weight, height, BMI, waist circumference, blood pressure, pulse and skin pigmentation by spectrophotometer (CM-700d/600d, Konica Minolta, Ramsey, NJ, USA).
[c] Body composition assessed by DXA to yield: total mass (g), fat mass (g), lean + BMC (g) % BF, Android/Gynoid ratio.
[d] DXA measures of bone to yield: bone area (cm^2), BMC (g), BMD (g/cm^3).
[e] pQCT measures both bone content [i.e., cortical density (mg/cm^3)] and geometry [i.e. cortical area (mm^2)].
[f] Force plate assessment: jumping (force, power), sit-to-stand (force, power) and balance.
[g] Basic Education: Performed by a registered dietitian; a basic review of nutrition and PA guide.
[h] A dietitian is always present to answer questions and deal with age changes if in control.
[i] Control group receives the 6 lifestyle interventions after completion of 12-mo of study.

Table 3.2 Differences among Standard (StnInt) and Modified (ModInt) treatment groups

Standard Intervention	Modified Intervention	Control Group
NUTRITION Recommendations [a]		
Food Servings per day [b][23]: • Vegetables and Fruit: 5 • Grain Products: 4 • Milk and Alternatives: 2 • Meat and Alternatives: 1	Food Servings per day [b][23]: • Vegetables and Fruit: 5 • Grain Products: 4 • Milk and Alternatives: 4 • Meat and Alternatives: 1	Food Servings per day: • Will be based on the Canada Food Guide for their age.
PHYSICAL ACTIVITY Recommendations [a]		
Frequency: 7 days **Intensity:** moderate to vigorous **Type:** cardiovascular **Time:** 60 minutes **Frequency:** 3 days **Intensity:** light **Type:** strength **Time:** 30 minutes	**Frequency:** 7 days **Intensity:** moderate to vigorous **Type:** cardiovascular **Time:** 60 minutes **Frequency:** Daily **Intensity:** light **Type:** strength **Time:** 30 minutes	**Frequency:** 7 days **Intensity:** moderate to vigorous **Type:** cardiovascular **Time:** 60 minutes **Frequency:** 3 days **Intensity:** light **Type:** strength **Time:** 30 minutes
SEDENTARY ACTIVITY		
< 2 hours screen time per day	< 2 hours screen time per day	< 2 hours screen time per day

[a] Children are encouraged to meet these recommendations for nutrition and physical activity. These recommendations are based on Canada's Food Guide and the Canadian Physical Activity Guide. Children in the StnInt are reminded to engage in strength straining activities, but the discussions are not as in depth as ModInt group.

[b] If a child turns 9 their recommended to consume 6 Vegetables and Fruit; 5-6 Grains; 3 Milk and alternatives; 1 Meat and alternative.

Table 3.3 Intervention protocol for Standard (StnInt) and Modified (ModInt) treatment groups[a]

Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
EDUCATION					
1. Review CFG and PA guide [b]	1. Understanding Food labels [c]	1. Eating "out and about" (eating in other environments other than home)	1. Making a meal plan 2. Identifying hunger cues	1. Tricky situations	1. Staying on track
Each session will also include discussions concerning:					
• Eating behaviours • Reviewing the dairy intervention and strategizing how to stay on track • Evaluating healthy food choices (Traffic light evaluation) • Physical activity (frequency, type, time and intensity) discussion • Sedentary activity (screen time) and provide alternative activities • Relapse prevention through identification of a "tricky situation" (i.e., birthday party, vacation, holiday, sleepovers, rainy/ snowy days) • Three SMART Goals					

CFGHE: Canada's Food Guide to Healthy Eating, PA: Physical Activity

[a] Control group will receive the same visit format but at the end of 12-months of the study.

[b] According to randomization, children are instructed to consume 2 or 4 servings of milk and milk alternatives per day.

[c] Parents are encouraged to bring in food labels from home to ensure discussions are individualized.

Bridge Statement 2

The study protocol described in chapter 3 was a detailed overview of all assessments performed during the MY LIFE study. It provides an outline as to how each measurement was obtained which could then be used for interpretation in statistical analyses. This paper also reviewed the intervention platform used in this trial and included a discussion of the conceptual theories and models which allows for reproduction in subsequent studies. The MY LIFE study is comprehensive: it features many different themes, ranging from biological to physical to psychological. This study includes assessment methods that are valid; others were tested for validity.

The MY LIFE study's primary objectives were to assess the overall impact on changing adiposity over 1 year participation. The modified intervention group were guided to increase, or double, their recommended servings of milk and milk products per day compared to standard treatment, who were asked to consume as per current Canadian guidelines. In addition, the modified group were also encouraged to perform daily weight-bearing types of activities, whereas the standard treatment group were guided to participate in these forms of activities 3x/ wk. The control group participated in study visits every 3 months. The following chapter aims to address the differences among groups on changes in body composition.

The literature review of this dissertation discussed studies that included a milk intervention using standard methods of dietary assessment to test compliance (i.e., 24-h recalls, 3-day food diaries, food frequency questionnaires); objective methods of assessment would provide greater insight to the changes in dietary practices as they are free of measurement bias possibly associated with dietary recall error or analysis. Although there are studies that have includes these dairy biomarkers in their study designs, majority test compliance to changing milk fat intakes and none report its use in children with obesity. The following chapter explores the impact of a family-centered lifestyle intervention that includes a biochemical analysis to test compliance to increased milk and milk product intakes in addition to standard methods of dietary assessment.

CHAPTER 4

Manuscript 2: Main Study Outcomes

Published: *Canadian Journal of Public Health*, 2016; 107(4-5): e453–e460
Reprinted with permission of the Canadian Public Health Association

A family-centered lifestyle intervention for obese six- to eight-year-old children: Results from a one-year randomized controlled trial conducted in Montreal, Canada

Tamara R. Cohen, RD, MSc,[1] Tom J. Hazell, PhD,[2] Catherine A. Vanstone, RN, MSc,[1] Celia Rodd, MD, MSc,[3] Hope A. Weiler, RD, PhD[1]

Author's Affiliation:

[1] School of Human Nutrition, McGill University, Montreal, QC

[2] Department of Kinesiology and Physical Education, Wilfrid Laurier University, Waterloo, ON

[3] Children's Hospital, University of Manitoba, Winnipeg, MB

Corresponding Author:
Hope A. Weiler
McGill University, School of Human Nutrition
21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Québec H9X 3V9 Canada
Tel: (514) 398-7905
Fax: (514) 398-7739
E-mail: hope.weiler@mcgill.ca

4.1 ABSTRACT

Background: Childhood obesity interventions should be family-centered and focused on lifestyle behaviors that achieve sustainable reductions in adiposity. The primary objective of this randomized controlled trial was to test a family-centered lifestyle intervention using Canada's Food and Physical Activity (PA) Guidelines to reduce body mass index-for-age z-scores (BAZ) in overweight and obese (OW/OB) children.

Methods: Children (n=78; ages 6–8.5 years) were randomized to standard (StnTx) or modified (ModTx) interventions or control (Ctrl). Measurements at baseline and every three months for one year included: anthropometry, BAZ, waist circumference (WC), and dual-energy x-ray absorptiometry scans for percent body fat (%BF), fat mass (FM) and trunk fat mass. Fatty acids measured by gas chromatography were used to assess compliance to the milk and alternatives interventions during the first six months. Six intervention sessions were based on Canada's Food and PA Guidelines and individualized to meet the needs of the family. ModTx were advised to consume four milk and alternatives/day versus the recommended two (StnTx) and to preferentially engage in daily weight-bearing PA. Ctrl were provided the guidelines.

Results: Baseline anthropometry did not differ among groups. At 12 months (n=73), all groups increased height ($p<0.001$) and lean mass ($p<0.001$). ModTx decreased BAZ ($p<0.001$); %BF decreased in ModTx ($p=0.02$), but not in StnTx ($p=0.99$) or Ctrl ($p=0.99$). FM, WC and trunk fat mass all significantly increased in Ctrl ($p<0.001$). At baseline and three months, erythrocyte fatty acids did not differ among groups, however they did decrease in ModTx at six months [C14:0 (−0.07%, $p=0.05$), C15:0 (−0.04%, $p=0.05$), C17:0 (−0.09%, $p=0.04$)].

Conclusion: Participating in a family centered-lifestyle intervention that focused on Canadian dietary and PA Guidelines and emphasized increasing milk and alternatives and weight-bearing PA had positive effects on reducing adiposity in OW/OB children. Guidelines are appropriate for the obese pediatric population but need to be individualized to meet the needs of the family. Additional studies are warranted to test the use of biochemical indices to assess compliance to milk and alternative intakes in OW/OB children participating in lifestyle interventions.

4.2 BACKGROUND

Childhood obesity is a public health concern in Canada [36]. Obese children are at a greater risk of health complications later in life, including premature death [329], however treatment is challenging due to its complex etiology [218]. Evidence demonstrates that intervention programs should be family-centered and focus on physical activity (PA), diet and lifestyle behaviors [218]. Furthermore, these programs should include discussions concerning self-monitoring, goal setting, problem solving, and relapse prevention [218].

Current Canadian clinical practice guidelines for the management of childhood obesity suggest that interventions be administered by primary care providers or through a weight management program [330]. Readily available educational resources include Canada's Food Guide [23] and PA guidelines [35]. However, a recent review of Canadian primary care providers estimates that only 50% of providers discuss weight management with families and that information is simply offered, not individualized [48]. Furthermore, there exist few family-centered weight management programs that have utilized or evaluated these Canadian resources as the primary treatment of childhood obesity [48].

In Canada, it is recommended that children 4–8 years of age consume two servings of milk and alternatives/day [23], however only one third meet this recommendation [119]. While a milk serving is defined as 250 mL, servings of alternatives such as yogurt are dependent on the product. Increasing servings of milk to 3–4 servings per day has positive effects on weight management in children [227-229, 253]. However, these randomized controlled trials ranged from 16 weeks to six months in duration and thus the sustained benefits are not clear [227-229]. Physical activity recommendations are 60 minutes of moderate-to-vigorous PA seven days per week, with weight-bearing activities performed during three of those days [35]. Physical activity, specifically weight-bearing PA (i.e., skipping, dancing or soccer), not only affects body composition, but also strengthens muscles and bones [35].

To date, there are no trials that use current dietary and PA recommendations as a basis of an intervention program and explore the effects of increased milk products and weight-bearing PA on changes in adiposity in children. The primary objective of the randomized controlled trial (RCT) was to test the effects of a family-centered lifestyle intervention using Canada's Food Guide and PA guidelines to reduce body mass index (BMI)-for-age z-scores (BAZ) in overweight and obese (OW/OB) children. It is hypothesized that children randomized to

increased milk and alternatives and focus on daily weight-bearing PA will have a lower BAZ at 12 months compared to children counselled to meet current Canadian dietary and PA recommendations, and the control group.

4.3 METHODS

Ethics statement

Ethics approval was obtained from the McGill University Faculty of Medicine Institutional Review Board, Lester B. Pearson School Board (Montréal, QC) and the English Montreal School Board (Montréal, QC) [Trial registration: ClinicalTrials.gov: NCT01290016].

Setting

Participants were recruited (January 2011–January 2013) for the McGill Youth Lifestyle Intervention with Food and Exercise (MY LIFE) from Montréal (QC). The study was conducted at the Mary Emily Clinical Nutrition Research Unit (Sainte-Anne-de-Bellevue, QC). Details on the study protocol, including recruitment strategy, are published elsewhere [331].

Study population

Eligible participants included healthy children 6–8 years of age with no known illnesses, classified as overweight or obese according to the World Health Organization (WHO) BMI cut-off criteria [38]. At baseline, parents completed a consent form; children completed an assent form.

Study design

At baseline, children were randomized (allocation ratio 1:1:1) to one of three groups (Standard [StnTx], Modified [ModTx] or Control [Ctrl]) by a computer-generated list and stratified by sex and BMI percentile-for-age (overweight: 85–97 percentile; obese: >97 percentile) [38]. Study measurements occurred every three months for one year. Pubertal stage was reported by caregiver using Tanner Staging images [332] and confirmed using fasting serum concentrations of luteinizing hormone and estradiol. Caregivers completed a socio-demographic questionnaire and self-reported their height and weight.

Intervention

All families received the same standard teaching of Canada's Food and PA Guidelines at baseline. StnTx and ModTx participated in six monthly interventions with a dietitian; details concerning the interventions have been published elsewhere [331]. Briefly, interventions were family-centered and focused on overall lifestyles, including discussions concerning both diet and activity. Specifically, all sessions included different educational components that covered various diet and PA topics. Despite each family receiving the same teachings, dietitians focused on individualizing goals and relapse prevention techniques using Health Canada's SMART Goals approach. Further, the dietitian facilitated discussions concerning self-monitoring and problem solving in order to successfully meet diet and PA recommendations. All sessions were conducted in either English or French and were designed to be appropriate for the Level 1 literacy level to ensure that participants and all family members irrespective of education would be able to actively participate in and understand the sessions. This ensured that goals were attainable and realistic given the family-specific needs and capabilities.

The dietitian guided families to either provide their child with two servings (StnTx) or four servings (ModTx) of milk and alternatives/day, preferably consuming products with lower percentage of milk fat (%MF) (i.e., 1%MF milk, 15%–18%MF cheese, 1%–2%MF yogurt). Both groups were encouraged to meet current PA guidelines (60 minutes of moderate-to-vigorous activity/day) and limit screen time (<2 hours/day); daily weight-bearing activities (i.e., skipping rope, jumping types of activities) were emphasized in ModTx. The Ctrl received the same interventions after completing the study.

Main measurements and outcomes

At each visit, children presented having fasted the previous 12 hours. Weight was measured using a standard balance beam scale (Detecto, Webb City, MO, USA); height was measured to 0.1 cm using a stadiometer (model 213, SECA Medical Scales and Measuring Systems, Hamburg, Germany). BMI (kg/m^2), BMI-for-age z-scores (BAZ) and height-for-age z-scores (HAZ) were computed using the WHO AnthroPlus Software [333]. Waist circumference (WC) was measured to the nearest 0.1 cm at the umbilicus [334].

Body composition was assessed using whole body dual-energy x-ray absorptiometry (DXA) (Hologic Discovery A fan beam with APEX software [version 13.3:3], Hologic Inc.,

Bedford, MA, USA) for fat mass (FM; kg), lean mass (LM; kg), percent body fat (%BF; %), trunk fat mass (kg), android/gynoid ratio and fat mass index (FMI; kg/height^2). FMI estimates excess fat versus the conventional BMI that measures excess weight [335]. Values were compared to the Hologic normative database (National Health and Nutrition Examination Surveys). Quality assurance scans using Hologic lumbar spine phantom no.14774 were performed at each visit, with coefficients of variability of 0.5% for bone mineral content and 0.3% for bone mineral density.

Biochemistry

Every three months, blood samples were obtained by venipuncture between 8 am and 12 pm, following 12 hours fasted. Samples were analyzed for luteinizing hormone, estradiol, and other health indicators, via auto-analyzers (Beckman Access and Beckman DXC600, CA, USA) at the Montréal Children's Hospital Clinical Chemistry laboratory (Montréal, QC).

Valid biomarkers of dairy fat were used to complement dietary records as a measure of compliance to the increased milk and alternative intervention (ModTx) [235, 336]. Myristic (14:0), pentadecanoic (15:0), heptadecanoic (17:0) and stearic (18:0) acids were measured in red blood cells (RBC) using a gas chromatography (Varian CP-3800, Walnut Creek, CA, USA) with a flame-ionization detector. RBC lipids were prepared using direct methylation [337]; recovery was determined to be 99.8% based on added C21:0 (Nu-Chek Prep, Inc., Elysian, MN, USA). Chromatogram peaks were identified against standard GLC 461 (Nu-Chek Prep, Inc., Elysian, MN, USA); fatty acids were expressed as percentage of total fatty acids, with coefficients of variation ranging from 4.9% to 15.4%.

Dietary intake and physical activity

Dietary intakes were assessed using three-day food diaries (3DFD). Caregivers recorded dietary intake for three non-consecutive days including a weekend day, as instructed by a registered dietitian. The first 3DFD was recorded the week after the study visit; all other 3DFD reflected diet prior to study visits. Data were analyzed using Nutritionist Pro software (Axxya Systems, Stafford, TX, USA) and the Canadian Nutrient File 2010b. Intakes were analyzed according to Canada's Food Guide (CFG) food groups. Children were classified as not meeting, meeting or exceeding CFG recommendations. Physical activity was captured using the Physical

Activity Questionnaire for Children [PAQ-C] [303] modified to include measures of time and intensity [mPAQ-C]. The mPAQ-C, completed by caregivers, reflected the child's PA the week prior to study visits and included questions concerning total screen time per week.

Statistical analysis

Data were analyzed using SAS version 9.3 (SAS Institute, Cary, NC, USA). Based on previous studies [227, 338] 116 overweight or obese children were needed to provide 80% power at a 5% significance level (two-sided) to detect a mean change in BAZ of −0.2 (SD 0.3) at 12 months. Estimating a 10% drop-out rate, 39 children were needed per group. Differences at baseline among groups were assessed using analysis of variance (ANOVA). Mixed-model ANOVAs were used to determine group and time interactions for anthropometry, body composition, dietary and PA measures with Tukey-Kramer adjustments. Similar models excluding time were examined to test changes (Δ) at six months and 12 months from baseline for BAZ, %BF, WC, trunk fat mass and FM. All models were tested for covariance structure using best-fit statistics. Fixed effects included group, time, BMI classification (i.e., overweight or obese) and gender; random effects included age, subject nested in group, family income, and parent education. Associations between measured RBC fatty acids and milk and alternative intakes were analyzed by Spearman correlations. Analyses were performed as intent-to-treat and presented as mean ± standard deviation, unless otherwise noted. Significance was set at <0.05.

4.4 RESULTS

Seventy-eight children (7.8±0.8 years of age) participated in the study (**Figure 4.1**). StnTx had families with lower household incomes ($p=0.02$) and fathers with lower education ($p<0.05$) compared to ModTx and Ctrl (**Table 4.1**). Children were prepubescent (Tanner Stage 1) with normal luteinizing hormone and estradiol. The majority of caregivers had self-reported BMI classified as overweight/obese (**Table 4.1**).

At baseline, PA reported by caregivers revealed that 21% of children met while 46% exceeded recommendations (n=71). Weight-bearing PA was performed on average 4.6±3.3 times/week and screen time was 6.9±3.2 hours/week.

Intervention effects on anthropometry and body composition

In all groups, HAZ increased at 12 months ($p<0.001$); ΔHAZ was greater in StnTx ($p<0.01$) and Crtl ($p=0.01$) compared to ModTx (**Figure 4.2A**). Both intervention groups decreased BAZ at six months (StnTx: $p=0.02$; ModTx: $p<0.001$) and 12 months (StnTx: $p<0.001$, ModTx: $p<0.001$). However, ModTx resulted in greater reductions in ΔBAZ at six months ($p=0.001$) and 12 months ($p=0.001$) compared to Ctrl (**Figure 4.2B**).

ModTx decreased %BF at six months ($p=0.03$) and 12 months ($p=0.02$). The Δ%BF from baseline to 12 months showed greater reductions in ModTx ($p=0.03$) compared to Ctrl (**Figure 4.2C**). At 12 months, Ctrl increased FM ($p<0.001$); there was a greater decrease in ΔFM in ModTx compared to Ctrl at six months ($p=0.05$) and 12 months ($p<0.001$) (**Figure 4.2D**). At 12 months, WC increased in both StnTx ($p<0.01$) and Ctrl ($p<0.001$); the ΔWC in Ctrl was greater at 12 months ($p<0.01$) compared to ModTx (**Figure 4.2E**). In Ctrl, trunk fat mass increased at 12 months ($p<0.01$); Δtrunk fat mass was greater in Ctrl compared to ModTx at 12 months ($p=0.01$) (**Figure 4.2F**). At 12 months, Ctrl gained 0.29 kg FM in the android region ($p=0.03$); there were no other significant group-by-time interactions for FM in the gynoid region or android/gynoid ratio. Fat mass index did not significantly change in any group at 12 months. Lean mass increased in all groups at 12 months ($p<0.001$) (n=68; median 3.3 kg, range: −1.0 to 7.6 kg); however, Δlean mass was greater in StnTx ($p<0.01$) and Ctrl ($p<0.0001$) compared to ModTx at 12 months.

Intervention effects on lifestyle: Diet and physical activity

Fifty (64%) week-1 3DFD were returned. There were no differences for anthropometric or body composition measures between participants who returned 3DFD versus those who did not. Diet did not conform to CFG or differ among groups in proportions below, meeting or exceeding recommendations (**Figure 4.3**). However, week-1 intakes differed (1×1 comparisons): ModTx consumed ~340 kcal/day less compared to Ctrl ($p=0.02$) (**Table 4.2**) while StnTx ($p=0.03$) and ModTx ($p=0.01$) consumed less protein compared to Ctrl (**Table 4.2**).

Only 41% and 33% of 3DFD were returned at six months and 12 months respectively (**Table 4.2**); there were no differences among groups for macronutrient intakes. Similarly, diet analyzed by food group servings did not differ among or within groups over time after age adjustments. Data analyzed categorically to describe dietary intakes as percentage below,

meeting or exceeding the food groups are presented in Figure 3. At 12 months (n=24), fruit and vegetable intake recommendations were met by 30% of StnTx and 29% ModTx, but remained at 0% for Ctrl (**Figure 4.3**). Grain product intakes were met by 20% StnTx, 14% ModTx and 0% Ctrl (100% exceeded recommendations in Ctrl). Thirty percent StnTx, 43% ModTx and 29% Ctrl met milk and alternative recommendations. Finally, 50% StnTx, 57% ModTx and 57% Ctrl met the meat and alternative recommendations.

Saturated fatty acids were analyzed at baseline (n=65), three months (n=35) and six months (n=52) as an objective measure of compliance to the milk and alternative intervention. At three months, there were no differences in C15:0 among groups. At six months, there was a decrease (0.04%) in C15:0 in ModTx ($p<0.05$). Paired C15:0 values to 3DFD (n=8) at six months showed no relationship to total dairy intake (rho=0.26; p=0.55). Although C17:0 decreased (0.09%) in ModTx from baseline to six months (p=0.04), C17:0 did not associate with dairy intakes at any time. C14:0 decreased (0.07%) in ModTx at six months (p=0.05); at three months, C14:0 positively associated with dairy intake in Ctrl (n=4; rho=1.0; $p<0.0001$). Finally, C18:0 decreased in Ctrl at three months (1.38%, $p<0.01$) and six months (0.93%, p=0.04); C18:0 did not associate with dietary intake.

PA assessed by caregivers did not change from baseline. At six months (n=68), 25% of children met while 54% exceeded recommendations. Similarly, at 12 months (n=72), 26% met while 53% exceeded PA targets. There were no significant differences throughout the study for activities classified by intensity or type (weight-bearing) as well as time spent engaged in screen time. Average screen time per week at 12 months was 6.5±3.1 hours/week.

4.5 DISCUSSION

This study demonstrated that children who participated in a family-centered lifestyle intervention that was based on current health guidelines sustained losses in adiposity: StnTx and ModTx attained greater decreases in BAZ compared to the control group. However, ModTx also achieved losses in %BF, whereas at 12 months, Ctrl increased WC and trunk fat mass. Simply providing dietary and physical activity guidelines with a brief education at baseline to the Ctrl group did not reduce obesity in any way.

The interventions used in this study were based on current Canadian guidelines but allowed for individualization and included caregivers. After baseline education sessions, both

StnTx and ModTx had lower energy intakes compared to Ctrl, showing early signs of success. Furthermore, irrespective of intervention group, at 12 months both StnTx and ModTx were closer to meeting CFG recommendations compared to Ctrl. This study yielded similar results to those of other studies: when diet and PA goals are realistic and attainable, treatment groups experience a decrease in BAZ [227, 242] while control groups experience an increase [321]. Similarly, the magnitude of reductions in BAZ were greater in children randomized to higher dairy intakes [227], however unique to this study was the addition of weight-bearing PA.

To our knowledge, this was the first intervention in obese children to include a biochemical analysis of saturated fatty acids to complement dietary intake assessments [231, 336]. Although ModTx were guided to increase servings of dairy, C15:0 decreased at six months. The interventions in this study focused on choosing lower %MF options, and although the percentage of children meeting the milk and alternative recommendations in the ModTx increased from 21% to 43% at 12 months, the decrease in C15:0 suggests children were possibly consuming diets with lower %MF [231]. Furthermore, C15:0 is found in ruminant meat [336]. At 12 months, all children in the ModTx were either below (43%) or meeting (57%) the meat and alternative recommendation compared to 74% exceeding the recommendation at baseline. Other studies have shown moderate correlations (r=0.45–0.46) of C15:0 with dairy fat from dietary assessment in adults [236] and children [336]. In this study, the dietary assessments were underpowered, thus limiting similar analyses. Future RCTs are warranted to evaluate these biomarkers and associations with different percentages of milk fat [232].

Strengths and limitations

Interventions aimed at obese children often face many challenges with recruitment and follow-up. There is evidence to suggest that caregivers may be reluctant to enroll their OW/OB child in an intervention that targets weight due to possible adverse effects [338]. Our study focused on developing a safe, non-judgmental environment for children, which is evident by the high retention rate (94%). Nevertheless, this study has a small sample size below target despite various recruitment efforts and a lengthy recruitment period. Additionally, there were imbalances in family income and father's education. However, we accounted for these imbalances in our statistical analyses. Further, we did not survey number of siblings in our questionnaires, which may impact or influence the child's success. However, the interventions were designed to

address any barriers or limitations to meeting goals that may arise specifically due to the challenges of the family dynamics (i.e., single parent versus dual; single child versus siblings). As seen in studies of OW/OB children [232], this study had a low return of diet records by parents, limiting dietary intake analyses. Although mean intakes at 12 months appear similar, dietary data presented as below, meeting or exceeding the CFG recommendations suggest that monthly interventions helped children attain diets closer to Canadian guidelines. Similarly, PA was subjectively assessed by caregivers. Given the obesogenic nature of the parents participating in the study, it is possible that PA levels were overestimated, and/or children's perceptions of intensity or duration of activity were not accurate [339]. The relationships between PA and anthropometry thus require confirmation using more objective assessments. We therefore caution interpretation of our PA data. Objective measures of PA by accelerometer or pedometer are more valid and should be used with OW/OB children [340]. Finally, the study design did not permit for distinguishing the effects of either dietary (increased milk and alternatives) or PA (weight-bearing PA) on benefits of reducing adiposity as we did not use a step-wise approach to adding these during the interventions.

4.6 CONCLUSION

This study suggests that Canadian dietary and PA guidelines are suitable to form the basis of treatment programs for OW/OB children. In order to achieve and sustain goals, interventions need to be realistic for the participating children and caregivers. A follow-up study is warranted to evaluate whether these changes were sustained, an important first step in achieving healthy habits prior to adolescence and adulthood.

4.7 ACKNOWLEDGEMENTS

The authors acknowledge Sarah-Eve Loiselle and Popi Kasvis for their help with the interventions; Nicolas Kim for his assistance with DXA measures; Caitlin Ellery for sample processing and Sandra Dell'Elce for assisting with blood sampling. Dr. Hugues Plourde is acknowledged for his guidance with conceptualizing the behavioral components of the interventions in the study

Table 4.1 Participant baseline characteristics according to randomization

Variable		StnTx	ModTx	Ctrl	Total Sample
Age (years)		7.7±0.8 (25)	8.1±0.7 (25)	7.7±0.8 (28)	7.8±0.8 (78)
Ethnicity, White (%, n)		71% (17)	92% (22)	81% (22)	81% (78)
Gender, Female (%, n)		56% (14)	60% (15)	57% (16)	57% (78)
Family Income >$75,000.00/y (%, n) [a]		38% (8)	81% (17)	57% (15)	59% (68)
Education, Higher Education [b]	Mother (%, n)	79% (19)	84% (21)	85% (23)	83% (76)
	Father (%, n)	40% (9)	77% (17)	81% (17)	65% (66)
Parental Self-Report BMI [c]	Mother, BMI >25kg/m^2 (%, n)	68% (13)	70% (14)	61% (13)	70% (61)
	Father, BMI >25kg/m^2 (%, n)	93% (13)	83% (15)	94% (16)	88% (49)
Waist Circumference (cm)		81.3±8.4 (25)	81.6±8.7 (25)	80.7±9.1 (28)	81.2±8.6 (78)
Weight z-score		3.4±1.3 (25)	3.0±1.2 (25)	3.1±1.0 (28)	3.2±1.2 (78)
Height z-score		1.1±0.9 (25)	1.1±0.9 (25)	1.2±0.9 (28)	1.1±0.9 (78)
BMI (kg/m^2)		25.2±3.9 (25)	24.1±2.8 (25)	24.1±3.2 (28)	24.4±3.3 (78)
BAZ		3.6±1.4 (25)	3.1±1.0 (25)	3.2±1.1 (28)	3.3±1.2 (78)
BMI Classification OW/OB [c]		2/ 25	1/ 25	3/ 28	6/ 78
Lean Body Mass (kg)		25.5±3.8 (25)	26.1±4.2 (25)	25.6±4.3 (28)	25.7±4.0 (78)
Total Fat Mass (kg)		17.2±5.6 (25)	15.9±3.5 (25)	15.6±4.4 (28)	16.2±4.6 (78)
Percent Body Fat (%)		38.5±5.5 (25)	36.8±5.6 (25)	36.4±4.9 (28)	37.2±4.9 (78)
Fasting Glucose (mmol/L)		4.9±0.5 (18)	4.8±0.4 (21)	4.8±0.3 (24)	4.8±0.4 (63)
Insulin (pmol/L)		53.1±28.5 (18)	44.8±24.9 (19)	36.2±15.5 (24)	43.9±26.3 (61)
Total Cholesterol (mmol/L)		4.2±0.7 (19)	5.5±6.2 (21)	4.2±0.9 (24)	4.6±3.6 (64)
HDL (mmol/L)		1.2±0.4 (18)	1.2±0.3 (21)	1.3±0.3 (24)	1.2±0.3 (63)
LDL (mmol/L)		2.5±0.7 (18)	2.5±0.7 (21)	2.6±0.9 (24)	2.5±0.8 (63)
Triglycerides (mmol/L)		1.1±0.7 (19)	0.9±0.5 (21)	0.8±0.4 (24)	0.9±0.5 (64)

Note: mean ± standard deviation (n) unless stated otherwise; Abbreviations: StnTx: Standard treatment group; ModTx: Modified treatment group; Ctrl: Control group; BMI: Body mass index (kg/m^2); BAZ: Body mass-for age-z-score; OW: overweight; OB: obese; HDL: High-density lipoprotein; LDL: Low-density lipoprotein. [a] Total family income was less in StnTx compared to ModTx and Ctrl ($p=0.02$). [b] Higher education included university, college or Cégep. Father's self-reported education was less in StnTx compared to ModTx and Ctrl ($p<0.01$). [c] BMI classifications are denoted as n/total

Table 4.2 Macronutrient intakes[a] and Canada's Food Guide Food Groups[b] assessed by 3-day food diary at week-1, 6-months and 12-months

	StnTx			ModTx			Ctrl		
	Week-1	**6-mo**	**12-mo**	**Week-1**	**6-mo**	**12-mo**	**Week-1**	**6-mo**	**12-mo**
n=	*19*	*10*	*10*	*19*	*12*	*7*	*12*	*8*	*7*
Total energy (kcal)	1669±379	1661±384	1635±325	1567±271 [c]	1598.7±337	1449.8±173	1909±312 [c]	1947±443	1938±463
Protein (g)	69.7±13.8[d]	69.6±13.6	71.7±7.7	68.4±10.5[d]	72.9±19.2	62.6±8.2	83.5±18.3[d]	88.0±26.3	87.3±27.7
Fat (g)	57.5±23.0	52.6±15.9	53.6±13.5	53.1±14.4	59.3±14.6	48.59±13.6	63.9±13.6	59.4±17.5	68.8±17.8
Carbohydrate (g)	223.9±49.6	232.7±56.3	223.2±49.6	213.0±47.3	199.1±48.4	198.6±29.5	253.8±42.3	268.7±59.2	248.0±65
Fruits and Vegetables (servings/d)	4.9±1.9	6.6±2.4	4.9±1.3	4.4±2.0	4.9±2.4	4.0±1.8	4.6±1.9	5.6±2.3	4.9±1.7
Grain Products (servings/d)	6.8±2.0	6.1±1.8	6.1±1.8	6.2±1.8	6.0±1.8	6.2±2.0	7.2±2.2	6.9±1.9	7.0±2.0
Milk and Alternatives (servings/d)	2.2±1.2	1.6±0.8	1.9±0.7	2.0±0.9	2.3±1.2	2.8±1.1	2.8±1.6	3.3±2.5	3.6±2.6
Meat and Alternatives (servings/d)	1.8±0.7	2.3±1.0	2.1±0.6	1.9±0.6	1.7±0.7	1.0±0.4	2.2±0.9	2.0±0.6	2.2±0.6

Note: mean ± standard deviation

[a] Week-1 food diaries were completed the week after visit; all subsequent diaries were completed and reflect the week prior to study visit.

[b] Canada's Food Groups: servings/day

[c] p-values for comparison of total energy (kcal) were lower at week-1 between ModTx and Ctrl (p=0.02)

[d] p-values for comparison of total protein (g) were higher at week-1 in Ctrl compared to StnTx and ModTx (p=0.01)

Figure 4.1 CONSORT diagram for the MY LIFE Study

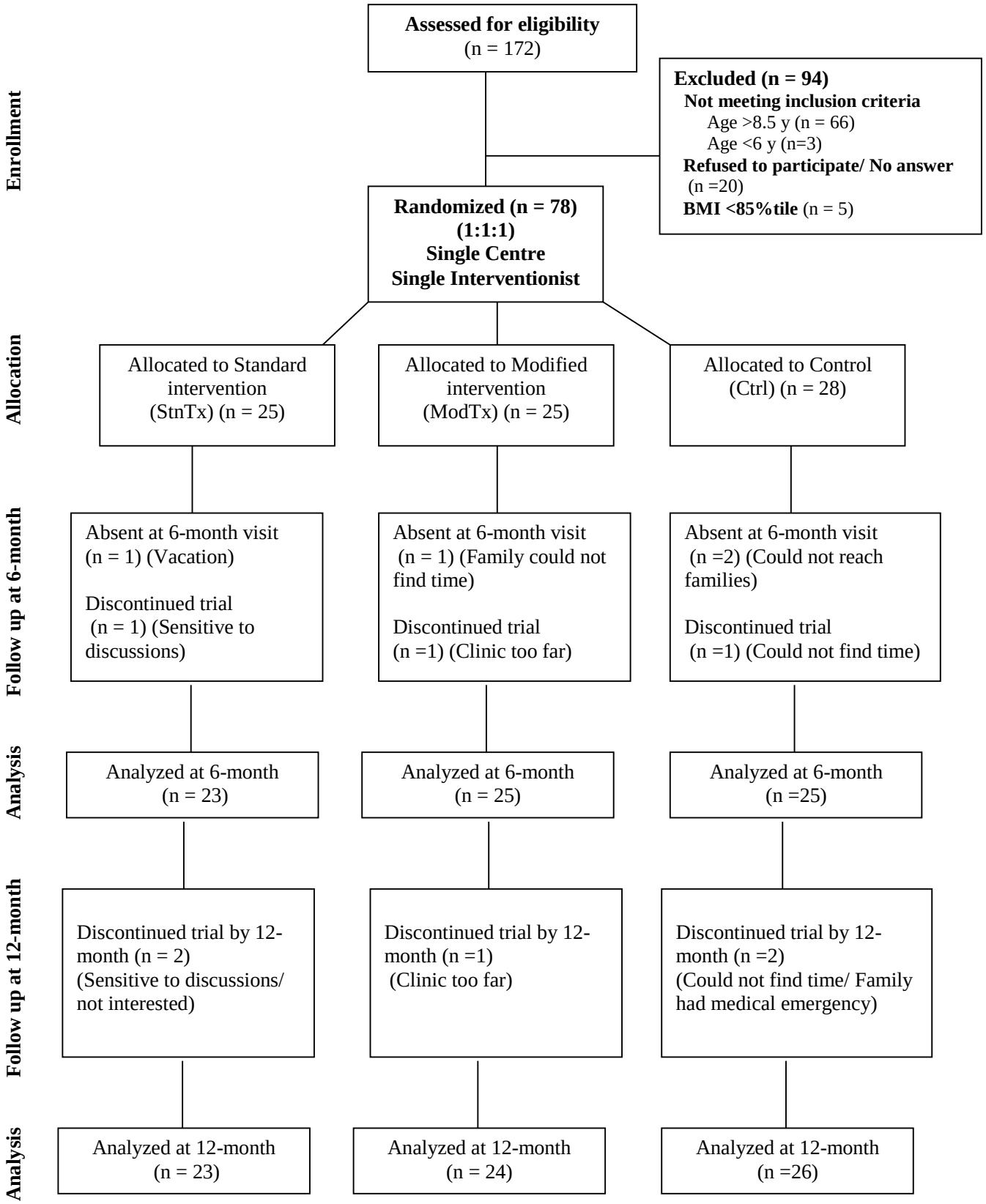


Figure 4.2 Anthropometric and body composition changes (Δ) from baseline (BL) at 6-month and 12-month [median, range] [+ mean]; **Panel A**: Height-for-age z-scores (HAZ); **Panel B**: BMI-for-age z-score (BAZ); **Panel C:** Percent body fat (%BF); **Panel D**: Total fat mass; **Panel E**: Waist circumference; **Panel F**: Trunk fat mass. *p<0.05, **p<0.001

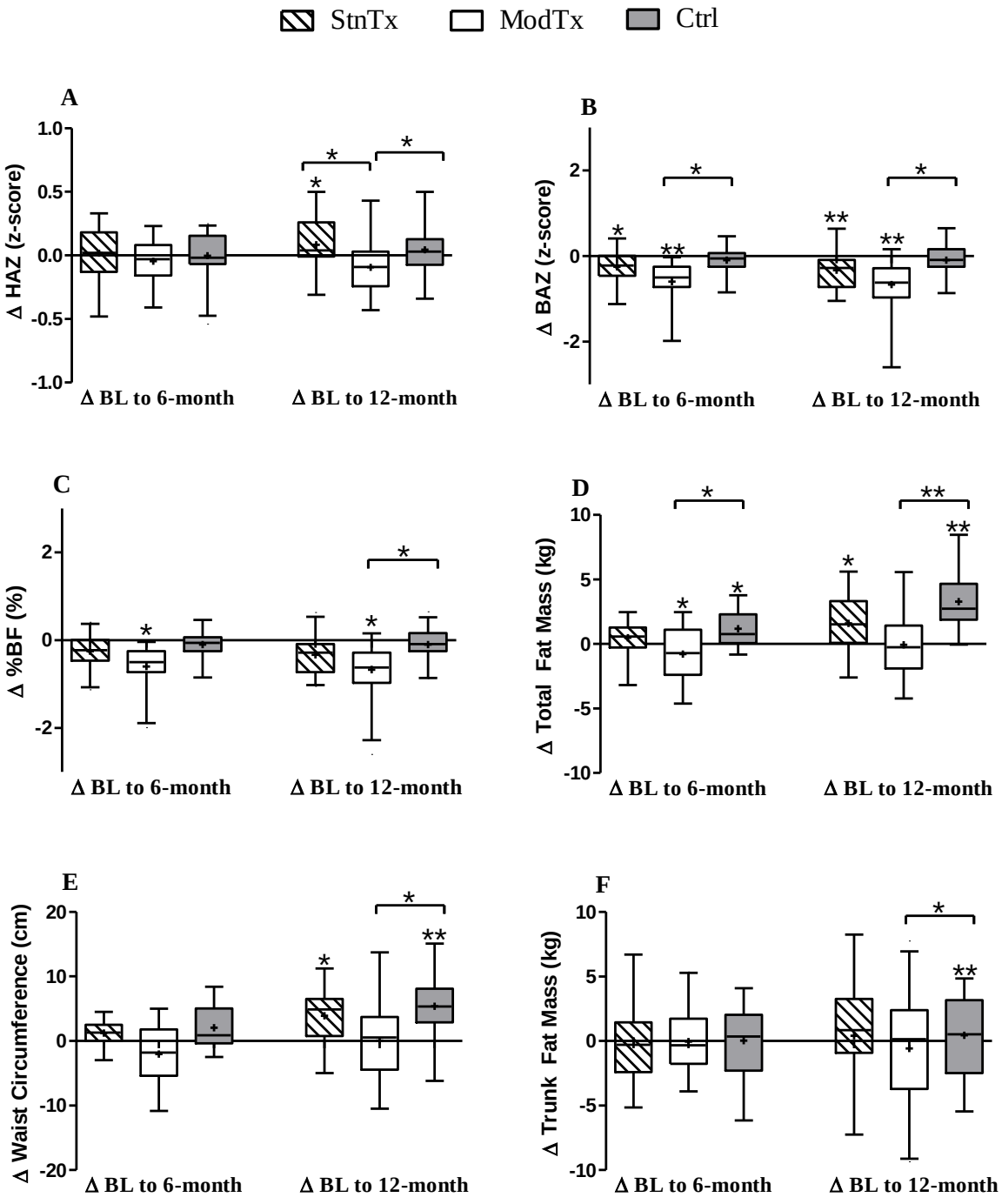
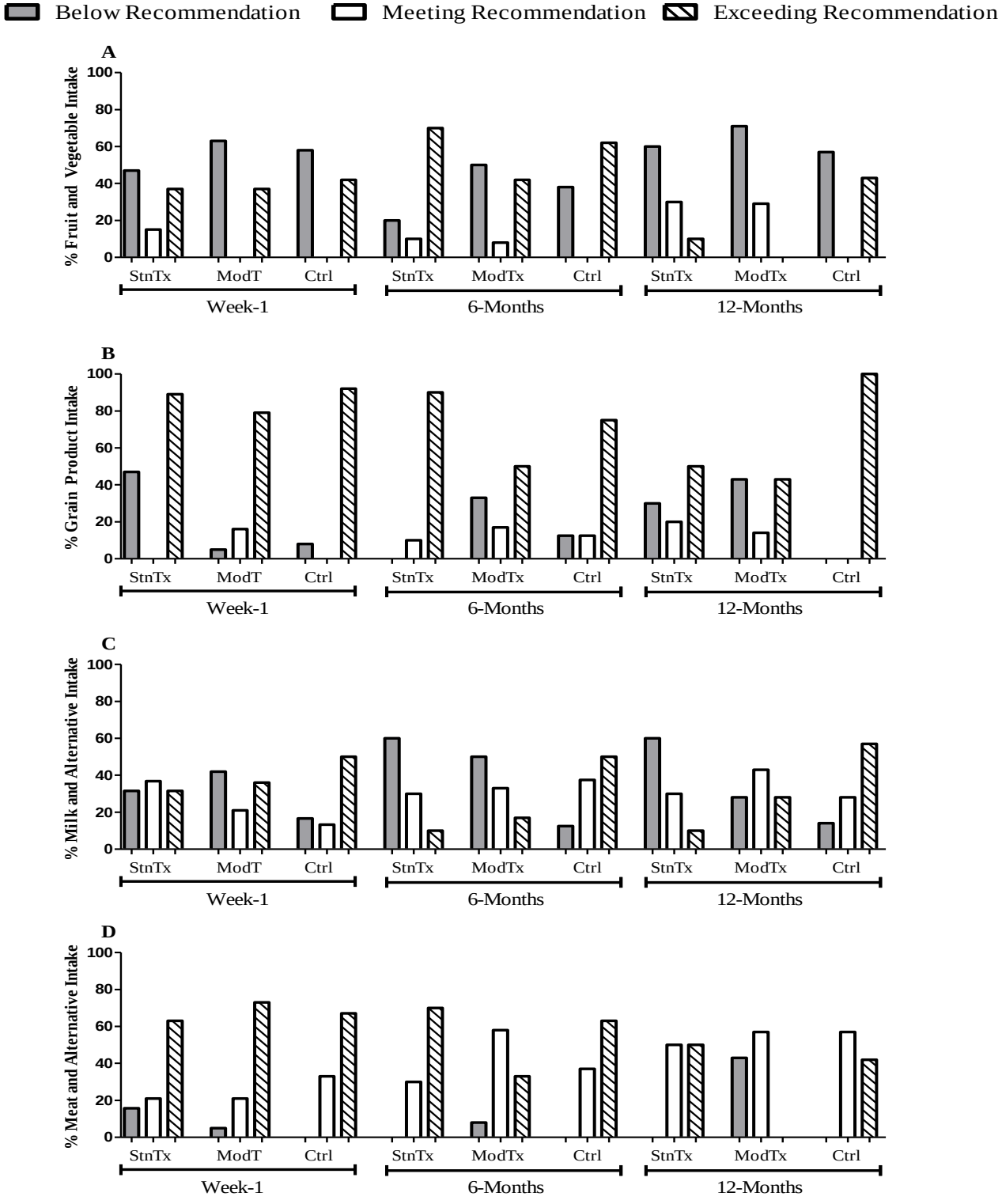


Figure 4.3 Dietary assessment of 3-day food diaries by percentage below, meeting or exceeding Canada Food Guide food groups adjusted for age at week-1, 6-months and 12-months; **Panel A**: Fruits and vegetables; **Panel B**: Grain products; **Panel C**: Milk and alternatives; **Panel D**: Meat and alternatives.



Bridge Statement 3

The results presented in manuscript 2 are promising: children randomized to the intervention groups reduced adiposity while unfortunately, children in the control group significantly increased not only whole body fat mass, but also waist circumference by 12 months, suggesting that the intervention platform used in the MY LIFE Study was successful.

The literature review of this thesis discussed the importance of considering bone health in this pediatric population: children with obesity are at greater risk of fractures which may be attributed to increased fat mass despite having more lean mass. Further, fat mass may exert different effects on bone depending on bone site. A potential consequence of weight loss is bone loss, an outcome often see in adult weight loss trials. Although significant reductions in fat mass in obese adolescents who participate in lifestyle interventions have not shown unfavorable changes in bone outcomes, it is currently unknown if similar results would be seen in pre-pubertal children with obesity who also significantly reduce fat mass. Moreover, if decreases in total body fat impact bone health on weight and non-weight bearing sites has not been explored in children with obesity.

The effects of milk and milk products and weight-bearing types of activities on bone health are established. However, if there are any benefits to increasing the recommended daily servings of milk and milk products and weight-bearing types of activities above current guidelines to counteract the potential loss of bone mass during a childhood obesity intervention have not been explored. The following chapter aims to address these uncertainties by assessing not only whole body, but also including weight-bearing and non-weight bearing bone outcomes. Additionally, the inclusion of bone health biomarkers to further appreciate the impact of reductions of fat mass on bone formation or bone resorption are discussed.

CHAPTER 5

Manuscript 3: Bone Health

Published: *Calcified Tissue International*, 2017 Dec;101(6):612-622.

Bone health is maintained while fat mass is reduced in pre-pubertal children with obesity participating in a 1-year family-centered lifestyle intervention

Tamara R. Cohen, RD, MSc,[1] Tom J. Hazell, PhD,[2] Catherine A. Vanstone, RN, MSc,[1] Celia Rodd, MD, MSc,[3] Hope A. Weiler, RD, PhD[1]

Author's Affiliation:

[1] School of Human Nutrition, McGill University, Montreal, QC

[2] Department of Kinesiology and Physical Education, Wilfrid Laurier University, Waterloo, ON

[3] Children's Hospital, University of Manitoba, Winnipeg, MB

Corresponding Author:
Hope A. Weiler
McGill University, School of Human Nutrition
21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Québec H9X 3V9 Canada
Tel: (514) 398-7905
Fax: (514) 398-7739
E-mail: hope.weiler@mcgill.ca

5.1 ABSTRACT

Background: Diet and physical activity (PA) influence bone health in children. This study tested whether increasing milk and milk products and weight-bearing types of PA favorably changed bone outcomes assessed by dual-energy X-ray absorptiometry (DXA) and bone biomarkers in children with obesity participating in a 1-y family-centered lifestyle intervention.

Methods Children were randomized to 1 of 3 groups: Control (Ctrl: no intervention), Standard treatment (StnTx: 2 servings milk products/d; meet PA guidelines plus weight-bearing PA 3 x/wk), or Modified treatment (ModTx: 4 servings milk products/d; meet PA guidelines plus daily weight-bearing PA). Baseline (BL) and 12-mo measurements included DXA scans for whole body (WB), lumbar spine (LS), lumbar lateral spine (LLS), and ultra-distal (UD) ulna+radius for bone mineral content (BMC), areal bone mineral density (aBMD) and BMD z-scores. Fat mass index (FMI) and fat-free mass index (FFMI) and biomarkers of bone metabolism were assessed.

Results: Seventy-eight children 6-8 y were recruited (mean body mass index for-age z-score: 3.3±1.2). Compared to BL, all groups increased BMC of WB, LS, and LLS ($p<0.001$) whereas only StnTx increased UD ulna+radius BMC at 12-mo ($p<0.05$). At 12-mo, WB BMD z-scores were significantly lower in Ctrl ($p<0.05$), whereas WB and LLS aBMD increased in StnTx and ModTx ($p<0.001$) but not Ctrl. All groups increased FFMI ($p<0.001$) while only Ctrl increased FMI ($p<0.001$). Bone biomarkers did not change over time.

Conclusion: Participating in a family-centered lifestyle intervention based on Canadian diet and PA guidelines maintained bone health and reduced FMI in obese children.

5.2 BACKGROUND

As childhood is linked to health in adulthood, it is crucial that children be given optimal opportunity to establish skeletal health. The positive effects of diet, specifically milk and milk products, and physical activity, particularly weight-bearing types of activities, on bone health are well established [341]. Further, healthy bone development is also influenced by skeletal muscle, which in turn is also affected by nutrition and the mechanical loading effects of physical activity [342].

The childhood obesity epidemic is an ongoing public health concern and its etiology is complex [56]. Unbalanced dietary intakes, particularly inadequate calcium intakes, coupled with an increasing sedentary lifestyle, not only affect adiposity but also bone health. Research is now recognizing that excessive body fat may negatively affect the musculoskeletal health of children with obesity [343]. Specifically, that they experience more joint pain [344], skeletal dysfunction [344] and may be at an increased risk of fractures [343], among other health-related issues [56].

Despite these clinical manifestations, deciphering the effects of excessive body fat on their growing skeletal systems is a challenge [172, 343], particularly since the effects of adipose tissue on bone may be site specific [24]. Studies comparing bone outcomes in children with different body mass indices by dual-energy x-ray absorptiometry (DXA) suggest there are positive associations between fat mass and bone size [175, 345], however children with obesity may have lower bone mineral content (BMC) [171, 248], lower whole body bone mineral density (BMD) [171, 172], lower bone mass relative to bone size [248] and reduced radial-cortical diameter [177] and tibia volumetric BMD [27] compared to normal-weight peers. Together, these results suggest that alterations in cortical and trabecular bone are not adapting to sufficiently compensate for the increases in total body weight of obese children, thereby increasing their risk of fractures [171].

In addition to differences in musculoskeletal systems, bone biomarkers may also be altered in children with obesity. Although normally elevated due to skeletal growth velocity [189], this population tends to have even higher concentrations of bone-specific alkaline phosphatase (BAP) [191], lower osteocalcin [198, 346] and either the same [201] or increased [202] C-terminal telopeptide of type 1 collagen (CTX-1) concentrations compared to normal-weight children. Though biomarker concentrations may be altered with reductions in adiposity; children who reduce fat mass have shown decreases in BAP within 3-months [191] while others

have shown an increase in osteocalcin within 1-year of reducing adiposity [198]. Similarly, 25-hydroxyvitamin D (25(OH)D) concentrations are often lower in children with obesity [206] and reductions in adiposity have increased 25(OH)D concentrations [207]. Therefore, interventions aimed at reducing adiposity in children with obesity may benefit both body composition and bone markers [207], and possibly BMD over time.

Currently, it is unknown if a lifestyle intervention that focuses on increasing milk and milk products in combination with weight-bearing types of physical activities will result in changes in both bone outcomes assessed by DXA and bone biomarkers. In our previous analyses of this data set, we showed that children randomized to a group that were advised to consume 4 servings/d of milk and milk products and to participate in daily weight-bearing types of activity compared to standard treatment (i.e., consume 2 servings/d milk and milk products and engage in weight-bearing activities 3 times/week) or control (no treatment), significantly reduced percentage body fat by 12-mo following a 1 y family-centered lifestyle intervention [347]. Whether these children also favorably changed bone outcomes would be of interest to those who work with children that are obese. Therefore, this re-analyses of this data set tested whether increasing milk and milk products and weight-bearing types of physical activities resulted in favorable changes in bone outcomes assessed by DXA and favorably changed bone biomarkers in children with obesity participating in a 1-y family-centered lifestyle intervention.

5.3 METHODS

Ethical consideration

Ethics approvals were obtained from the McGill University Faculty of Medicine Institutional Review Board, Lester B. Pearson School Board and the English Montreal School Board (Trial Registration: ClinicalTrials.gov: NCT01290016).

Participants and study design

The McGill Youth Lifestyle Intervention with Food and Exercise (MY LIFE) Study was a randomized controlled trial (2011-2013) for healthy children from Montréal (Québec, Canada). Eligibility included healthy 6- to 8-year-old children with no known illness, who were overweight (85-97 percentile; body mass index (BMI) +1 standard deviation scores (SDS)) or

obese (>97 percentile; BMI +2 SDS) as per the World Health Organization (WHO) weight-for-height body mass index (BMI) cut-off criteria [38].

This study reports on secondary outcomes of a previously published Canadian randomized control trial [347]. Details concerning study protocol, recruitment strategy and main outcomes are published [331, 347]. At baseline, parents completed a consent form; children completed an assent form. Briefly, children were randomized to one of three groups: Control (Ctrl), Standard treatment (StnTx), or Modified treatment (ModTx). Assessments occurred at the Mary Emily Clinical Nutrition Research Unit of McGill University (Sainte-Anne-de-Bellevue, QC, Canada) every 3-months for 1 year. This paper reports on baseline and 12-mo (end of study) data for bone outcomes.

At baseline, sociodemographic information were surveyed and all families participated in a basic teaching of Canada's Food Guide [23] and physical activity (PA) guidelines [35]. Canada's Food Guide recommends children 4-8 y to consume 5 servings/d of fruit and vegetables (e.g., 1 serving= ½ cup (125 mL) fruit; 1 cup (250 mL) leafy vegetable), 4 servings/d of grain products (e.g., 1 serving= 1 slice of bread (35g) or ½ cup (125 mL) cooked pasta, rice), 2 servings/d of milk and milk products (e.g., 1 serving= 1 cup (250 mL) milk; 50 g (1.5 oz.) cheese; ¾ cup (175 g) yogurt) and 1 serving/d of meat and alternatives (e.g., 1 serving= 75g meat, fish or poultry; 2 eggs) [23]. Canadian children are encouraged to engage in 60 minutes of moderate-to-vigorous physical activity per day, with weight-bearing types of activity being performed 3 times/week [35].

StnTx and ModTx were seen by a registered dietitian for 6 sessions: once a month for 5-months and one time at the end of their 8th month for a follow-up session. The first intervention session occurred at the end of the 1st-month from starting the study. StnTx were encouraged to meet the current Canadian recommendations for diet and PA whereas ModTx were guided to follow Canada's Food Guide but consume an extra 2 servings of milk products per day and to participate in daily weight-bearing activities versus 3 times/week (StnTx). Children randomized to the Ctrl were assessed every 3 months identical to the treatment groups but received interventions only after 1 year.

Anthropometry and pubertal stage

Research-trained nurses and dietitians performed all anthropometric measurements. Weights were measured at each visit using a standard balance beam scale (Detecto, Webb City, MO, USA). Heights were measured to the 0.1 cm using a SECA 213 portable stadiometer (SECA Medical Scales and Measuring Systems, Hamburg, Germany) and used to calculate height velocity (height at 12-mo minus height at baseline divided by age at 12-mo minus age at baseline). BMI (kg/m^2) were calculated and BMI for age z-scores (BAZ) were computed using the WHO AnthroPlus Software version 3.2.2. [333]. Using this software, weight-for-age z-scores (WAZ) and height-for-age z-scores (HAZ) were also computed. Parents were asked to identify their child's pubertal stage using Tanner Staging images [332] which were confirmed with fasting serum concentrations of luteinizing hormone and estradiol.

DXA assessment

At baseline and 12-mo, DXA (Hologic Discovery A fan beam with APEX software [version 13.3:3], Hologic Inc, Bedford, MA, USA) were used to assess whole body (WB), appendicular regions, lumbar spine (LS) 1-4, lumbar lateral spine (LLS) vertebrae 3 and non-dominant forearm for analyses of the ultra-distal (UD) region of both ulna and radius (ulna+radius). Forearm dominance was established by asking the child which hand they color with; forearm length was measured prior to scans at baseline and 12-mo.

All children were scanned in light clothing with no metal, zippers or jewelry. Values were compared to the Hologic normative database (U.S. National Health and Nutrition Examination Surveys). Quality assurance was conducted at each visit using Hologic lumbar spine phantom no. 14774; coefficients of variability were 0.5% for BMC and 0.3% for BMD.

WB, LS, LLS and UD ulna+radius was analyzed for areal BMD (aBMD) and BMC; BMD z-scores were available for WB and LS only. LS were also analyzed as bone mineral apparent density (BMAD; $BMAD= BMC/BA^{1.5}$) as per Carter *et al* [348] to produce a 'volumetric' density from areal results. WB and appendicular regions were assessed for fat mass (FM) and fat-free mass (FFM, lean mass (kg) +BMC (kg)). To normalize body composition relative to body size, WB-FM and WB-FFM were log-transformed and regressed against log-height (m) to yield: FMI ($kg/m^{0.29}$) and FFMI ($kg/m^{0.67}$).

Biochemistry

Children presented to each study visit 12-h fasted. A venous blood sample was collected between 0800-1200h by a registered nurse. Plasma samples (BAP, PTH, osteocalcin, and 25(OH)D) were immediately centrifuged while serum samples (CTX-1) were spun 30 min after collection. All samples were stored in -80ºC freezers until biochemical analyses.

Manual assays were used to measure serum CTX-1 (Serum CrossLaps ELISA, Cat# AC-02F1, Intermedico, Markham, ON, CA) which yielded coefficients of variation (CV) of 7.8% intra-assay and 9.6% inter-assay. Plasma BAP, plasma PTH, plasma (total) osteocalcin and plasma 25(OH)D (measured as total 25-hydroxyvitamin D) were measured by chemiluminescence immunoassay (Liaison, DiaSorin, Ontario, Canada), with the following CV and accuracies: BAP (intra-assay CV: <6.0 %, inter-assay CV: 3.9%, accuracy: 78-86%), PTH (intra-assay CV: <5.0 %, inter-assay CV: 8.6%, accuracy: 83-111%), osteocalcin (intra-assay CV: <5.1%, inter-assay CV: 9.5%, accuracy: 89-106%). In addition, total calcium was analyzed from whole blood plasma samples using auto-analyzers (Beckman Access and Beckman DXC600, CA, USA) at the Montréal Children's Hospital Clinical Chemistry laboratory (Montréal, QC).

The CV for 25(OH)D were 2.0% (39.6 ± 0.3) for low and 1.9% (113.1 ± 2.3) for high controls of 25(OH)D resulting in a 2% inter-assay CV between low and high controls. Other quality control measures included participating in the Vitamin D External Quality Assurance Scheme and using the National Institute of Standards and Technology (NIST) guidelines. Samples from the NIST resulted in inter-kit CV of 2.6% for the high control and 4.0% for the low control. Accuracy for the high and low controls was 104% and 98% respectively.

Physical activity and dietary intake

Physical activity levels were assessed by the parent-surveyed physical activity questionnaire [PAQ-C] [303], which was modified to include measures of time and intensity. This questionnaire reflected activities performed the week prior to study visit. Activities were analyzed for type (i.e., weight-bearing versus non-weight-bearing) and duration (min/d). Children's participation in weight-bearing types of activities were compared on an individual basis, specifically if they increased or decreased the number of times per week performing

weight-bearing types of activities from questionnaires completed at the baseline and the 6-mo visit and at baseline and the 12-mo visit.

Dietary intakes of the children were assessed using 3-day food diaries, which reflected 3 non-consecutive days of intake (i.e., 2 weekdays and 1 weekend) and were completed every 3-mo. At the baseline visit, registered dietitians taught families how to complete the food diaries. After the baseline visit, parents were instructed to mail back completed food diaries to the study center and were provided with a stamped addressed envelope; these food diaries reflected dietary intakes 1-week post baseline visit and prior to the first intervention, which was held at the end of month 1 of the study. All other food diaries (i.e., 3-mo, 6-mo, 9-mo and 12-mo) were brought back to the research unit during the study visit.

Food items were entered and analyzed in the Nutritionist Pro software (Axxya Systems, Woodinville, WA, USA), a software that uses the Canadian Nutrient File 2010b. An average of the three days from food diaries returned at 3-mo, 6-mo, 9-mo and 12-mo were used to estimate the dietary intakes of the children over the course of the study. Specifically, mean values for total energy intakes (kcal/d), macronutrients and micronutrients of interest (i.e., calcium and vitamin D). In addition, food items from the food diaries were manually grouped according to Canada's Food Guide [23] into one of the 4 food groups; the mean of each food group from food diaries collected between 3-mo and 12-mo were also computed.

Statistical analysis

Data were analyzed using SAS version 9.3 (SAS Institute, Cary, NC, USA). Graphs were generated using GraphPad Prism (version 5.04; GraphPad Software, La Jolla, CA, USA). Differences at baseline among groups for anthropometry, body composition including bone outcomes, and biomarkers were assessed using analysis of variance (ANOVA).

Mixed-model ANOVAs were used to determine if there were significant effects of group, time or group-by-time interactions for bone and body composition outcomes, with time reflecting baseline and 12-mo. All models were tested for covariance structure, using the best-fit statistics for each appropriate model. Reported p-values are those after adjustment for multiple comparisons using Tukey-Kramer; modeling included random effects (i.e., age, subject nested in group, ethnicity, family income and parental education) and fixed effects (group, time, BMI classification as overweight or obese and sex). Dietary data were also analyzed by ANOVA for

mean dietary intakes from 3-mo, 6-mo, 9-mo and 12-mo food diaries and compared among groups for differences for total energy, macronutrient, dietary calcium and vitamin D intakes as well as food groups. Proportional data were calculated to assess the if children increased or decreased their frequency in participating in weight-bearing types of activities using the questionnaires completed by parents at the baseline and 6-mo visits and at the baseline and 12-mo visits. Significance among groups were tested using separate chi-square analyses for baseline and 6-mo data versus baseline and 12-mo data. All analyses were performed as intent-to-treat and presented as mean ± standard deviation (SD), unless otherwise noted.

5.4 RESULTS

Baseline

1. Lifestyle characteristics

At baseline, 33 boys and 45 girls were measured (7.8±0.8 y); 32 boys and 41 girls completed the 12-mo visit. Most the children were white (82%) from families with annual household incomes greater than $75,000 Canadian dollars (58%). At baseline, StnTx had families with lower household incomes ($p=0.02$) and fathers with lower education ($p<0.001$) compared to ModTx and Ctrl. Those who did not complete the 12-mo visit (1=boy; n=4 girls) did not differ in terms of age, sex or socioeconomic status compared to children who were analyzed at 12-mo.

Fifty families (64%) returned 3-day food diaries at baseline. Dietary intake 1-week post baseline visit differed for total energy intake between ModTx (1567±271 kcal/d) and Ctrl (1909±312 kcal/d) ($p=0.02$) while protein intakes were significantly greater in Ctrl (83.5±18.3 g/d) compared to StnTx (69.7±13.8 g/d) and ModTx (68.4±10.5 g/d) ($p<0.05$). At baseline, the mean intakes of milk and milk products were 2.3±1.2 servings/day; mean dietary calcium intakes were 925±366 mg while dietary vitamin D intakes were 188±123 IU/d, all of which were not different among groups (See [347] for additional dietary data). Children participated in 3±2 types of weight-bearing activities per week, lasting 28±28 min/d, with no differences among groups.

2. DXA and bone biomarkers

Baseline anthropometric measurements were similar among groups [WAZ: 3.2±1.2 ($p=0.51$), HAZ: 1.1±0.9 ($p=0.95$), BMI: 24.4±3.3 kg/m^2 ($p=0.38$), BAZ: 3.3±1.2 ($p=0.33$)]. Bone

outcomes measured by DXA for WB, LS, LLS and UD ulna+radius did not differ among groups for BMC (**Figure 5.1**), and aBMD (**Table 5.1**); BMAD were also not different among groups at baseline. Body composition analyzed as FMI and FFMI were not different among groups at baseline (**Figure 5.2**).

There were no differences among groups for concentrations of 25(OH)D (n=71, 64.7±16.1 nmol/L, p=0.06), total osteocalcin (n=72, 54.6±20.6 ng/mL, p=0.59), BAP (n=67, 85.5±21.2 µg/L, p=0.46) or CTX-1 (n=58, 12.9±3.6 nmol/L, p=0.75) (**Figure 5.3**). PTH (n=62, 15.2±5.1 pg/mL, p=0.96) and total calcium (n=64, 2.3±0.1 mmol/L, p=0.49) did not differ among groups at baseline.

Changes from baseline to 12-months

1. Growth and whole-body bone outcomes

Throughout the study pubertal status were measured and did not change, whereby Tanner stage remained ≤2. All children grew in height ($p<0.001$). However, from baseline to 12-mo, both height velocities ($p<0.05$) and changes (Δ) in HAZ ($p<0.01$) were greater in StnTx and Ctrl compared to ModTx. Specifically, the height velocities were significantly greater in StnTx (6.5±1.2 cm/y) and Ctrl (6.3±1.1 cm/y) compared to ModTx (5.6±1.2 cm/y). Among all groups, values for WB area (**Table 5.1**) and WB BMC (Figure 5.1A) were greater at 12-mo ($p<0.001$). However, compared to baseline, 12-mo values of WB aBMD were greater in StnTx ($p<0.001$) and ModTx ($p<0.001$) versus Ctrl; whereas WB BMD z-scores were lower in Ctrl ($p=0.02$) compared to StnTx and ModTx (**Table 5.1**).

2. Lumbar spine, lateral lumbar spine and forearm analyses

Lumbar spine total area and aBMD (**Table 5.1**) as well as BMC (**Figure 5.1B**) increased by 12-mo in all groups. There were no group-by-time differences in LS z-scores at 12-mo (**Table 5.1**). Similarly, LLS total area (**Table 5.1**) and BMC (**Figure 5.1C**) increased in all groups whereas only StnTx ($p<0.001$) and ModTx ($p<0.001$) increased aBMD (**Table 5.1**); these outcomes did not differ among groups-by-time. When analyzing the LS as BMAD, there were no significant changes among groups over time (**Table 5.1**).

Total forearm length did not significantly increase in children from baseline or 12-mo among groups ($p=0.39$). By 12-mo, UD ulna+radius BMC increased in all groups by 12-mo

($p<0.001$) (**Figure 5.1D**), whereas only StnTx increased aBMD (**Table 5.1**). Forearm outcomes were not different among groups by 12-mo.

3. Body composition

At 12-mo, BAZ changed by -0.10±0.35 in Ctrl ($p=0.93$), -0.33±0.39 in StnTx ($p<0.001$) and -0.67±0.59 in ModTx ($p<0.001$) compared to baseline. BAZ values were significantly lower in ModTx compared to Ctrl by 12-mo ($p<0.01$). Fat mass significantly increased in Ctrl (ΔFM: 3.3±2.0 kg) compared to ModTx (ΔFM: -0.1±2.5 kg) ($p<0.001$) as did trunk fat mass (Ctrl: Δtrunk FM: 1.1±1.0 kg versus ModTx: Δtrunk FM: -0.1±1.1 kg, $p<0.01$) at 12-mo compared to baseline. By 12-mo, % body fat (%BF) measures were significantly decreased in ModTx (Δ%BF: -2.1±3.0%) compared to Ctrl (Δ%BF: 0.7±2.2%) ($p<0.05$). Ctrl significantly increased FMI ($p<0.001$) (**Figure 5.2A**); both Ctrl ($p<0.001$) and StnTx ($p=0.04$) significantly increased total appendicular fat mass (**Figure 5.2C**).

The changes in WB-lean mass (LM) from baseline to 12-mo were greater in StnTx (ΔLM 3.8±1.4 kg) and Ctrl (ΔLM 4.1±1.5 kg) compared to ModTx (ΔLM 1.9±1.3 kg) ($p<0.001$). However, when data were normalized for height, all groups significantly increased FFMI ($p<0.001$) with no differences among groups (**Figure 5.2B**). Appendicular lean mass analyzed as lean mass index also increased among all groups ($p<0.001$) with no group differences (**Figure 5.2D**).

4. Bone biomarkers

At 12-mo, 25(OH)D (**Figure 5.3A**), osteocalcin (**Figure 5.3B**), BAP (**Figure 5.3C**), CTX-1 (**Figure 5.3D**), PTH, and calcium did not differ among groups or compared to baseline.

5. Diet and activity

Only 33% (n=24/73) of the 3-day food diaries were returned at 12-mo. However, when comparing mean dietary intakes from 3-mo to 12-mo among groups, both StnTx (1699±273 kcal/d) and ModTx (1579±199 kcal/d) had significantly lower total energy intakes per day from 3-mo to 12-mo compared Ctrl (1962±376 kcal/d) ($p<0.05$). Similarly, the mean protein intakes from 3-mo to 12-mo were significantly lower in both intervention groups (StnTx: 73.9±9.5 g/d; ModTx: 70.2±12.1 g/d) compared to Ctrl (87.4±24.2 g/d) ($p<0.05$). Trending towards

significance were the lower mean dietary intakes of dietary fat (p=0.08) and meat and alternative intakes (p=0.07) in ModTx compared to Ctrl from 3-mo to 12-mo.

Both time spent (h/wk) and frequency (number/wk) of participating in weight-bearing activities did not significantly change among groups by 12-mo. However, when comparing the change in frequency in participating in weight-bearing types of activity on an individual-basis, the proportion of children who increased their frequency from baseline at 6-mo or from baseline to 12-mo were different among group. Specifically, 43%, 61% and 81% of children from Ctrl, StnTx, and ModTx, respectively, increased weight-bearing types of activity from baseline to 6-mo, whereby the difference was significant between ModTx and Ctrl (p=0.01). The percentage of children who increased weight-bearing types of activity from baseline to end of study were 43%, 58% and 56% in Ctrl, StnTx and ModTx, respectively; these proportions were not significantly different (p=0.61).

5.5 DISCUSSION

This study examined changes in bone outcomes measured by DXA and included an assessment of bone biomarkers in pre-pubertal children with obesity who participated in a 1-y family-centered lifestyle intervention. In this study, the independent effects of increasing servings of milk and milk products and weight-bearing types of activities cannot be determined, however, this study resulted in positive changes in whole body and lumbar spine assessments in BMC among all groups. These results suggest that despite decreases in adiposity, bone health outcomes were not compromised in pre-pubertal children with obesity, an important result as this pediatric population are at greater risk of bone alterations due to higher fat mass.

Unique to this study were the inclusion and distinction of weight-bearing (i.e., lumbar spine) versus non-weight bearing (i.e., UD ulna+radius) skeletal sites of assessment, as the effects of lean mass and fat mass on bone differ depending on site, age and sex [24]. To our knowledge, this is the first report of DXA assessment of lumbar spine in children with obesity participating in an intervention. As children grow, maturation of lower limbs occurs earlier than the spine [349]. This may be problematic in the obese pediatric population; as children with obesity continue to increase fat mass, their spines may not adapt at the same rate, potentially increasing their risk of damaging intervertebral joints and thereby causing back pain [293]. In

this study, BMD z-scores of the lumbar spine were within appropriate ranges for age and sex and did not significantly change among groups by 12-mo.

The use of family-centered lifestyle interventions to safely reduce adiposity in children has been reviewed in the literature [215], however, few report on its effects on changes in lean mass and/ or bone outcomes. In this study, compared to baseline, children randomized to intervention groups significantly decreased BAZ by 12-mo, while all groups significantly increased lean mass and BMC by 12-mo. However, the increase in fat mass were not similar among groups, suggesting that the effects of lean mass may have been more pronounced on bone mass than fat mass. Studies that include more sophisticated assessments of bone, such as peripheral quantitative computed tomography, are warranted to examine the direct effects of lean mass and fat mass on bone outcomes [24]. Additionally, this study also resulted in differences in height velocities among groups: both StnTx and Ctrl had significantly greater changes in HAZ and height velocities compared to ModTx at 12-mo. In StnTx and ModTx, aBMD significantly increased from baseline to 12-mo, whereas the increase in aBMD were not significantly different in Ctrl. Therefore, these results suggest that Ctrl grew in height but as aBMD remained relatively constant over the course of the study, this group presented with significantly lower WB BMD z-scores at 12-mo compared to baseline.

The interventions used in this study were based on Canadian diet and PA guidelines. At baseline, StnTx and ModTx had significantly lower dietary intakes of total energy and protein compared to Ctrl. However, when evaluating the mean dietary intakes from 3-mo to 12-mo from available food diaries, although dietary calcium, dietary vitamin D and mean daily servings of milk and milk products were not significantly different among group, children randomized to the intervention groups continued to consume less total energy and total protein compared to Ctrl. These results suggest that the intervention had a positive effect on changing dietary intakes in children.

Similarly, the physical activity intervention in this study was offered through counselling to families to either meet (StnTx) or exceed (ModTx) Canadian recommendations and were monitored by parent-completed survey. At baseline, parents reported children were meeting Canadian recommendations. Although this may be plausible, these results contradict Canadian national surveys that suggest only 10% of overweight children meet the current Canadian guidelines [350]. Therefore, we caution interpretation of our PA data, particularly since the PA

questionnaires were completed by parents who were asked to survey children's activity levels outside of the home environment; objective assessments are needed and therefore we used lean mass and FFMI as proxies for activity to circumvent this challenge. Despite not seeing significant differences in PA participation in general by group, when evaluating the percentage who increased weight-bearing types of PA from baseline to 6-mo or baseline to 12-mo, it is apparent that the interventions had positive effects on changing activity behaviors in children compared to the smaller percentage increases seen in Ctrl.

In addition to exploring interrelationships among body composition assessments and DXA-based bone outcomes, this study evaluated the effects of participating in an intervention on changes in bone biomarkers. Despite changes in fat mass, there were no significant changes in bone biomarkers by 1 y among all groups. Generally, these results conflict with comparable trials [198], with the exception of CTX-1, which as seen in a 3-mo lifestyle intervention [191], did not significantly change. Although CTX-1 may increase in obese adult weight-loss trials of short duration (3-months), research supports our findings in that CTX-1 did not differ from baseline in trials of 6-months to 24-months duration [184].

This study did not result in any statistical differences in osteocalcin, 25(OH)D and BAP by 1 y, however the patterns of change seen in this trial follow similar directions of change reported by others [191, 198, 207]. Specifically, that by 12-mo, ModTx showed a decreasing level of BAP while osteocalcin and 25(OH)D concentrations slightly increased. Conversely, Ctrl showed decreasing concentrations of osteocalcin and 25(OH)D with no apparent changes in BAP. Despite these opposing-patterns of change, both groups did not negatively impact overall bone health, suggesting that perhaps these biomarkers were not at sufficient concentrations to alter bone formation. It is important that studies that include assessment of bone biomarkers in obese children consider sex-differences [198, 351, 352]. Further, the degree of adiposity at baseline and actual loss of fat mass by end of the trial should be considered as biomarkers may respond differently to changes in adiposity [191, 198, 353].

As with all studies, this study presents with limitations. Despite a lengthy (2 years) recruitment period, this trial was powered to detect changes in BAZ not bone outcomes. As a secondary outcome, this study sought to test the effects of increasing milk and milk products in combination with weight-bearing physical activities above standard guidelines on changes in bone mass and bone biomarkers. A different study design, such as a step-wise approach to

introducing milk and milk products or weight-bearing types of activities, would have been needed to evaluate their independent effects on bone outcomes. Unfortunately, as seen in other childhood obesity interventions [354], compliance for returning 3-day food diaries were poor and therefore dietary intake variables were not accounted for in the statistical analyses. Often reported in obesity-related research is the underreporting of dietary intakes [232] and overreporting of physical activity [339]. Future studies should consider objective measures of PA and novel approaches to measuring dietary intake in children.

5.6 CONCLUSION

In summary, this study reports on the effects of body composition and bone parameters in overweight and obese children who participated in a 1 y family-centered lifestyle intervention. Children who were randomized to intervention groups successfully and safely reduced adiposity while maintaining bone outcomes. Despite changes in adiposity, there were no changes in bone biomarkers, likely due to study power or the rate at which fat mass changed. This study highlights the need for childhood obesity clinical trials to include bone outcomes. Future studies that include larger sample sizes are necessary to test the effects of lifestyle interventions on changes in bone health outcomes.

5.7 ACKNOWLEDGEMENTS

The authors would like to thank Sherry Agellon for her work with the biomarkers.

Table 5.1 Characteristics of participants at baseline and 12-mo assessed by DXA

	Control			Standard Intervention			Modified Intervention		
	Baseline	**12-mo**	**p-value**	**Baseline**	**12-mo**	**p-value**	**Baseline**	**12-mo**	**p-value**
Whole Body									
Area (cm^2)	(28)1267.76±128.66	(23)1427.12±148.04	**<0.001**	(25)1252.99±116.87	(22)1372.49±148.61	**<0.001**	(25)1257.24±95.30	(24)1377.11±116.07	**<0.001**
aBMD (g/cm^2)	(28) 0.901±0.063	(23) 0.921±0.058	0.23	(25) 0.888±0.054	(22) 0.913±0.645	**<0.001**	(25) 0.875±0.052	(24) 0.907±0.049	**<0.001**
z-score	(28) 3.2±1.1[a]	(23) 2.5±0.9	**0.02**	(25) 2.3±1.2	(22) 2.4±1.2	0.65	(25) 2.3±1.1 [a]	(24) 2.0±0.9	0.56
Lumbar Spine 1-4									
Area (cm^2)	(28) 37.18±3.66	(24) 39.68±4.53	**<0.001**	(25) 37.48±3.04	(23) 40.09±3.47	**<0.001**	(25) 38.39±3.93	(24) 40.73±4.34	**<0.001**
aBMD (g/cm^2)	(28) 0.636±0.069	(24) 0.666±0.087	**0.03**	(25) 0.644±0.096	(23) 0.677±0.096	**<0.001**	(25) 0.626±0.076	(24) 0.650±0.084	**<0.001**
BMAD (g/cm^3)	(28) 0.106±0.013	(24) 0.105±0.011	0.96	(25) 0.105±0.014	(23) 0.107±0.015	0.26	(25) 0.101±0.013	(24) 0.102±0.013	0.73
z-score	(28) 1.2±1.0	(24) 1.1±1.1	0.95	(25) 1.3±1.1	(23) 1.3±1.2	0.99	(25) 1.1±1.3	(24) 0.8±1.2	0.92
Lateral Lumbar Spine 3									
Area (cm^2)	(25) 16.59±2.13	(20) 19.58±2.54	**<0.001**	(23) 17.03±5.52	(19) 19.84±2.61	**<0.001**	(22) 16.72±1.94	(21) 19.78±2.19	**<0.001**
aBMD (g/cm^2)	(25) 0.543±0.082	(20) 0.572±0.078	0.18	(23) 0.544±0.096	(19) 0.592±0.080	**<0.001**	(22) 0.514±0.057	(21) 0.574±0.085	**<0.001**
Ultra-Distal Ulna+Radius									
Area (cm^2)	(28) 4.02±0.52	(25) 4.24±0.46	0.57	(25) 3.79±0.33	(23) 4.05±0.43	**<0.001**	(25) 3.92±0.58	(24) 3.99±0.47	1.00
aBMD (cm^2)	(28) 0.301±0.034	(24) 0.310±0.033	0.52	(25) 0.294±0.030	(23) 0.299±0.031	0.54	(25) 0.291±0.033	(24) 0.299±0.028	0.29

[a] Baseline WB BMD z-scores: Difference between ModTx and Ctrl p=0.03. Data presented as (number of participants) mean±SD; p-values represent differences within a group from baseline to 12-mo. z-scores not available for lateral lumbar spine and ultra-distal ulna+radius measures.

Figure 5.1 **Changes in bone mineral content (BMC) of whole body, lumbar spine, lumbar lateral spine and ultra-distal ulna+radius at baseline (BL) and 12-mo by group (mean ± SD); p-values denoted difference from baseline; *p<0.05; **p<0.001**

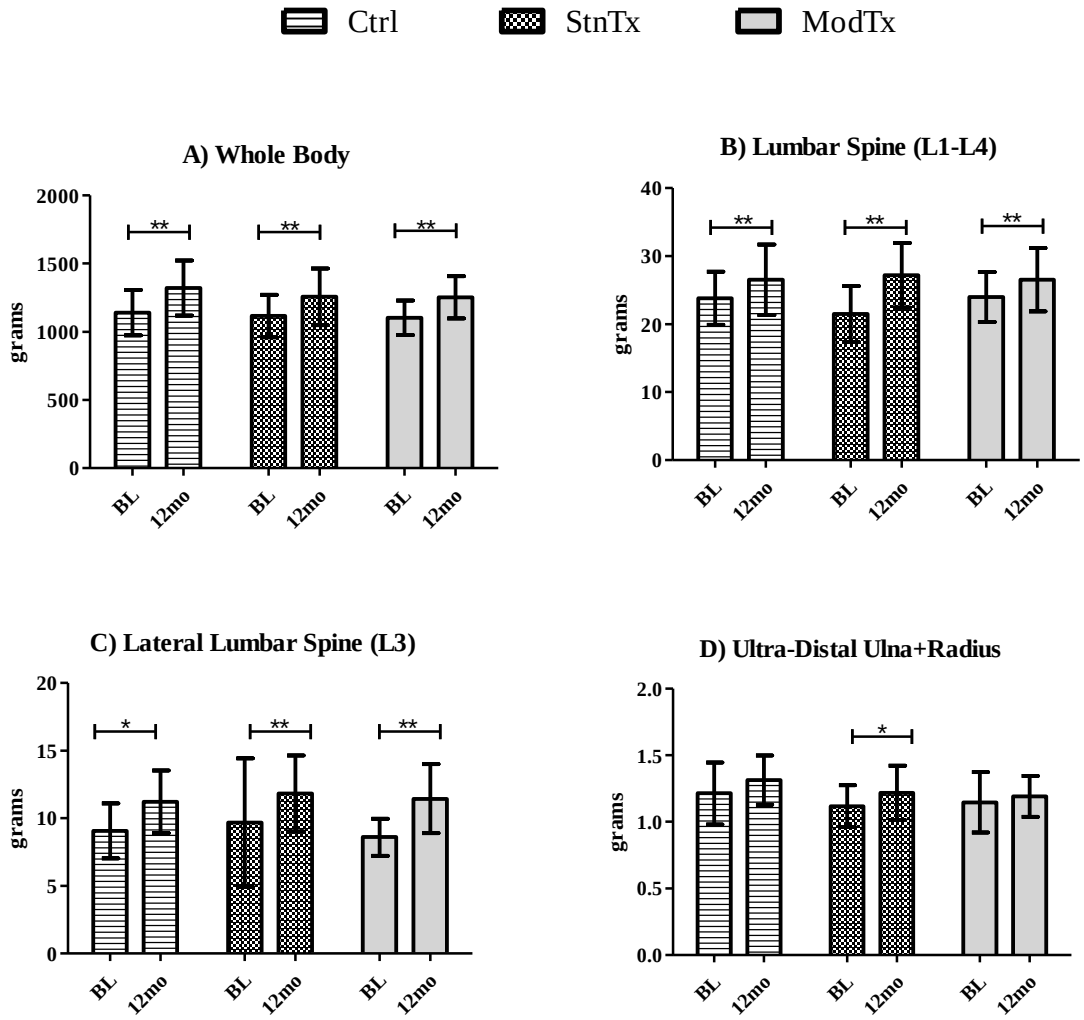


Figure 5.2 Changes in fat mass and fat-free mass of whole body (WB) and appendicular sites at baseline and 12-mo by group (mean ± SD); *p<0.05, **p<0.001

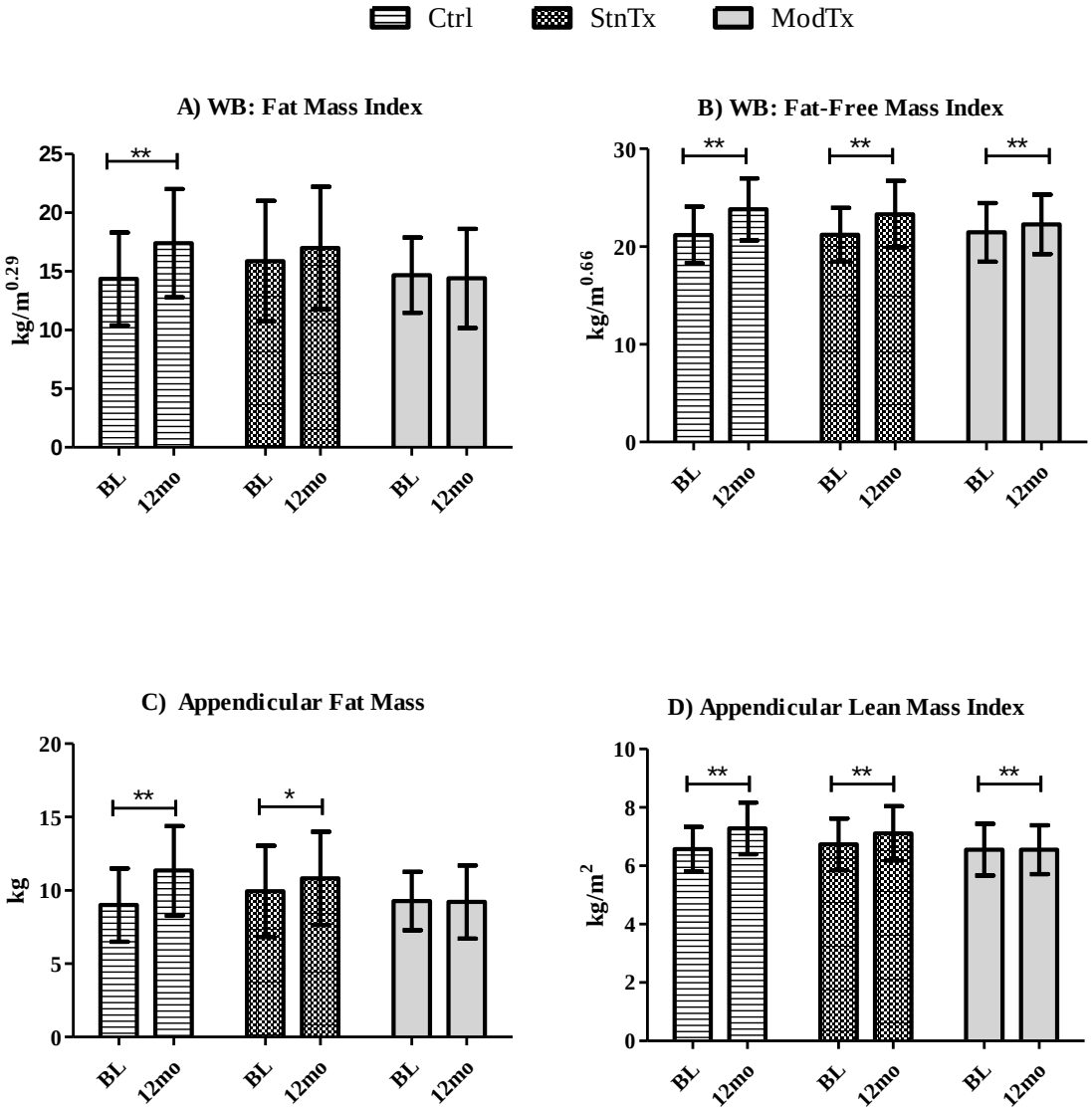
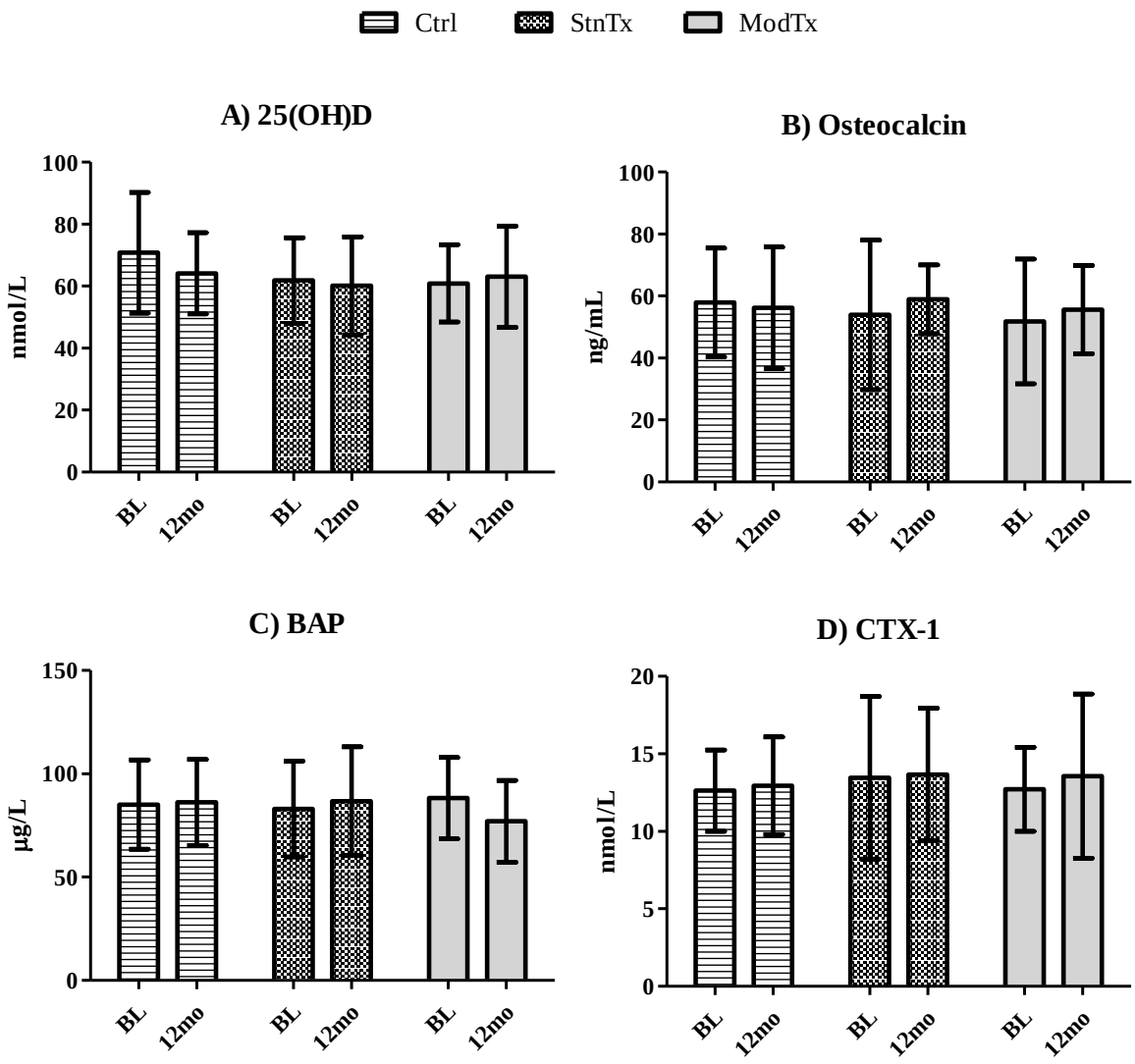


Figure 5.3 Changes in 25(OH)D, osteocalcin, BAP and CTX-1 values among groups from baseline and 12-mo (mean ± SD)



Bridge Statement 4

Manuscript 3 describes the changes in bone outcomes and biomarkers of bone health in children with obesity participating in the 1 year trial. Despite changes in adiposity in the intervention groups, bone health was maintained while bone biomarkers were unchanged; the results of this study are encouraging and suggest that changes in adiposity did not negatively affect bone health.

To date, this dissertation has reviewed physiological changes in children with obesity who participated in the MY LIFE Study. The literature review in Chapter 2 highlighted that a child's weight status is governed by many factors, one of which are eating behaviors. The research has acknowledged that children with obesity are more likely to be emotional eaters, are greater responders to external cues of food and are more likely to override feelings of fullness compared to normal weight peers.

The literature suggests that milk and milk products have a satiating effect and have been shown to decrease appetitive scores in children, which result in decreases in energy intakes. However, these trials are short and are not designed to test the long-term effects on appetite, or satiety. Further, few are tested in children with obesity. Should children with obesity favorably change eating behaviors in response to a milk intervention would be beneficial to those who work with this population in directing future dietary interventions.

Further, this literature review of this thesis suggested that energy intake is governed by different peptides: the hormone leptin has been shown to affect long-term regulation of energy balance. In children with obesity, leptin is often elevated and changes with fat mass. However, it is not known if changes in leptin are associated with changes in eating behaviors. As childhood obesity is a multifactorial disease, if changes in leptin concentrations are associated with changes in eating behaviors may be important to understand when designing childhood obesity interventions.

This next chapter aims to explore the impact of the family-centered lifestyle intervention on changes in eating behaviors and plasma leptin, and will provide findings that may be beneficial to future dietary interventions aimed at children with obesity.

CHAPTER 6

Manuscript 4: Eating Behavior and Leptin

Currently under peer-review

Changes in eating behavior and plasma leptin in children with obesity participating in a family-centered lifestyle intervention

Tamara R. Cohen, RD, MSc,[1] Tom J. Hazell, PhD,[2] Catherine A. Vanstone, RN, MSc,[1] Celia Rodd, MD, MSc,[3] Hope A. Weiler, RD, PhD[1]

Author's Affiliation:

[1] School of Human Nutrition, McGill University, Montreal, QC

[2] Department of Kinesiology and Physical Education, Wilfrid Laurier University, Waterloo, ON

[3] Children's Hospital, University of Manitoba, Winnipeg, MB

Corresponding Author:
Hope A. Weiler
McGill University, School of Human Nutrition
21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Québec H9X 3V9 Canada
Tel: (514) 398-7905
Fax: (514) 398-7739
E-mail: hope.weiler@mcgill.ca

6.1 ABSTRACT

Background: Eating behaviors can be modulated by different factors, such as the hormone leptin and foods that promote satiety, such as milk products. If increasing milk and milk products will favorably change eating behaviors and leptin concentrations in children with obesity is not known. This study examined the effects of increasing milk products in combination with daily weight-bearing types of activity on changes in eating behaviors and leptin in children with obesity who participated in a 1-y family-centered lifestyle intervention.

Methods: Interventions were based on Canadian diet and physical activity (PA) guidelines. Children were randomized to 1 of 3 groups: Control (Ctrl; no intervention), Standard treatment (StnTx: 2 servings milk products/day (d), 3x/wk weight-bearing PA), or Modified treatment (ModTx: 4 servings milk products/d; daily weight-bearing PA). Study visits occurred every 3-mo for 1-y; interventions were held once a month for 6-mo with one follow-up visit at 8-mo. Ctrl received counselling after 1-y. Caregivers completed the Children's Eating Behavior Questionnaire (CEBQ) and reported on diet and activity. Plasma leptin were measured from morning fasted blood samples.

Results: Seventy-eight children (mean age 7.8 ± 0.8 y; mean BMI 24.4 ± 3.3 kg/m^2) participated; 94% completed the study. Compared to baseline, at 6-mo StnTx reduced Emotional Overeating and Desire to Drink scores ($p<0.05$) while Food Responsiveness scores were reduced in both StnTx and ModTx ($p<0.05$). At 1-year, scores for Desire to Drink in StnTx and Food Responsiveness in ModTx remained reduced compared to baseline ($p<0.05$). Plasma leptin concentrations were significantly lower in ModTx at 6-months compared to baseline ($p<0.05$).

Conclusion: This study resulted in intervention groups favorably changing eating behaviors, supporting the use family-centered lifestyle interventions using Canadian diet and PA recommendations for children with obesity.

6.2 BACKGROUND

Eating behaviors are related to a child's weight status [355] and are influenced by personal preference, parental influences and environment [140]. Assessment of eating behaviors is often done by questionnaire [355], such as the Children's Eating Behavior Questionnaire (CEBQ) [92]. The CEBQ has been validated and used to assess eating behaviors in children from different countries [18-20, 356], including Canadian preschool children [17]. CEBQ scores are positively associated with body mass index (BMI) [15] and compared to normal-weight peers, children with obesity score differently in their responses to satiety cues, rate of meal consumption, sensitivity to environmental cues (i.e., emotional stress), and desire to drink [17]. It is important that interventions aimed at children with obesity consider these differences in eating behaviors when guiding children to adapt healthier lifestyles.

Long term food intakes can be mediated by hormones, such as the tonic protein leptin [357]. Lepin is released into circulation by adipose tissue among other sites [67]. Compared to normal-weight children, children with obesity often present with higher circulating leptin [67] that declines with decreases in BMI after dietary [86], physical activity [358], or lifestyle [359] focused interventions. However, whether changes in leptin concentrations are associated with changes in eating behaviors in children with obestiy has not been reported.

There has been growing interest in the effects of milk products on modulating body weight in children [360] as their effects induce satiety therefore reducing energy intake [77]. While there are many appetite hormones involved in the episodic control of food intake, the tonic signal leptin is known for its long-term regulation of dietary intake [67]. Therefore, should changes in leptin concentrations be associated with reductions in adiposity due to increased servings of daily milk products in addition to being associated with changes in eating behaviors would be of interest to interventions conveying realistic dietary messages to children with obesity.

In Canada, children 4-8 years are recommended to consume 2 servings of milk and milk products per day. In previous work, we showed that children randomized to consume 4 servings per day of milk and milk products plus participate in daily weight-bearing types of activity compared to children randomized to standard treatment (i.e., follow Canadian diet [23] and activity guidelines [361]) and control (no intervention), significantly reduced percentage body fat by 12-months following a 1-year family-centered lifestyle intervention [347]. Whether these

children also favorably changed eating behaviors and decreased plasma leptin concentrations has not been reported. Therefore, this paper aimed 1) to examine changes in eating behaviors and plasma leptin concentrations in overweight and obese children participating in a 1-year family-centered lifestyle intervention and 2) to determine if children randomized to increase milk and milk product intakes and participate in daily weight-bearing types of activity resulted in favorable changes in eating behaviors and leptin concentrations after 1-year. This study reports on secondary outcomes from a previously published randomized controlled trial [331, 347].

6.3 METHODS

Recruitment and Inclusion Criteria

Recruitment for the McGill Youth Lifestyle Intervention with Food and Exercise (MY LIFE) Study took place from 2011- 2013 in greater Montréal (Québec, Canada). Eligibility included healthy 6- to 8-year-old children with no known illness, who were overweight (85-97 percentile) or obese (>97 percentile) as per the World Health Organization (WHO) weight-for-height body mass index (BMI) cut-off criteria [38]. This study has been reviewed and approved by McGill University Faculty of Medicine Institutional Review Board (A09-M52-10B), Lester B. Pearson School Board and the English Montreal School Board (Trial Registration: ClinicalTrials.gov: NCT01290016).

The recruitment strategy has been published elsewhere [331]. At baseline (BL), parents consented and children assented to participate. Using a computer-generated list, a dietitian randomized and allocated (1:1:1) children to 1 of 3 groups (Standard [StnTx], Modified [ModTx] or control [Ctrl]) by sex and BMI percentile for age (overweight or obese) [38]. Socio-demographics were also surveyed at BL. Follow-up visits occurred every 3 months for 1-year at the Mary Emily Clinical Nutrition Research Unit (Sainte-Anne-de-Bellevue, QC, Canada). This paper reports on data collected when plasma leptin was analyzed, specifically at baseline, 6-months (end of intervention phase) and 12-months (end of study).

Interventions

At BL, all groups participated in a standard teaching of Canada's Food Guide [23] and physical activity (PA) [361] guidelines by a registered dietitian. StnTx and ModTx participated in a total of 6 interventions which were held at the end of each month for the first 5-months of

the study, then a final "relapse prevention" session at the end of the 8th month. The Ctrl group received the interventions after 1-year.

All interventions followed the same educational platform, but were tailored to meet the needs of the families and children by identifying barriers to meeting goals and strategizing how to achieve them considering all members of the family. Discussions concerning eating behaviors were included in each intervention as deemed fit by the dietitian. The only differences between StnTx and ModTx were the amount of milk and milk product intakes they were guided to consume (i.e., StnTx: 2 servings/day; ModTx: 4 servings/day) and the time allotted to weight-bearing physical activities per week (i.e., StnTx: 3 times/week; ModTx: daily). The goal of 60 minutes of moderate-to-vigorous PA and maximum of 2-hours of screen time per day were encouraged to in both StnTx and ModTx, as were Canada's Food Guide recommended servings for fruit and vegetables, grain products and meat and alternatives. Canadian nutrition guidelines are based on similar principals as those for children in the United States [362]. Specifically, Canada's Food Guide to Healthy Eating recommends children 4-8 years of age to consume 5 servings/day of fruit and vegetables (e.g., 1 serving= ½ cup (125 ml) fruit; 1 cup (250 ml) leafy vegetable), 4 servings/day of grain products (e.g., 1 serving= 1 slice of bread (35g) or ½ cup (125 ml) cooked pasta, rice), 2 servings/day of milk and milk products (e.g., 1 serving= 1 cup (250 ml) milk; 50 g (1.5 oz.) cheese; ¾ cup (175 g) yogurt) and 1 serving/day of meat and alternatives (e.g., 1 serving= 75g meat, fish or poultry; 2 eggs) [23]. Details concerning the education and intervention topics are published elsewhere [331].

Outcomes

Eating Behaviors

The Child Eating Behavior Questionnaires (CEBQ) [92] consists of eight scales that reflect different eating styles. Questions are surveyed using a 5-point Likert scale where eating behaviors are identified as: never, rarely, sometimes, often, and always. This questionnaire contains 35 questions that are categorized using 8 subscales (n= # of questions): 1. Food Responsiveness (n=5); 2. Enjoyment of Food (n=4); 3. Slowness in Eating (n=4); 4. Food Fussiness (n=6); 5. Satiety Responsiveness (n=5); 6. Emotional Overeating (n= 4); 7. Emotional Undereating (n=4); and 8. Desire to Drink (n=3) (**Table 6.1**). Questions were scored per Wardle and colleagues [92]. Specifically, for each participant, the score for each subscale was calculated

as the sum of the individual questions divided by the total number of questions. These subscales were further grouped into 2 categories: (1) Food Approach, or pro-intake scores, which comprises of the total scores of Food Responsiveness, Enjoyment of Food, Emotional Overeating and Desire to Drink questions; and (2) Food Avoidance, also called anti-intake scores, which sums up scores identified from the headings Slowness in Eating, Food Fussiness, Satiety Responsiveness and Emotional Undereating. The CEBQ was completed during each study visit by the caregiver and analyzed at the end of the trial.

Anthropometry

Research-trained nurses, dietitians and kinesiologists performed all anthropometric measurements. Height was measured to 0.1 cm using a SECA 213 portable stadiometer (SECA Medical Scales and Measuring Systems, Hamburg, Germany). Weight was measured using a standard balance beam scale (Detecto, Webb City, MO, USA). Body mass index (BMI) (kg/m^2) were calculated; BMI for-age and-sex z-scores (BAZ) were computed using the WHO AnthroPlus Software version 3.2.2. [333].

Body Composition

Body composition, specifically whole body lean mass (LM), fat mass (FM), percent body fat (%BF) were assessed using whole-body dual-energy x-ray absorptiometry (DXA) (Hologic Discovery A fan beam with APEX software [version 13.3:3], Hologic Inc, Bedford, MA, USA). For consistency across and within subjects, children were measured in light clothing with no metal, zippers or jewelry. Values were compared to the Hologic normative database (U.S. National Health and Nutrition Examination Surveys). Quality assurance was conducted at each visit using Hologic lumbar spine phantom no. 14774; coefficients of variability were 0.5% for bone mineral content and 0.3% for bone mineral density.

Biochemistry

Children presented to each study visit 12-hours fasted. A venous blood sample was collected between 8:00 am and 12:00 pm by a registered nurse. Samples were stored in -80ºC freezers and thawed for analyses. Plasma leptin was analyzed using an immunoassay (Millipore

Milliplex panel Cat # HMHMAG-34K, Millipore Corp., St. Charles, Mo, USA), with a 4.0% intra-assay and 15.4% inter-assay coefficient of variation.

Diet

Dietary intakes were assessed using 3-day food diaries. At baseline, registered dietitians instructed parents on how to properly complete the diaries, by reporting their child's food consumption on 3 non-consecutive days. The food diaries were collected 1-week post BL visit (mailed back to research unit by Canada Post); all subsequent food diaries were completed by parents 1-week prior to study visit and were returned to study unit during the visit. Food diaries were analyzed using Nutritionist Pro Software (Axxya Systems, Stafford, TX, USA) using the Canadian Nutrient File 2010b; food items were categorized into food groups described by Eating Well with Canada's Food Guide to Healthy Eating [23]. More specifically, milk products were grouped as mean servings per week to reflect intakes at BL, 6-mo and 12-mo, but also broken down to specific food items (i.e., milk, cheese, yogurt and milk product alternatives (i.e., soy beverage)) for descriptive analyses.

Physical Activity

Physical activity was caregiver reporting using the Physical Activity Questionnaire for Children [PAQ-C] [303] and modified to include measures of time and intensity [mPAQ-C]. This questionnaire reflected the child's PA the week prior to study visits. Weight-bearing PA were categorized by frequency (number of times per week) and duration (minutes per week). The mPAQ-C included questions related to sedentary behavior, specifically asking parents about total screen hours/week (i.e. average time in hours/week that the child watched television or played on the computer/video games while sitting down).

Statistical Analysis

Data were analyzed using SAS version 9.3 (SAS Institute, Cary, NC, USA). Graphs were generated using GraphPad Prism (version 5.04; GraphPad Software, La Jolla, CA, USA); multidimensional charting were performed on Microsoft Excel (Microsoft Office 2017). Data were tested for normality; leptin was the only variable not normally distributed and was therefore log-transformed prior to analysis. Sample size calculation has been published elsewhere [331].

Aligned with the recommendations of CONSORT, differences at baseline among groups for anthropometry, body composition, CEBQ and leptin were not tested [363-365]. Baseline data were merged to test for associations among leptin, body composition and CEBQ subscales using Pearson correlations.

Mixed-model ANOVAs were used to determine if there were significant effects of group, time or group-by-time interactions for CEBQ subscales and leptin, with time analyzed at 6-months (end of intervention phase) and 12-months (end of study). Similarly, the percentage change from baseline to 6-months and baseline to 12-months in body composition outcomes (i.e., changes in BAZ, FM and %BF) were analyzed for group-by-time interactions. All models were tested for covariance structure, using the best-fit statistics for each appropriate model and included both fixed effects (i.e., age, group, time, BMI, and sex) and random effects (i.e., subject nested in group).

Models were also analyzed with BMI as a category (i.e., classification [overweight or obese]); there were no differences in best-fit statistics or interpretation of the results. Models did not include milk and milk products as a random effect as the return of 3-day food diaries were only 41% and 33% at 6 and 12-months, respectively, which would limit the number of observations accounted for in the analyses. **Supplement Table 6.1** reports on models that accounted for both diet (milk products) and activity (weight-bearing activity) as random effects. Reported p-values are those after adjustment for multiple comparisons using Tukey-Kramer. All analyses were performed as intent-to-treat and presented as mean ± standard deviation (SD), unless otherwise noted.

6.4 RESULTS

This study enrolled 78 children (mean age: 7.8±0.8 year): 75 children completed the 6-month visit and 73 children completed the 12-month visit. The study's CONSORT diagram has been published [347]. Most participants were white (n=61, 81.3%) girls (n=45, 57.7%) who came from homes with total household incomes >$75,000/y (n=40, 58.8%).

At baseline, 92% of the children were classified as obese as per WHO BMI percentiles; BAZ ranged from 1.5 to 8.3 (mean BAZ: 3.3±1.2) (**Table 6.2**). Reasons for discontinuing the study at 12-month (n=5) were due to lack of time, or study unit being too far from home; these

children did not differ in terms of anthropometry or body composition compared to children who completed the 12-month visit.

Multidimensional charting shown in **Figure 6.1** outlines the mean baseline CEBQ scores from all children (n=78). Overall, children from this study exhibited behaviors associated mostly in the "Food Approach" domain with Enjoyment of Food (4.4±0.6) and Food Responsiveness (3.8±0.8) having the highest mean scores (**Table 6.2**).

Three-day food diaries returned to the study center 1-week post baseline visit (n=50) resulted in milk product consumption to be 2.3±1.2 servings/day which were not different among groups. Fluid milk was the most consumed product (1.1±0.9 servings/day) followed by cheese (0.8±0.5 servings/day), yogurt (0.3±0.3 servings/day) and milk alternatives (0.0±0.1 servings/day).

Associations between baseline leptin and body composition, CEBQ and lifestyle variables

At baseline, mean leptin concentrations (n=66) were 12.3±7.8 ng/mL (range: 3.0-42.0 ng/mL). Using the total baseline sample (n=66), plasma leptin significantly correlated with BAZ ($r=0.46$, $p<0.01$), and fat mass ($r=0.68$, $p<0.001$). Only one CEBQ score (i.e., Desire to Drink scores) was found to be moderately correlated with plasma leptin concentrations at baseline ($r=0.30$; $p=0.02$); there were no other significant correlations between baseline leptin and CEBQ scores. Additionally, there were no significant correlations between leptin concentrations and lifestyle behaviors, which included total servings of milk products, weight-bearing PA (time or frequency per week) or sedentary behaviors (screen hours/week).

Effects of the intervention on changes in CEBQ subscales

Changes in CEBQ scores from baseline to 6-months

Compared to baseline, at 6-months CEBQ scores were lower in both StnTx and ModTx for Food Responsiveness ($p<0.01$) (**Figure 6.2, Panel A**), whereas StnTx had significantly reduced scores for both Emotional Overeating ($p<0.05$) (**Figure 6.2, Panel B**) and Desire to Drink ($p<0.05$) (**Figure 6.2, Panel D**). Categorizing CEBQ scores as either Food Approach or Food Avoidance, Food Approach scores were significantly reduced in both StnTx (-0.5±0.1; $p<0.05$) and ModTx (-0.3±0.1; $p<0.05$); Food Approach scores remained unchanged in Ctrl (-

0.1±0.1; p=1.00). Scores analyzed under the Food Avoidance heading did not significantly change among groups.

Changes in CEBQ scores from baseline to 12-months

Compared to baseline, by 12-months, Desire to Drink scores were significantly reduced in StnTx ($p<0.05$) (**Figure 6.2, Panel D**). Food Responsiveness scores were significantly lower in ModTx compared to baseline ($p<0.05$) (**Figure 6.2, Panel A**). There were no other significant differences among groups at 12-months for changes in CEBQ scores. However, scores categorized as Food Approach were significantly reduced in StnTx (-0.5±0.1, $p<0.05$); ModTx reduced scores categorized as Food Approach (-0.4±0.1, p=0.22) whereas Ctrl resulted in relatively no change (0.0±0.1; p=1.00) by 12-months. Food Avoidance scores were not significantly different within or between groups from baseline to 12-months (data not shown).

Effects of the intervention on changes in leptin

Compared to baseline, mean leptin concentrations at 6-months were significantly lower in ModTx (-3.88±0.35 ng/mL, $p<0.05$) and had decreased in StnTx (-1.48±0.76 ng/mL, p=1.00); at 6-months leptin concentrations remained unchanged in Ctrl (-0.10±0.31 ng/mL, p=1.00) (**Figure 6.3)**. At 12-months, leptin concentrations were significantly lower in ModTx compared to Ctrl ($p<0.05$) but not StnTx (p=0.07). There were no differences between leptin concentrations at 12-months between StnTx and Ctrl (p=1.00).

Effects of the intervention on changes in body composition

Body composition variables were analyzed at the percentage change (%Δ) from baseline to 6-months and from baseline to 12-months.

Changes in body composition from baseline to 6-months (end of intervention phase)

The %Δ in BAZ was significantly less in Ctrl (mean %Δ in BAZ: -1.1±9.3) compared to ModTx (mean %Δ in BAZ: -19.5±14.9) (p=0.01); the mean %Δ in BAZ in StnTx was -5.1±8.9. Similarly, ModTx resulted in a significant reduction in FM (mean %Δ FM: -6.5±11.2%) compared to StnTx (mean %Δ FM: 4.6±7.5%) (p=0.01) and Ctrl (mean %Δ FM: 7.6±7.9%) ($p<0.001$); the %Δ FM between StnTx and Ctrl were also significantly different (p=0.03) at 6-months. The %Δ in %BF were significantly greater in ModTx (mean %Δ %BF: -6.1±7.1%)

compared to Ctrl (mean %Δ %BF: -0.3±4.7%) ($p<0.01$) but not compared to StnTx (mean %Δ %BF: -1.2±4.5%) ($p=0.13$) at 6-months.

Changes in body composition from baseline to 12-months (end of study)

Compared to baseline, at 12-months all groups significantly increased whole body lean mass ($p<0.001$) whereby LM increased by 16.5%, 14.8% and 7.3% in Ctrl, StnTx and ModTx, respectively. By 12-months, although not significantly different among groups, the %Δ in BAZ were greater in ModTx (mean %Δ BAZ: -22.3±15.1%) compared StnTx (mean %Δ in BAZ: -8.2±13.3%) and Ctrl (mean %Δ in BAZ: -1.0±10.1%). The %Δ in FM were significantly greater in Ctrl (20.8±12.3%) compared to ModTx (-2.2±15.6%) ($p<0.001$); StnTx increased FM by 10.2±12.3% which was significantly greater to ModTx ($p<0.05$) but not to Ctrl ($p=0.51$). Finally, the %Δ in %BF was significantly greater in Ctrl (2.2±5.8%) compared to ModTx (-6.5±8.3%) at 12-months ($p<0.01$). The %Δ in %BF in StnTx at 12-months was -2.3±7.2% which was significantly less to ModTx ($p=0.02$) but not statistically different compared to Ctrl ($p=0.85$).

Associations between changes in body composition and CEBQ

Data were analyzed for associations between %Δ in BAZ, FM and %BF with CEBQ scores by group and time (i.e., 6-months and 12-months).

Changes in body composition at 6-months and CEBQ scores

Compared to baseline, the %Δ in BAZ were positively associated with Desire to Drink scores (rho=0.45; $p<0.05$) in ModTx. The %Δ in FM were negatively associated with Enjoyment of Food (rho=-0.48; $p<0.05$) and Food Fussiness (rho=-0.49; $p<0.05$) scores in ModTx. Similarly, Food Fussiness scores were negatively associated with %Δ in %BF in ModTx (rho=-0.52; $p<0.05$). Compared to baseline, there were no significant associations in %Δ in BAZ, FM and %BF with CEBQ scores in Ctrl and StnTx.

Changes in body composition at 12-months and CEBQ scores

Compared to baseline, the %Δ in BAZ were negatively associated with Slowness in Eating scores (rho=-0.45; $p<0.05$) in StnTx. There were no significant associations found between the %Δ in FM and %BF and CEBQ scores among all groups at 12-months compared to baseline.

Effects of the intervention on diet and activity

Fifty food diaries were returned to study center at baseline; 41% and 33% were returned at 6 and 12-months. There were no differences between children who returned food diaries or not for anthropometric and body composition variables. Details concerning dietary analyses has been published. Milk product intake did not significantly differ at 6 or 12-months between groups (data published [347]); at 12-months, mean milk product intakes were 2.8±1.1 servings/day in ModTx. Similarly, parent-reported PA and sedentary behaviors did not significantly change among or between groups at 12-months [347].

6.5 DISCUSSION

Overall, this study showed that children randomized to family-centered intervention groups successfully changed their eating behaviors compared to Ctrl. Specifically, that CEBQ Food Approach scores were significantly decreased both intervention groups at 6-months with reductions sustained until the end of study whereas eating behaviors remained unchanged in children randomized to Ctrl, suggesting that a family-centered lifestyle intervention can mediate changes in eating behaviors in children with obesity. Further, this study evaluated the tonic satiety signal leptin to reflect changes in body composition in children with obesity and tested for associations between changes in leptin and eating behaviors: leptin concentrations were reduced in both intervention groups and remained at a lower concentration at 12-months compared to baseline. However, changes in leptin did not associate with changes in eating behaviors. Nonetheless, this study suggests that interventions based on Canadian diet and PA guidelines using the family-centered approach were successful at modifying eating behaviors and plasma leptin, which are favorable outcomes for children with obesity.

The results of this study are consistent with previous literature suggesting children with obesity are highly sensitive to environmental cues of food and tend to consume foods in response to negative emotions [18, 107]. However, they are also more likely to limit the range of food products that they consume [15]. In general, the children participating in this study had higher mean baseline scores for Food Approach subscales, which align with those who assessed Brazilian [19], Portuguese [18], Chilean [366], British [15] and Canadian [17] overweight and obese children. The findings in this study are consistent with cross-sectional studies; however, to our knowledge this is the first 1-year RCT in children with obesity that was based on Canadian

health guidelines that has included the CEBQ. In this study, children randomized to Ctrl were simply taught the Canadian recommendations at the baseline visit which resulted in no changes in eating behaviors over the course of the study, suggesting that eating behaviors can be positively changed if families are educated and guided to meet nutrition and PA recommendations versus simply given generic information on healthy lifestyle behaviors.

In addition to exploring changes in eating behaviors, this study evaluated the effects of participating in an intervention on changes in plasma leptin concentrations. The ranges of leptin seen in this study are aligned with others [86, 358, 359, 367, 368]. In previously published work, we showed that ModTx had significantly decreased %BF at 6-months [347], therefore supporting the literature whereby changes in adiposity mediated changes in leptin concentrations [367]. In this study, the percentage change in body composition outcomes favored intervention groups over no changes or increases in fat mass in Ctrl. In comparable weight-loss trials in children with obesity, short-term losses of body fat resulted in reductions in leptin; however, leptin concentrations do not remain suppressed despite changes in body composition [369]. However, leptin concentrations remained reduced at 12-months compared to baseline measures in both of our intervention groups; changes in leptin were not associated with changes in eating behaviors, suggesting that changes in body composition were mediating the effects on leptin.

This study included an intervention arm that encouraged increased milk and milk product consumption in addition to engaging in daily weight-bearing activity. However, due to the low return of 3-day food diaries, our statistical analyses and modeling did not include dietary variables and therefore our results did not analyze for associations between diet and changes in leptin. A recent meta-analysis of clinical trials suggests that consumption of dairy has beneficial effects on satiety, specifically fullness and reduced hunger [370]. Research in adult studies has found that leptin concentrations differ with nutritional intake [83] specifically that in low-fat/high carbohydrate diets, leptin concentrations increase whereas high-protein diets do not mediate changes [67]. To our knowledge, only one other study has published work that examines the effects of milk products on changes in body composition in obese adolescents and includes measures of leptin. They found a 30% and 50% increase in leptin in adolescents randomized to casein and whey groups, respectively [371], however these groups also increased fat mass by the end of the trial. This study tested for associations between changes in leptin and CEBQ scores and resulted in no significant correlations. Leptin is considered a tonic signal based on fat mass

and overall energy balance whereas the CEBQ can be viewed as a descriptive measure of current (acute) appetite-regulation that includes assessments of hunger and fullness. Future work that examines the effects of episodic signals involved in appetite-regulation should consider other valid markers, such as ghrelin and evaluate their associations with CEBQ scores in children with obesity.

Strengths and Limitations

While this is the first 1-year family-centered lifestyle intervention in children with obesity that used Canadian health guidelines as its intervention platform [331], there are important strengths and limitations that should be addressed. Firstly, the main limitation of this study was the limited number of 3-day food diaries at 6 and 12-mo; statistical models did not include milk products as a random effect, however, results presented in Supplemental Table 1 that include both diet and activity resulted in similar findings. Dietary assessment in children is a challenge and the literature suggests individuals classified as obese often underreport dietary intake [372], further adding to the issue of return of food diaries as majority of parents in this study were self-identified as overweight or obese. Secondly, we acknowledged that the lack of effects or relationships between CEBQ and leptin may be due to statistical power rather than to true lack of relationship; this study was powered on changes in BAZ not changes in CEBQ scores or leptin. Nonetheless, this study used the CEBQ to capture changes in eating behaviors over the course of a year, an outcome that has not been previously reported, and showed that the family-centered lifestyle approach which aims to reduce adiposity in children with obesity has the potential to mediate favorable changes in eating behaviors. In this study, the CEBQ were completed during each visit by parents, and were analyzed at the end of the study for statistical purposes and not for clinical use; future work may wish to consider evaluating the CEBQ during interventions and incorporating standard methods of addressing and documenting methods of changing eating behaviors as they arise. The subjectivity of the CEBQ offers many advantages: it is completed by caregivers thus provides a global understanding to the underlying behavioral issues that the child may experience. Furthermore, compared to other dietary assessments methods, the CEBQ allows for reflection of different environments that the children may encounter [20]. However, a limitation of this questionnaire, particularly for the Desire to Drink scores, is that it does not describe the type of beverage that children are consuming: an increase

in water and decrease in sugary drinks would be favorable choices, which are not reflected when answering questions from this subscale [15]. To account for changes in choices of beverages, researchers should include other dietary assessment techniques, such as 3-day food diaries and new platforms/technology for capturing the information.

Nonetheless, the CEBQ a valuable assessment tool that should be included in study designs or family-centered interventions for obese children. It should be stressed that the use of the CEBQ is not for diagnostic purposes of eating disorders, but rather should be used to characterize eating behaviors and thus mediate discussions by trained professionals during interventions. Future studies are warranted to test the effects of analyzing the CEBQ during interventions and tailoring intervention sessions to address specific subscales. Finally, future trials should consider follow-up assessments beyond 1-year to asses if changes in either CEBQ scores, leptin or body composition persist in the long term. Finally, aligned with the recommendations of randomized controlled trials that follow CONSORT [364], our results are not adjusted for differences in sociodemographic variables that may differ among groups. We previously reported that children randomized to StnTx had more father's with lower education and came from households with lower total family income compared to Ctrl and ModTx [347]; mixed-models that include total household income and parental education resulted in similar findings (**Supplemental Tables 6.2 and 6.3**).

6.6 CONCLUSION

The use of the family-centered lifestyle approach to reduce adiposity in children with obesity resulted in favorable changes in eating behavior and plasma leptin concentrations after 1-year. This study supports previous research demonstrating that children with obesity exhibit higher scores for Food Approach subscales and suggests that interventions need to include discussions tailored to individual families. Future studies that include larger sample sizes are necessary to test the effects of changing diet on eating behaviors and leptin.

6.7 CONFLICT OF INTEREST

None of the authors have a conflict of interest to declare.

6.8 FUNDING AND ACKNOWLEDGMENTS

This study was supported by an operating grant from the Dairy Research Cluster Initiative (Agriculture and Agri-Food Canada, Dairy Farmers of Canada and the Canadian Dairy Commission. TC is supported by Frederick Banting and Charles Best Canada Graduate Doctoral Award (Canadian Institutes of Health Research). HW is supported by a Canada Research Chair and the Canada Foundation for Innovation.

The authors would like Sherry Agellon for conducting the immunoassays and all the families who participated in the study.

Table 6.1 Eight subscales of eating behavior measures from the CEBQ [92, 366]

Subscale	Definition	Example Question
Food Approach (Pro-Intake)		
Enjoyment of Food	Condition positively associated with hunger, desire to eat and pleasure by the food	• "My child loves food" • "My child looks forward to mealtimes"
Food Responsiveness	Eating in response to environmental food cues	• "My child is always asking for food" • "If given the chance, my child would always have food in his/her mouth"
Emotional Overeating	Tendency to increase the intake in response to negative emotional contexts	• "My child eats more when worried" • "My child eats more when anxious" • "My child eats more when s/he has nothing else to do"
Desire to Drink	Desire to drink and generally tends to choose sugary drinks	• "My child is always asking for a drink" • "If given the chance, my child would drink continuously throughout the day" • "If given the chance, my child would always be having a drink"
Food Avoidance (Anti-Intake)		
Satiety Responsiveness	Decreased sense of hunger caused by food consumption	• "My child can not eat a meal if s/he has had a snack just before" • "My child leaves food on his/her plate at the end of a meal"
Slowness in Eating	Tendency to eat more slowly over a meal and prolong meal duration	• "My child eats more and more slowly during the course of a meal" • "My child takes more than 30 min to finish a meal"
Emotional Undereating	Tendency to reduce intake as a result of negative emotional contexts	• "My child eats less when angry" • "My child eats less when upset"
Food Fussiness	Limits the range of food products that are acceptable	• "My child refuses new foods at first" • "My child decides that s/he doesn't like a food, even without tasting it"

Table 6.2 Baseline characteristics of children participating in a family-centered lifestyle intervention

	Ctrl	StnTx	ModTx	Total
Weight z-scores	(28) 3.1 ± 1.0	(25) 3.4 ± 1.3	(25) 3.0 ± 1.2	(78) 3.2 ± 1.2
Height z-scores	(28) 1.2 ± 0.9	(25) 1.1 ± 0.9	(25) 1.1 ± 0.9	(78) 1.1 ± 0.9
Body mass index (BMI) (kg/m^2)	(28) 24.1 ± 3.2	(25) 25.2 ± 3.9	(25) 24.1 ± 2.8	(78) 24.4 ± 3.3
BMI for-age and-sex z-score (BAZ)	(28) 3.2 ± 1.1	(25) 3.6 ± 1.4	(25) 3.1 ± 1.0	(78) 3.3 ± 1.2
Percent body fat (%BF) (%)	(28) 36.4 ± 4.9	(25) 38.5 ± 5.5	(25) 36.8 ± 5.6	(78) 37.2 ± 4.9
Fat mass (FM) (kg)	(28) 15.6 ± 4.4	(25) 17.2 ± 5.7	(25) 16.0 ± 3.6	(78) 16.3 ± 4.6
Leptin (ng/mL)	(24) 11.9 ± 8.0	(20) 15.2 ± 9.8	(22) 10.0 ± 4.8	(66) 12.3 ± 7.8
Food Approach	(26) 3.3 ± 0.5	(24) 3.6 ± 0.5	(24) 3.5 ± 0.6	(74) 3.5 ± 0.5
Food Responsiveness	(26) 3.7 ± 0.9	(24) 3.7 ± 0.9	(24) 3.8 ± 0.8	(74) 3.8 ± 0.8
Emotional Overeating	(26) 2.6 ± 0.9	(24) 2.9 ± 0.8	(24) 2.9 ± 1.1	(74) 2.8 ± 0.9
Enjoyment of Food	(26) 4.4 ± 0.7	(24) 4.3 ± 0.7	(24) 4.5 ± 0.5	(74) 4.4 ± 0.6
Desire to Drink	(26) 2.6 ± 0.8	(24) 3.2 ± 0.9	(24) 2.9 ± 1.0	(74) 2.9 ± 0.9
Food Avoidance	(26) 2.7 ± 0.2	(24) 2.8 ± 0.3	(24) 2.6 ± 0.3	(74) 2.7 ± 0.3
Satiety Responsiveness	(26) 2.7 ± 0.4	(24) 2.8 ± 0.4	(24) 2.6 ± 0.6	(74) 2.7 ± 0.5
Slowness in Eating	(26) 2.5 ± 0.4	(24) 2.6 ± 0.4	(24) 2.4 ± 0.5	(74) 2.5 ± 0.4
Emotional Undereating	(26) 2.6 ± 0.6	(24) 2.8 ± 0.6	(24) 2.3 ± 0.6	(74) 2.6 ± 0.6
Food Fussiness	(26) 3.0 ± 0.3	(24) 3.1 ± 0.3	(24) 3.0 ± 0.3	(74) 3.0 ± 0.3

NOTE: Data presented as (n) mean ± standard deviation

Figure 6.1 Multidimensional chart of mean CEBQ scores grouped as Food Approach and Food Avoidance at baseline for all participants (n=78)

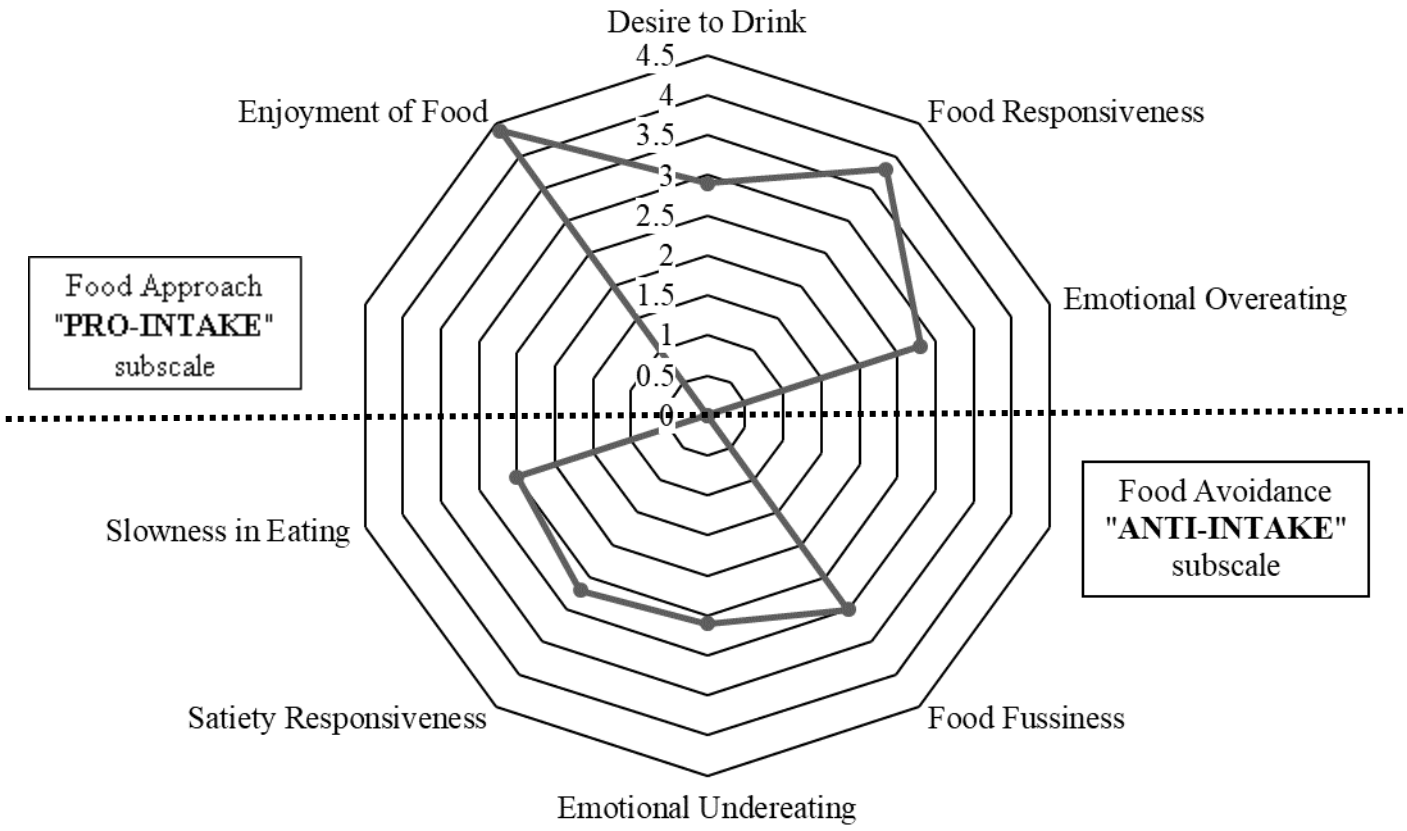


Figure 6.2 CEBQ Subscales categorized by group at baseline, 6-month and 12-month, mean ± standard deviation; *p<0.05

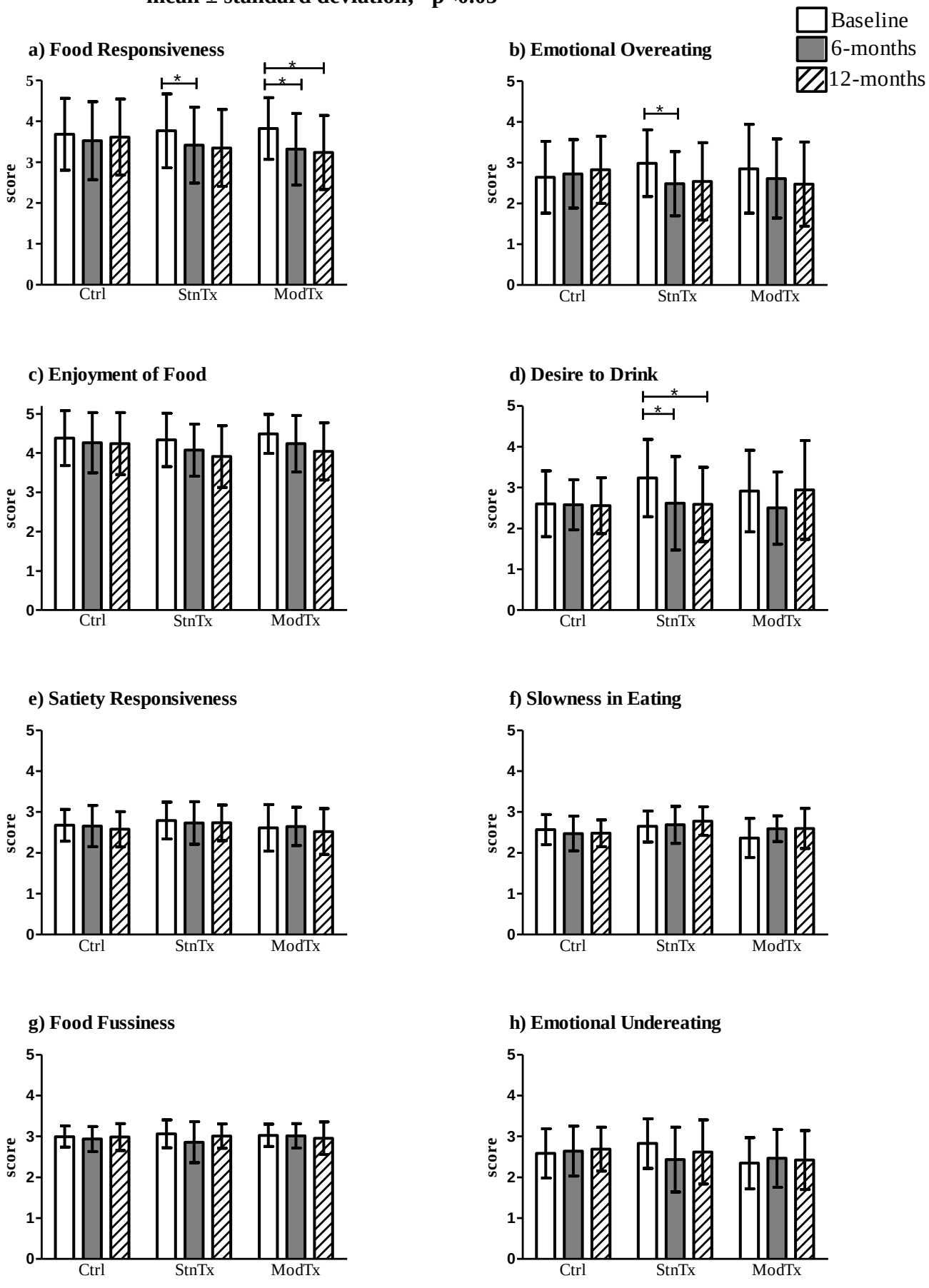
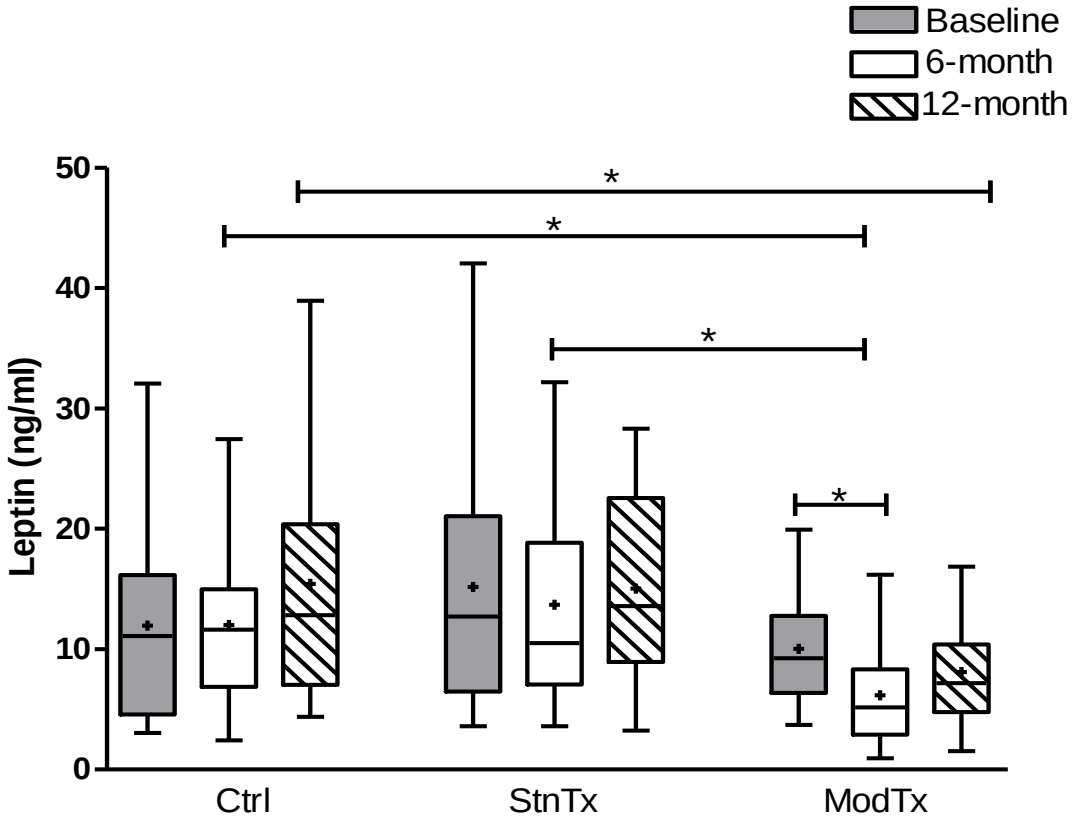


Figure 6.3 **Mean plasma leptin concentrations by group at baseline, 6-months and 12-months. Data were log-transformed prior to analyses and are presented in normal units as mean ± standard deviation; *p<0.05.**



Supplemental Table 6.1: Differences among groups for CEBQ scores at baseline and 6- or 12-months accounting for milk and milk product intake and participating in weight-bearing types of physical activities in mixed-models*

	Comparison between baseline and 6-months		Comparison between baseline and 12-months	
Dependent Variable	**Estimate (SE)**	**Adjusted p-value**	**Estimate (SE)**	**Adjusted p-value**
Food Approach**				
Ctrl	-0.02 (0.13)	1.00	-0.11 (0.18)	0.99
StnTx	0.39 (0.11)	**0.05**	0.57 (0.16)	**0.02**
ModTx	0.45 (0.11)	**0.01**	0.34 (0.18)	0.59
Food Responsiveness				
Ctrl	0.15 (0.22)	0.99	-0.09 (0.29)	1.00
StnTx	0.33 (0.19)	0.74	0.54 (0.26)	0.52
ModTx	0.67 (0.19)	**0.03**	0.74 (0.29)	0.22
Emotional Overeating				
Ctrl	-0.10 (0.23)	0.99	-0.28 (0.31)	0.99
StnTx	0.49 (0.20)	0.30	0.46 (0.27)	0.75
ModTx	0.38 (0.20)	0.63	0.14 (0.29)	0.99
Enjoyment of Food				
Ctrl	-0.15 (0.19)	0.99	-0.28 (0.31)	0.99
StnTx	0.14 (0.17)	0.99	0.45 (0.22)	0.56
ModTx	0.33 (0.17)	0.57	0.45 (0.24)	0.57
Food Avoidance†‡				
Ctrl	-0.05 (0.12)	1.00	0.03 (0.12)	1.00
StnTx	0.11 (0.07)	0.98	0.02 (010)	1.00
ModTx	-0.15 (0.09)	0.79	-0.19 (0.12)	0.82
Satiety Responsiveness				
Ctrl	-0.16 (1.33)	0.94	0.28 (0.15)	0.66
StnTx	0.05 (0.11)	1.00	-0.02 (0.14)	1.00
ModTx	-0.03 (0.11)	1.00	0.12 (0.15)	0.99
Slowness in Eating				
Ctrl	-0.11 (0.14)	0.99	-0.04 (0.15)	1.00
StnTx	0.06 (0.13)	0.99	0.04 (0.13)	1.00
ModTx	0.31 (0.19)	0.99	-0.28 (0.14)	0.56

Supplemental Table 6.1: Differences among groups for CEBQ scores at baseline and 6- or 12-months accounting for milk and milk product intake and participating in weight-bearing types of physical activities in mixed-models* *(continued)*

	Comparison between baseline and 6-months		Comparison between baseline and 12-months	
Dependent Variable	**Estimate (SE)**	**Adjusted p-value**	**Estimate (SE)**	**Adjusted p-value**
Emotional Undereating				
Ctrl	-0.22 (0.20)	0.97	-0.08 (0.26)	1.00
StnTx	0..25 (0.18)	0.91	0.05 (0.23)	1.00
ModTx	-0.13 (0.17)	0.99	-0.30 (0.25)	0.94
Food Fussiness				
Ctrl	0.13 (0.14)	0.98	-0.04 (0.12)	1.00
StnTx	0.22 (0.12)	0.64	0.08 (0.11)	0.99
ModTx	-0.22 (0.11)	1.00	0.03 (0.11)	1.00

Abbreviations: SE: standard error; Ctrl: Control group; StnTx: Standard Treatment; ModTx: Modified Treatment

* Models accounted for fixed effects (age, group, time, BMI, and sex) and random effects (subject nested in group, **total servings of milk and milk products per day** and **time engaged in weight-bearing activity per day**); n=100 total observations used in the models. Reported p-values are post-hoc adjusted using Tukey-Kramer.

****** Food Approach: Mean score of Food Responsiveness, Emotional Overeating, Enjoyment of Food and Desire to Drink

† Food Avoidance: Mean score of Satiety Responsiveness, Slowness in Eating, Emotional Undereating and Food Fussiness

‡ Food Avoidance: Convergence criteria were not met when testing for fit statistics using different types of covariance structures when both "total servings of milk products" and "weight-bearing activity" were included as random effects. Models were tested for best fit statistics by considering both variables separately as a random effect. The fit statistics favored the model that included the following random effects: subject nested in group, and total servings of milk products.

Supplemental Table 6.2: Differences among groups for CEBQ scores at baseline and 6- or 12-months accounting for income and parental education in mixed-models *

	Comparison between baseline and 6-months		Comparison between baseline and 12-months	
Dependent Variable	**Estimate (SE)**	**Adjusted p-value**	**Estimate (SE)**	**Adjusted p-value**
Food Approach**				
Ctrl	0.04 (0.11)	1.00	0.07 (0.14)	0.99
StnTx	0.44 (0.11)	**0.01**	0.49 (0.14)	**0.03**
ModTx	0.41 (0.11)	**0.02**	0.42 (0.15)	0.11
Food Responsiveness				
Ctrl	0.10 (0.17)	0.99	0.13 (0.21)	0.99
StnTx	0.40 (0.17)	0.37	0.39 (0.21)	0.67
ModTx	0.44 (0.17)	0.21	0.56 (0.22)	0.22
Emotional Overeating				
Ctrl	-0.09 (0.14)	0.99	-0.10 (0.20)	0.99
StnTx	0.65 (0.16)	**<0.01**	0.48 (0.19)	0.29
ModTx	0.39 (0.15)	0.23	0.44 (0.20)	0.44
Enjoyment of Food				
Ctrl	-0.01 (0.13)	1.00	0.17 (0.17)	0.98
StnTx	0.10 (0.14)	0.99	0.36 (0.17)	0.44
ModTx	0.46 (0.13)	**0.02**	0.56 (0.17)	**0.04**
Food Avoidance†				
Ctrl	0.01 (0.06)	1.00	0.07 (0.08)	1.00
StnTx	0.07 (0.07)	0.98	0.08 (0.08)	0.97
ModTx	-0.04 (0.07)	0.99	-0.03 (0.08)	1.00
Satiety Responsiveness				
Ctrl	0.04 (0.09)	0.99	0.16 (0.12)	0.91
StnTx	0.02 (0.10)	1.00	0.12 (0.12)	0.98
ModTx	0.03 (0.09)	1.00	0.11 (0.12)	0.99
Slowness in Eating				
Ctrl	0.04 (0.09)	0.99	0.16 (0.12)	0.91
StnTx	0.22 (0.10)	1.00	0.12 (0.12)	0.98
ModTx	0.03 (0.09)	1.00	0.11 (0.12)	0.99

Supplemental Table 6.2: Differences among groups for CEBQ scores at baseline and 6- or 12-months accounting for income and parental education in mixed-models * *(continued)*

	Comparison between baseline and 6-months		Comparison between baseline and 12-months	
Dependent Variable	**Estimate (SE)**	**Adjusted p-value**	**Estimate (SE)**	**Adjusted p-value**
Emotional Undereating				
Ctrl	-0.09 (0.22)	1.00	0.00 (0.19)	1.00
StnTx	0.29 (0.16)	0.67	0.29 (0.18)	0.83
ModTx	-0.14 (0.16)	0.99	-0.14 (0.19)	0.99
Food Fussiness				
Ctrl	0.04 (0.08)	0.99	0.06 (0.10)	0.99
StnTx	0.11 (0.08)	0.92	0.12 (0.09)	0.94
ModTx	0.12 (0.09)	0.90	0.16 (0.01)	0.79

Abbreviations: SE: standard error; Ctrl: Control group; StnTx: Standard Treatment; ModTx: Modified Treatment

* Models accounted for fixed effects (age, group, time, BMI, and sex) and random effects (subject nested in group, **family income, parental education**); n=161 total observations used in the models. Reported p-values are post-hoc adjusted using Tukey-Kramer.

****** Food Approach: Mean score of Food Responsiveness, Emotional Overeating, Enjoyment of Food and Desire to Drink

† Food Avoidance: Mean score of Satiety Responsiveness, Slowness in Eating, Emotional Undereating and Food Fussiness

Supplemental Table 6.3: Differences among groups for CEBQ scores at baseline and 6- or 12-months accounting for income, parental education, milk and milk product intake and participating in weight-bearing types of physical activities in mixed-models*

	Comparison between baseline and 6-months		Comparison between baseline and 12-months	
Dependent Variable	**Estimate (SE)**	**Adjusted p-value**	**Estimate (SE)**	**Adjusted p-value**
Food Approach**				
Ctrl	-0.00 (0.12)	1.00	-0.04 (0.19)	1.00
StnTx	0.33 (0.13)	0.27	0.47 (0.18)	0.19
ModTx	0.41 (0.12)	**0.03**	0.31 (0.19)	0.80
Food Responsiveness				
Ctrl	0.16 (0.17)	0.99	-0.05 (0.25)	1.00
StnTx	0.19 (0.18)	0.98	0.41 (0.23)	0.72
ModTx	0.53 (0.16)	**0.04**	0.43 (0.26)	0.75
Emotional Overeating				
Ctrl	-0.06 (0.23)	1.00	-0.17 (0.33)	0.99
StnTx	0.74 (0.23)	*0.06*	0.42 (0.31)	0.90
ModTx	0.43 (0.21)	0.54	0.17 (0.34)	0.99
Enjoyment of Food†				
Ctrl	-0.23 (0.28)	0.99	-0.27 (0.30)	0.99
StnTx	-0.17 (0.27)	0.99	0.37 (0.28)	0.92
ModTx	0.41 (0.24)	0.76	0.37 (0.32)	0.96
Food Avoidance††				
Ctrl	-0.09 (0.11)	0.99	0.09 (0.07)	0.96
StnTx	0.13 (0.11)	0.96	0.10 (0.07)	0.83
ModTx	-0.14 (0.09)	0.89	-0.31 (0.09)	**0.01**
Satiety Responsiveness‡‡				
Ctrl	-0.15 (0.13)	0.96	0.26 (0.18)	0.88
StnTx	0.09 (0.13)	0.99	0.10 (0.17)	0.99
ModTx	0.03 (0.12)	0.99	0.25 (0.19)	0.92
Slowness in Eating				
Ctrl	-0.13 (0.14)	0.99	-0.07 (0.15)	0.99
StnTx	-0.00 (0.14)	1.00	-0.00 (0.14)	1.00
ModTx	-0.24 (0.12)	0.57	-0.38 (0.16)	0.33

Supplemental Table 6.3: Differences among groups for CEBQ scores at baseline and 6- or 12-months accounting for income, parental education, milk and milk product intake and participating in weight-bearing types of physical activities in mixed-models* *(continued)*

	Comparison between baseline and 6-months		Comparison between baseline and 12-months	
Dependent Variable	**Estimate (SE)**	**Adjusted p-value**	**Estimate (SE)**	**Adjusted p-value**
Emotional Undereating				
Ctrl	-0.19 (0.21)	0.99	0.18 (0.26)	0.99
StnTx	0.31 (0.21)	0.86	0.18 (0.26)	0.99
ModTx	-0.26 (0.19)	0.91	-0.40 (0.29)	0.91
Food Fussiness				
Ctrl	0.03 (0.14)	1.00	-0.04 (0.13)	1.00
StnTx	0.24 (0.14)	0.70	0.11 (0.12)	0.99
ModTx	0.05 (0.12)	1.00	0.04 (0.14)	1.00

Abbreviations: SE: standard error; Ctrl: Control group; StnTx: Standard Treatment; ModTx: Modified Treatment

* Models accounted for fixed effects (age, group, time, BMI classification [overweight or obese], and sex) and random effects (subject nested in group, **family income, parental education**, **total servings of milk and milk products per day** and **time engaged in weight-bearing activity per day**); n=80 total observations used in the models. Reported p-values are those adjusted by post-hoc Tukey-Kramer.

****** Food Approach: Mean score of Food Responsiveness, Emotional Overeating, Enjoyment of Food and Desire to Drink

† Convergence criteria were not met when testing for fit statistics using different types of covariance structures when the variable "household income" was included as random effects; the results presented for Enjoyment of Food include the following random effects: subject nested in group, parental education, total servings of milk products and time engaged in weight-bearing activity per day.

†† Food Avoidance: Mean score of Satiety Responsiveness, Slowness in Eating, Emotional Undereating and Food Fussiness

‡ Convergence criteria were not met when testing for fit statistics using different types of covariance structures when the variable "weight-bearing activity" was included as random effects; the results presented for Satiety Responsiveness include the following random effects: subject nested in group, parental education, and total servings of milk products.

CHAPTER 7

General Discussion and Conclusions

7.1 General Discussion

The overall objective of this dissertation was to assess the impact of a 1 year family-centered lifestyle intervention that was based on current Canadian dietary and physical activity (PA) guidelines on changes in: (1) adiposity; (2) bone health outcomes; and (3) eating behaviors, in children 6-to 8-year old children with obesity. This thesis also thesis explored the potential benefits of increasing components of current Canadian diet and PA recommendation, specifically doubling the recommending daily serving of milk and milk products and suggesting daily engagement in weight-bearing types of activities on favorably changing these 3 outcomes.

This dissertation included 4 manuscripts: 1 protocol paper and 3 original research papers. The study design was based on theoretical frameworks that considered different factors that affect a child's weight status. As discussed in the main literature review, childhood obesity is multifaceted and stems from many different etiologies. The questionnaires used in this study were designed to capture different components that impact a child's weight status. To date, subsequent works (3 Master's theses and 2 Master's applied projects) have been published, all reviewing important outcomes from the MY LIFE study.

Overall, the literature review presented in this dissertation has identified numerous research gaps, some of which were examined in preceding chapters. This chapter will highlight the general findings from the thesis objectives and discuss additional considerations as they relate to the study findings. Despite some limitations, this dissertation has important public health implications and is positioned to extend recommendations for future related investigation.

***Hypothesis 1*: Adiposity**

The first hypothesis of this dissertation stated children randomized to increase milk and milk products and focus on daily weight-bearing PA (Modified Treatment; ModTx) would have a lower body mass index for-age z-score (BAZ) at 12-months (mo) compared to children randomized to meet current Canadian dietary and PA recommendations (Standard Treatment; StnTx), and the control (Ctrl) group. Compared to baseline (BL), at 12-mo, BAZ was significantly reduced in ModTx (-0.7±0.6) compared to Ctrl (-0.1±0.3); further, ModTx resulted in significantly greater reductions in percentage body fat (%BF) compared to BL. Conversely, Ctrl significantly increased fat mass, waist circumference and trunk fat mass. These findings support current literature that suggest that interventions based on the family-centered lifestyle

approach which include behavioral discussions result in favorable changes in body composition in children with obesity compared to control.

In children with obesity, lean mass is often increased compared to normal weight peers [373]. In this trial, all groups significantly increased lean mass at 12-mo compared to BL ($p<0.001$), however, the change in lean mass from BL to 12-mo were greatest in StnTx ($p=0.001$) and Ctrl ($p<0.0001$) compared to ModTx. In general, the MY LIFE study aimed to decrease fat mass without compromising lean mass; an outcome that was achieved. In adult trials, the effects of increased milk products on lean mass have shown to protect muscle mass during energy restriction and increase muscle mass when diets are balanced [374]. These positive effects of milk products on lean mass may be attributed to branched-chain amino acids, specifically leucine, that are present in milk products and are related to protein synthesis [374].

In this study, the general message was to meet current dietary and PA guidelines, not to engage in energy restriction, alter macronutrient compositions or perform excessive resistance training, all of which may modulate different effects on lean mass. Results from a meta-analysis examining the effects of diet-only interventions compared to those of diet plus exercise or exercise only on weight loss in overweight children resulted in greater muscle gains in interventions that included diet plus resistance training compared to diet-only interventions (pooled difference, 0.44 kg; 95% CI [0.04, 0.84]) [375]. However, these trials were carried out for an average of 4 months. In the MY LIFE Study, children were guided to perform daily PA, not exercise *per se*. A discussion concerning the importance of distinguishing between exercise and PA when directed to children is explored later in this chapter.

Nonetheless, PA also mediates changes in lean mass depending on the nature of the activity performed; and in one study, were found to be positively associated with fitness scores in both boys and girls [376]. In the present study, parents reported PA practices of children which resulted in no significant changes in frequency or time among groups. This was a surprising outcome considering children randomized to interventions were educated on the importance of activity and each session included a PA goal. The MY LIFE Study assessed PA using a questionnaire; objective measures, such as pedometers or accelerometers, would have provided a more accurate description of PA performed. However, these devices when used in the pediatric obese population present with some limitations, as previous studies have resulted in low

compliance to wearing the devices due to the children's general insecurities or fears of social acceptance [340].

As mentioned, adult trials suggest that milk product intakes may mediate changes in lean mass, however, their effects on changes in adiposity are conflicting. In this study, the milk intervention group (ModTx) resulted in significant reductions in adiposity at 12-mo; a discussion as to how milk products may have mediated these changes in body composition is worthy.

Potential mechanisms

To date, proposed mechanisms as to how milk products may mediate changes in adiposity or affect weight status are limited to *in vitro* studies using human adipocytes [377] and *in vivo* studies in mice [378]. *In vitro* studies examining the effects of the *agouti* gene in regulating adipocyte metabolism have provided a theoretical framework explaining how intracellular calcium (Ca^{2+}) may mediate lipid metabolism and triacylglycerol storage [379]. Specifically, in response to calcium concentrations, both parathyroid hormone (PTH) and 1,25-dihydorxyvitamin D ($1,25(OH)_2D$) effect human adipocyte lipogenic and lipolytic systems by mediating intracellular Ca^{2+}; intracellular Ca^{2+} responds to low- and high-calcium diets, whereby low-calcium diets stimulate expression of these hormones causing an increase in lipid storage; conversely, suppression of PTH and $1,25(OH)_2D$ mediated by high-calcium diets inhibits adiposity [379].

Figure 7.1 is a well cited figure that reviews the potential mechanisms involved with intracellular Ca^{2+} and adipose tissue due to a low-calcium diet. Briefly, intracellular Ca^{2+} promotes energy storage by stimulating the expression of fatty acid synthase resulting in *de novo* lipogenesis; at the same time, lipolysis is inhibited [377], resulting in phosphorylation of hormone sensitive lipase [380].

Despite the dual effect of PTH and $1,25(OH)_2D$, research involving human adipocytes suggest that $1,25(OH)_2D$ acts as the key regulator of adipocyte metabolism over PTH, as depicted in **Figure 7.1**. Specifically, when $1,25(OH)_2D$ acts on the membrane vitamin D receptors on adipocytes, a rapid increase of intracellular Ca^{2+} into the cell occurs, resulting in a decrease in uncoupling protein 2 (UCP2) [381]. These results have also been seen in animal studies, whereby mice exposed to high-calcium diets have shown an increase in adipose tissue

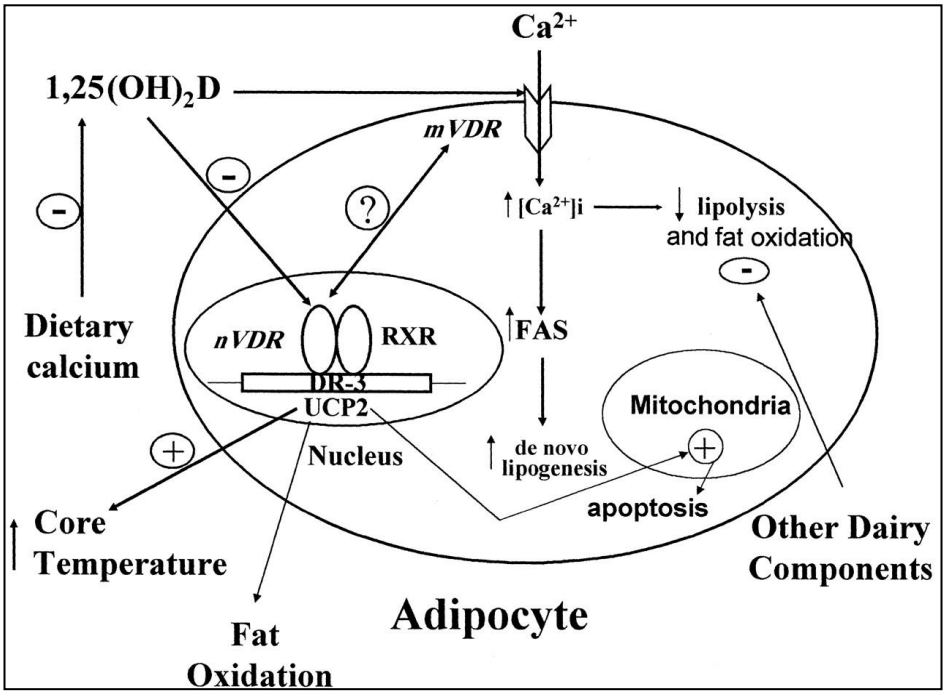
UCP2 expression and a decline in thermogenesis [378], suggesting that increased calcium intake may play a role in energy partitioning.

The effects of decreasing adipocytes as they relate to calcium intake have been explored in human adult feeding trials [381, 382]. In general, these trials suggest that calcium supplementation may enhance long term fat oxidation, however these effects are more pronounced when participants are low-calcium consumers at baseline.

The positive effects of milk products on energy balance are not limited to calcium; the additional components in milk products may also be beneficial. Milk proteins have been associated with body weight regulation by increasing satiety [74] and preserving or increasing lean mass [31]. It is suggested that leucine may be the key player, as it has effects on lean mass and may affect repartitioning of energy from fat mass to lean mass [383]. Milk also contains conjugated linoleic acid (CLA) which may have effects on energy metabolism and adipogenesis [374].

Thus, milk products, due to their calcium and protein component among other constituents, may have a potential role in managing energy balance. Increasing dietary calcium and/or milk products may decrease energy intake and increase energy utilization, in both adipocytes and potentially lean mass. However, these proposed mechanisms and this area of research in general is in its infancy; further investigation is needed before conclusions can be made.

Figure 7.1 **Mechanisms of dietary calcium and dairy modulation of adiposity** [379]
License agreement between *Am J Clin Nutr* and T. Cohen (June 1, 2017): 4120211258127



Legend: 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; [Ca^{2+}]i, intracellular Ca^{2+} concentration; *mVDR* and *nVDR*, mouse and nuclear vitamin D receptors, respectively; RXR, retinoic acid receptor; UCP2, uncoupling protein 2; FAS, fatty acid synthase; DR-3, D response element-3.

Biomarkers of milk fat

In this study, compliance to the dietary intervention of increasing the daily servings of milk and milk products was assessed using traditional and novel methods. Specifically, during each study visit 3-day food diaries were collected from parents for analyses. Nutritional biomarkers, specifically fatty acids from red blood cells not endogenously synthesized (i.e., myristic (14:0), pentadecanoic (15:0), heptadecanoic (17:0) and stearic (18:0) acids) were measured at baseline, 3-mo and 6-mo, to reflect the compliance during the intervention phase of the study.

Although the use of these biomarkers has previously been explored in pediatric studies [231, 384], to our knowledge, this was the first study to report on a trial that included a milk intervention. Nonetheless, the use of this novel intervention method raises important concerns when used in an intervention such as the one presented in the MY LIFE Study.

Firstly, the use of 3-day food diaries to assess dietary intake in children presents with both strengths and limitations. Despite the open-ended nature of food diaries, they represent high participant burden, in this case, the burden was on busy parents. Additionally, food diaries captured a child's dietary intake through the eyes of parents and not the children themselves, therefore may not be representative of actual dietary intakes outside the home setting. Children's bias and potential emotional responses to questions regarding food items should be considered [385], especially given children understood the food diaries would be reviewed during study visits. Currently, research is exploring novel methods of assessing dietary intakes; these are considered at the end of this chapter.

Secondly, the use of an objective measure to assess compliance to the milk intervention in this study was limited by the nature of the interventions themselves. Specifically, the interventions designed and used in this study were based on adapting healthy behaviors, which included reducing the intake of fat from milk products. For instance, if a child was consuming fluid milk with a fat content of 3.25 % milk fat (MF), they were guided to decrease to 2 %MF or if tolerated, 1 %MF, all while maintaining (or increasing) the servings of milk and milk products per day. Notably to the ModTx, if this were the case, an expected decrease in fatty acids concentrations due to the decrease in milk fat, not servings of fluid milk *per se,* would be observed. Simultaneously, the MY LIFE study interventions were based on meeting the recommended daily servings of the other food groups, and significant in this case, meat and

alternatives. In addition to milk products, low amounts of the fatty acids of interest are also present in fish, beef, veal and lamb [336]. Therefore, changes in fatty acid concentrations are mediated by both %MF and meat and alternative intake. **Table 7.1** details the meat and alternative intakes from 3-day food diaries. In general, beef and veal were the most consumed meat item. Although not statistically significant compared to baseline, at 6 months the consumption of beef and veal had slightly decreased in ModTx (p=1.00).

In this study, fatty acids were quantified using gas chromatography, which is considered a valid method of identifying fatty acid peaks, however, at times identification of peaks may be challenging as some may overlap or are small to quantify. Nonetheless, the use of a flame-ionization detector to help identify peaks aided in quality control, as did comparison of peaks using the manual peak reviews against historical averages from previous laboratory works; various internal quality assurance and quality control procedures were also included during analyses.

The use of traditional methods of dietary assessment, in this case 3-day food diaries, becomes paramount. In the MY LIFE Study, dietary records would have explained which food items were driving the changes in fatty acid concentrations. Regrettably, few food diaries were returned at study visits, thereby limiting the statistical analyses. Moreover, of the available food diaries, food items were not reviewed for specific details (i.e., %MF), but were rather categorized by food group. Future studies should consider additional categories of analyses, in this case %MF, to confirm findings.

Despite these limitations, the findings from this study are relevant to future trials that consider using saturated fatty acids as a milk product biomarker. This study highlights the importance of considering the population of interest and ensuring additional methods are used to associate and validate the findings. Should the population have a high consumption of milk products but low consumption of meat products, the use of the fatty acids biomarkers would be considered valid. However, those individuals with high servings from both food groups, the presence of these fatty acids should be correlated with other methods to avoid misleading conclusions. For instance, a method currently gaining momentum are the use of metabolomics for assessing milk product intakes [386]. To date, trials in children have used metabolomics to test short term interventions involving milk products [387]. However, this method of profiling low-molecular-weight metabolites is still in its infancy. The validation of biomarkers related to

milk products still needs to be established. Nonetheless, if feasible, future work may wish to consider using metabolomics in conjunction with other valid and reliable biomarkers of milk product intakes, such as fatty acids.

Table 7.1 Meat and alternative and milk and milk product intakes Canada's Food Guide food groups[b] assessed by 3-day food diary at week-1, 6-mo and 12-mo

	Ctrl			StnTx			ModTx		
	Week-1	**6-mo**	**12-mo**	**Week-1**	**6-mo**	**12-mo**	**Week-1**	**6-mo**	**12-mo**
n=	*12*	*8*	*7*	*19*	*10*	*10*	*19*	*12*	*7*
Meat and Alternatives									
Total	**2.2±0.9**	**2.0±0.6**	**2.2±0.6**	**1.8±0.7**	**2.3±1.0**	**2.1±0.6**	**1.9±0.6**	**1.7±0.7**	**1.0±0.4**
Poultry	0.3±0.3	0.4±0.3	0.4±0.2	0.5±0.5	0.8±0.8	0.4±0.4	0.6±0.5	0.4±0.4	0.2±0.2
Fish	0.3±0.5	0.2±0.7	0.2±0.3	0.1±0.2	0.3±0.2	0.1±0.4	0.0±0.2	0.2±0.4	0.0±0.1
Beef and veal	0.6±0.5	0.4±0.5	0.7±0.6	0.4±0.4	0.5±0.6	0.9±0.3	0.5±0.3	0.4±0.6	0.5±0.5
Pork	0.6±0.6	0.6±0.4	0.5±0.5	0.3±0.4	0.3±0.3	0.2±0.3	0.3±0.5	0.4±0.4	0.1±0.3
Eggs	0.3±0.4	0.1±0.2	0.0±0.1	0.2±0.2	0.1±0.2	0.1±0.1	0.2±0.3	0.1±0.2	0.1±0.2
Nut and seeds	0.1±0.2	0.0±0.1	0.0±0.2	0.2±0.5	0.2±0.3	0.0±0.2	0.2±0.4	0.0±0.1	0.0±0.0
Legumes	0.0±0.1	0.0±0.3	0.1±0.1	0.2±0.5	0.0±0.0	0.0±0.1	0.0±0.0	0.0±0.0	0.0±0.0
Tofu	0.0±0.1	0.0±0.0	0.0±0.1	0.0±0.1	0.1±0.5	0.1±0.3	0.0±0.0	0.0±0.0	0.0±0.1
Milk and Milk Products									
Total	**2.8±1.6**	**3.3±2.5**	**3.6±2.6**	**2.2±1.2**	**1.6±0.8**	**1.9±0.7**	**2.0±0.9**	**2.3±1.2**	**2.8±1.1**
Milk	1.5±1.4	1.9±2.1	2.1±1.9	1.1±0.7	0.7±0.4	0.9±0.5	1.0±0.6	0.9±0.9	1.5±0.9
Yogurt	0.3±0.4	0.3±0.2	0.4±0.3	0.3±0.4	0.2±0.4	0.2±0.3	0.4±0.2	0.4±0.4	0.6±0.6
Cheese	1.0±0.5	1.1±0.5	1.0±0.8	0.8±0.6	0.6±0.5	0.8±0.5	0.6±0.5	1.0±0.6	0.7±0.5
Alternatives	0.0±0.0	0.0±0.0	0.0±0.1	0.0±0.1	0.0±0.0	0.0±0.0	0.1±0.2	0.1±0.2	0.0±0.0

Note: mean ± standard deviation; Mixed-model ANOVA resulted in non-significant findings among and between groups for all variables-by-time.
[a] Week-1 food diaries were completed the week after visit; all subsequent diaries were completed and reflect the week prior to study visit.
[b] Canada's Food Groups: servings/day

<u>Hypothesis 2</u>: Bone Outcomes

The second aim of this dissertation was to determine the impact of the intervention on changes in bone outcomes assessed by dual-energy x-ray absorptiometry (DXA) and biomarkers of bone metabolism. It was hypothesized that children randomized to ModTx would increase bone mineral content (BMC) and bone mineral density (BMD), and favorably change bone biomarkers at 12-mo compared to children randomized to StnTx and Ctrl.

By 12-mo, all groups significantly increased BMC for whole body (WB), lumbar spine (LS), and lumbar lateral spine (LLS). StnTx and ModTx significantly increased WB and LS BMD; while only StnTx increased ultra-distal BMD by 12-mo. By 12-mo, WB BMD z-scores were significantly lower in Ctrl. Bone biomarkers did not significantly change throughout the study.

Effects of body composition on bone health

In this study, all groups maintained or increased bone outcomes at all 3 sites. The positive effects of body mass index (BMI) on bone are partly mediated by greater lean mass. The functional muscle-bone unit model suggests that skeletal muscles and the forces they exert on bone are determinants of bone accretion and strength [388]. As muscle mass increases, bone is increasingly exposed to mechanical loading and responds by modeling and remodeling leading to osteogenesis [388].

It has been suggested that in adults, a weight loss of ~10% may have a positive effect on comorbidities associated with obesity, but may also results in a 1% to 2% loss of BMC at the hip and whole body, with up to 4% loss in highly trabecular sites such as the radius [389]. Certainly, these types of losses could be detrimental in children and therefore studies suggest that interventions should include both diet and PA components to counteract potential losses [276]. The MY LIFE Study was not focused on weight loss, but rather reductions in adiposity through a lifestyle approach. As shown in previously cited adolescent studies [185, 186], interventions that involved both diet and exercise resulted in reductions in BMI and/or fat mass and increases in BMC after 1 y.

The adiposity-bone relationship in children remains unclear and conflicted as puberty, sex and distribution of fat mass may modulate different effects on bone [25]. To better understand the role of fat mass on bone, distribution of fat mass using different techniques have

been examined and although not measured in this study, are worthy discussion points. Research suggests that subcutaneous adipose tissue (SAT) has been shown to be positively while visceral adipose tissue (VAT) may be negatively associated with BMD [29]. Using computed tomography in healthy females (n=100; 15-25 y) to evaluate the associations between VAT and SAT with femoral bone outcomes, opposing effects were found given the type of adipose tissue and appendicular region [390]; SAT was positively associated while VAT was negatively associated with cortical bone area. These findings suggest that SAT may benefit bone structure whereas VAT may act as a pathogenic fat depot to bone. In general, these findings suggest that adipose tissue may exert different and potentially negative effects on bone depending on site. Magnetic resonance imaging is considered the most acceptable technique for differentiating VAT and SAT [391]; DXA, as used in this study, as well as ultrasounds and bioelectrical impendence are more accessible, however, are unable to measure VAT [51]. Despite this study not differentiating between VAT and SAT, this study measured waist circumference which significantly increased by 12-mo in Ctrl. Given the increased metabolic risks associated with higher waist circumferences in children, and the hormonal alterations that accompany VAT, the impact of adipose tissue on bone health should be considered in the pediatric obese population.

Health consequences of obesity on bone health and bone biomarkers

In children with obesity, it is important to consider the potential bone health consequences associated with hormonal alterations. Specifically, elevated circulating levels of pro-inflammatory cytokines and adipokines which favor fat mass accumulation may be potentially associated with loss of bone mass [389]. Tumor necrosis factor alpha, interleukin-1 and interleukin-6 can mediate osteoclast differentiation and promote bone resorption [25]. Children with obesity have been shown to have elevated cytokines compared to normal weight peers [392]; chronic exposure of these cytokines may in part explain the relationship between increased VAT and lower cortical bone thickness, as described [390]. The methods of the MY LIFE Study did not include measurement of these cytokines; future work may wish to consider their impact on bone health in children with obesity.

However, this study did measure plasma leptin which was measured at baseline, 6 and 12 months, and analyzed for associations with eating behaviors, not bone outcomes. This study found no associations between plasma leptin and eating behavior, however, plasma leptin

concentrations were significantly lower in ModTx at 6 months compared to baseline; leptin concentrations did not differ among or between groups by 12 months. In children with obesity, higher leptin concentrations are inversely related with BMD [393] as well as cortical porosity of the radius and trabecular thickness of the tibia [177]. *In vivo* and *in vitro* studies suggest leptin acts directly on human marrow stromato cells (osteoblast receptors) to promote osteoblast proliferation and differentiation; this inhibits adipocyte differentiation and osteoclastogenesis through generation of osteoprotegerin [394].

The MY LIFE Study focused on examining bone outcomes, and included bone biomarkers that reflected both formation by osteoblasts and resorption by osteoclasts. Specifically, this study assessed the bone formation markers bone-specific alkaline phosphatase and osteocalcin and C-terminal telopeptide of type 1 collagen, a bone resorption marker, in addition to 25-hydroxyvitamin D and parathyroid hormone. Previous studies suggest that these biomarkers may change in response to reductions in adiposity, however, this study did not see significant differences among groups by 12-mo. Suggested reasons for the non-significant findings were discussed; potential sex differences and magnitude of fat mass loss have been cited. Other considerations that may explain the null findings may be related to mode of handling of samples (i.e., number of freeze-thaws) as well as the time of which samples were collected (i.e., 8:00 am versus 11:00 am). Future work should consider biological considerations (i.e., age, sex and ethnicity) and temporal variations (i.e., season).

The biomarkers used in this study were appropriate, as previous work in children with obesity have used these markers, in addition to evaluating others (i.e., procollagen-1N-terminal propeptide [P1NP]) [395]; currently the literature on these bone biomarkers and changes in adiposity in children with obesity is limited. Despite this study resulting in no significant differences in the measured bone biomarkers at 12 months compared to baseline, these findings contribute to the ongoing and needed research area of bone health in children with obesity and are therefore important to publish.

Physical growth and timing of puberty

An interesting finding found in this study was that by 12-mo, Ctrl had significantly lower WB BMD z-scores compared to baseline; whereas WB BMD did not differ among StnTx or ModTx at 12-mo. All groups significantly increased height from baseline, however, the rate of height growth was faster in Ctrl and StnTx compared to ModTx.

Research suggests that children with obesity tend to be taller. Girls with obesity tend to have an earlier onset of puberty compared to normal weight peers while boys with obesity either have earlier or delayed pubertal onset [25]. However, this height advantage is lost during adolescence [396] as children with obesity often show a reduced growth spurt compared to lean subjects [397]. The MY LIFE Study assessed pubertal status using Tanner Staging images shown to parents and pubertal biochemical markers, including luteinizing hormone and estradiol. Despite not including a direct physical examination or measures of testosterone for boys, by 12-mo children remained in Tanner Stage 1 (pre-pubertal). As such, all statistical models used to analyze various study outcomes did not account for pubertal status.

Puberty, as well as gender, may affect bone density [29]; obese children have been shown to have higher BMD values for bone age compared to normal weight children before puberty, however after puberty, BMD values decrease [398]. The associations between childhood obesity and earlier pubertal onset may be related to adipose tissue, which prematurely activates gonadotropin-releasing hormone (GnRH)-gonadotropin axis [399]. Additional factors relevant to obesity may also contribute to pubertal development, such as leptin, which has been shown to stimulate GnRH and gonadotropin secretions [400] as well as luteinizing hormone and follicle-stimulating hormone thereby influencing gonadal functions [399]. The hormone ghrelin may also mediate effects on the hypothalamic-pituitary-gonadal axis and has been hypothesized to influence pubertal events [401]. However, research is still required to fully understand the precise role of these peptides on activation and maintenance of the gonadotropic axis in children with obesity and their role in early development of puberty.

Hypothesis 3: Eating Behaviors

The third aim of this dissertation was to determine if participating in the intervention favorably changed eating behaviors and plasma leptin concentrations. It was hypothesized that children randomized to ModTx would significantly decrease Food Approach types of eating

behaviors and decrease plasma leptin concentrations at 12-mo compared to children randomized StnTx and Ctrl. By 6-mo and 12-mo, both intervention groups significantly reduced scores categorized as Food Approach, whereas there were no changes in eating behaviors in Ctrl. Similarly, both groups reduced plasma leptin concentrations by 6-mo (ModTx: -3.88±0.35 ng/mL, $p<0.05$; StnTx: -1.48±0.76 ng/mL, $p=1.00$). At 12-months, leptin concentrations were significantly lower in ModTx compared to Ctrl ($p<0.05$) but not StnTx ($p=0.07$). There were no differences between leptin concentrations at 12-months between StnTx and Ctrl ($p=1.00$). Overall, these findings suggest that the interventions used in this study favorably modulated changes in eating behaviors, compared to Ctrl.

Assessment of eating behaviors in children

Despite the known associations between eating behaviors and body weight, few childhood obesity trials include assessments of eating behaviors to gain a better understanding as to what may be driving the changes in adiposity. It may be advantageous to assess these changes as interventions progress to ensure reductions in adiposity are sustained. In this study, eating behaviors were discussed throughout the interventions; one educational session was specifically devoted to Emotional Eating and Food Responsiveness. It was especially important to include these discussions using the family-centered approach as food intake and attitudes toward meals can depend on maternal influences [141], however, appetite traits may respond differently to diets that promote satiety, such as those high in milk products [86].

Eating behaviors are typically assessed by questionnaire. In this study, parents completed the Children's Eating Behavior Questionnaire (CEBQ) at each study visit. Parents have a great influence on their child's dietary intake and may affect children's ability to regulate energy intake and the amount of food consumed. An early decision to choose to breastfeed or formula-feed children may set the stage for a child's ability to self-regulate whereby breastfed children have more control over the size of the meal [141]. In this study, early feeding practices were not surveyed, which may have been a potentially interesting outcome to correlate with satiety scores of the CEBQ.

In this study, parents completed the CEBQ, which has its limitations as aspects of satiety and regulation of food intake are often subjectively assessed. Studies have confirmed that children can regulate their dietary intake based on energy content of foods not only within a meal

but over 24-hours [141]. However, their responsiveness to energy density may be diminished when adults use control strategies that focus on external cues to encourage intake (i.e., reward if plate is "clean") [402]. Further, in children with parents who have a high degree of control over their general diet, often result with low self-control; parental control has been shown to be linked with parent's own dieting and weight history [403]. In this study, most parents were classified as overweight or obese which could have affected their responses to the CEBQ. Parental weight status was self-reported at baseline only and parenting styles were not assessed. However, if randomized to StnTx or ModTx, these parents would have participated in the interventions and although interventions were tailored to meet the needs of the child, general discussions would have benefited parents in changing their lifestyles as well. Future work may wish to consider tracking a parent's weight status as they participate in interventions aimed at their child.

Biomarkers of satiety

A novelty of this study was the inclusion of plasma leptin to examine the potential associations with changes in eating behaviors. Leptin is considered a valid biomarker of satiety [67], however, in this study, leptin concentrations did not associate with eating behaviors at baseline nor did they significantly change among or between groups at 12 months.

To date, only one other study has included assessment of eating behaviors by the CEBQ and serum leptin in obese children (n=120; 6-12 y) [86]. This study examined the effect of carbohydrate (CHO) and fat restriction on appetite regulation (CEBQ) and leptin over 2-mo. By 2-mo, BMI and leptin significantly decreased in both groups, as did scores for Food Responsiveness and Enjoyment of Food; only the low-CHO diet decreased Emotional Overeating scores. Both groups had marginally increased scores for Satiety Responsiveness. Despite these favorable changes in eating behaviors, this study was limited with its short-duration, lack of dietary assessment and utilized an intervention protocol that may not be appropriate for children.

Studies have focused on the relationship between leptin and energy balance in children with obesity. In this study, changes in plasma leptin concentrations were following similar patterns of change in fat mass, but were not significantly different at 12 months. In children with obesity, leptin concentrations either decrease [404] or remain unchanged [405] following

exercise or dietary interventions. These inconsistencies are thought to be due to leptin resistance; however, the regulation of energy expenditure may also play a role [369].

As described in Chapter 3, additional biomarkers of satiety could have been considered, one of which was ghrelin. Ghrelin acts as a hunger signal; it rises before meals and falls after food intake, thereby playing a role in meal initiation. Lower ghrelin concentrations are seen in children with obesity compared to normal weight [67]. Lifestyle interventions designed for children with obesity have shown that with reductions in adiposity, ghrelin remains unchanged [406] or increases [407]. Other appetite-related peptides, such as peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) have also been explored in lifestyle interventions, but all, including ghrelin and leptin, respond differently depending on weight-loss and dietary intervention [408].

7.2 Strengths and Limitations

The research encompassed within this thesis has strengths and limitations as discussed. After 2 years, this study recruitment-phase closed with 78 families; this represented 67% of the estimated sample size originally set for this study (n=117; 39/group). The cited studies in this thesis have had attrition rates ranging from 9-46 % whereas the MY LIFE Study's attrition rate was 6%. Further, the sample size calculation for this study was set on attaining a mean change of BAZ of -0.2±0.3; compared to baseline, the mean change of BAZ at 12-mo was -0.1±0.3, -0.3±0.4 and -0.7±0.6 in Ctrl, StnTx and ModTx, respectively. Therefore, despite not achieving the estimated sample size, significant and meaningful results were seen.

The MY LIFE Study used a standard practice of randomization (computer-generated list) however, the use of milk and milk products as the intervention component may have posed an issue to some participants. At baseline, mean servings of milk and milk products were not different among groups. To truly test the effects of increasing milk products on changes in adiposity or bone, children would have had to been consuming the same number of milk and milk products at baseline. Further, although food preferences, such as specific likes towards milk and milk products, were discussed at screening, a dislike towards milk and milk products would have been especially problematic for children randomized to ModTx. However, the interventions of the MY LIFE Study were designed to meet the individual needs of children, therefore if this

were the case, the interventionist would have strategized with parents to ensure the goals were met.

This study focused on developing a safe, non-judgmental environment for children. The assessment tools used in this study were considered valid (i.e., anthropometric measures, biomarkers) and for some, gold-standard (i.e., DXA). This study considered the time commitment of participating, and conducted study visits at times that were convenient to families, all while maintaining a professional and clinical setting. This is particularly important when dealing with a population that are at a higher risk of emotional distress; children were always weighed facing the researcher and never shown the scale. DXA outcomes were always discussed with children, with an emphasis on developing strong bones and never on fat indices. Waist circumference measurements were described as putting on a belt, with an assistant recording the measurement so numbers were not disclosed out loud. These special measures were important and made the experience of participating enjoyable, a critical consideration for future work.

The interventions used in this study were designed at the grade 1 literacy level to ensure all participating members understood the education and evaluation methods. During each session, the interventionist completed a checklist: this insured consistency and that flow of the session, the topics of discussion (included handouts specifically designed for the study) and the methods of evaluation were uniform across all families, however, permitted for individualization. The use of SMART goals to create realistic and sustainable changes in health behaviors is considered favorable practice and was used in this study.

Despite these strengths, this study was limited by some unfortunate circumstances that are often reported in trials of such nature. First and foremost, the return of 3-day food diaries was minimal at both 6 and 12 months, which resulted in statistical models either not accounting for diet and if they did, results were limited by sample size. Reasons for minimal return of food diaries may have been due to time commitment of completing the task, not fully appreciating the importance of completing the food diaries, or embarrassment of disclosing what children were consuming. These limitations were not explored during the trial but should be considered and addressed in future studies. Secondly, PA measures were parent-reported; ideally, objective assessments would have been used to assess practices outside the home environment. An important characteristic of these families was that parents were predominately overweight or

obese; often seen in studies with obese adults is the overestimation of PA and underestimation of food intake [339]. Participants of this study were also predominately white from families of higher total household incomes. Childhood obesity rates are highest among lower sociodemographic families, therefore the findings from this study cannot be generalized to all children with obesity. Finally, and most importantly, this study was not designed to distinguish between the effects of either increased milk and milk products or weight-bearing PA on adiposity, bone or eating behaviors; a step-wise approach would have been needed to address these outcomes.

7.3 Important Considerations for Future Research

Throughout this dissertation, the need for future work has been discussed. This dissertation presents a unique opportunity to briefly explore additional methods of encouraging healthy behaviors, as shifts from conventional to more technological advances in data assessment and collection are gaining momentum. As children become more engaged in technology in the home and school settings, it is important that pediatric trials align themselves to account for the changes in society norms, to not only entice participation, but also encourage retention.

Recruitment efforts for trials assessing children with obesity

The recruitment period for the MY LIFE study was ~ 2 y and involved both active and passive methods of recruitment. More specifically, active methods included visiting >43 physician offices, including 1 dentist office; passive methods included bilingual newspaper advertisements (n=15), mailing bilingual study brochures by Canada Post (n=>26,000), online advertisements on childhood obesity websites (English) and visiting ~25 private and public schools from both French and English school boards where >4,900 study brochures were distributed directly to parents through teachers. These methods of recruitment were incredibly timely and costly; due to funding and time limitations, recruitment closed with n=78 families, 5 of which did not complete the 12-mo visit. Specifically, 51% were recruited passively (i.e., mass mailings, school, radio and newspaper), 42% actively through physician referral and 6% through word of mouth by enrolled participants.

Obesity is a sensitive topic and often recruitment of this population group is a challenge [409]. A parent's willingness but more importantly awareness that their child needs help for a

weight-related issue is a determining factor of participation. It is of upmost importance that recruiters appreciate the complexities and the sensitivities of this topic when discussing participation in such studies with parents. It has been documented that parents of overweight children often feel blamed and judged [410]. Therefore, discussions should focus on improving healthy lifestyles and not changing weight or meeting a goal weight for their child. Further, research supports the notion that more often than not, parents fail to recognize any weight-related issues and often underestimate their child's weight status [140], a more prevalent finding among overweight parents [411]. Additionally, and not surprising, that a child's self-perception may also be skewed by their environment; misconceptions of a child's own weight status have been shown to be correlated with their exposure to an overweight or obese parent [409].

For these reasons, it is important that both methods are used when recruiting children with obesity. Passive recruitment will typically attract parents who can identify with the outstanding issue and at a minimum, be at the pre-contemplating stage of change [412] . However, the most cost-effective method is active recruitment, and in the case of the MY LIFE Study, physician referral. Physicians can act as the gateway and help select eligible children, however, routine assessment of BMI may not be a priority during a doctor's visit; physician's time is limited and often visits are related to acute issues. In future studies, it is important to consider both passive and active methods of recruitment and consider the length of time the study requires, in addition to the potential financial costs.

In addition to recognizing a child's weight status and be willing to accept help, location of study should also be mentioned. The MY LIFE study was carried out in Ste-Anne-de-Bellevue, Quebec, approximately 35-38 km (~35 minutes) away from downtown Montreal. Despite being near public transportation and major highways, it is not considered a central location. Families were asked to visit the clinic 5-times per year, plus 7 additional times to receive counselling. To alleviate parental concerns with travelling time and overall time commitment of participating, the MY LIFE study visits were tailored to meet the family needs and offered at convenient times (weekends, after school). Whenever possible, intervention sessions were held on the same day as study visits. It is necessary that future studies consider following suit; timing of study visits to meet the needs of the family can act as an incentive to participate and therefore increase retention rates.

Assessment of body composition

In this study, DXA was used to assess body composition and bone outcomes and is considered valid to use in pediatric trials. Technical considerations such as size-related issues (i.e., weight limits of DXA), projection errors (i.e., increased tissue thickness may underestimate bone density), positional errors, precision (i.e., general potential of reduced precision in children due to movement) as well as tissue thickness (i.e., may result in overestimation of fat mass) are all worthy of mention [25]. Despite these potential limitations, DXA results permit fat mass index (FMI; fat mass (kg)/ height (m^2)) and lean mass index (LMI; lean mass + BMC (kg)/ height (m^2)) to be quantified; using FMI values for overweight and obese classifications has resulted in fewer misclassifications than BMI or %BF [413] and therefore should be considered whenever possible as an outcome in obesity trials.

In this study, FMI and LMI, or fat-free mass index (FFMI) were used in addition to other standard outcomes to describe the changes in body composition in this population. In *Chapter 4*, FMI results were not significantly different from BL to 12-mo among groups. However, in *Chapter 5*, FMI was significantly increased in Ctrl at 12-mo ($p<0.001$). Differences in these two outcomes was that in Chapter 5, height and fat mass were log transformed to normalize the data. Previously in this discussion, it was discussed that children with obesity tend to be taller. Normalizing height may be important given the differences in height velocities that this population may experience. Although height was not different among groups throughout the study, children randomized to StnTx and Ctrl had a higher rate of height (growth) velocity compared to ModTx. Future obesity research may wish to follow suit.

Novel methods of promoting health behaviors

Study designs that aim to improve health behaviors should consider current trends of everyday society. The use of technology and communication strategies have only recently been used to promote healthy behaviors in children. Specifically, the use of mobile phone short message service (SMS) [414] or fitness and nutrition applications (apps) [415] prove useful to enhance participation and self-monitoring in children. Additionally, web-based programs aimed at reducing obesity in children have shown to be successful, particularly when parents are also involved [416]. This area of research is still in its infancy; further work in web-based programs are necessary to assess their impact on maintaining reductions in BMI in the long term.

Without a doubt, engaging in PA during childhood is healthy and necessary to foster healthy development and disease prevention [417]. It is important that the research remains cognizant to the differences between physical activity and exercise, as they do not imply the same things. According to the Canadian Society of Exercise Science, physical activity is defined as: "*Any bodily movement produced by skeletal muscle that results in energy expenditure, and increases heart rate and breathing*" whereas exercise is defined as: "*Physical activity that is planned, structured, repetitive and purposive in the sense that improvement or maintenance of one or more components of physical fitness is an objective"* [418]. Both exercise and PA are important, however, when promoting healthy behaviors to children, PA objectives should be vague, described as "do-your-best" goals and most importantly, attainable [419], so that they are enjoyed and can be sustained when the trial is completed and beyond. Using SMART goals, as done in the MY LIFE Study, is a method to ensure PA objectives are met and are realistic thereby empowering the child to continue adopting new healthy habits.

Novel methods of increasing PA have been gaining momentum, particularly in research that aims to test the effects of active versus sedentary video gaming in children. Exergames, the new generation of active games, combine entertainment with physical exercise [420] and aim at reducing time engaged in sedentary behaviors. Short trials have assessed the impact of exergames on BMI and have resulted in improvements in BAZ (i.e., ΔBAZ -0.07±0.14) [421] and activity levels [419]; longer trials are needed. In general, there is a need for research and industry to develop video games that appeal to the new generation of Millennials, such as virtual pet games [422], and consider these methods as a means to reduce sedentary behaviors in future trials.

Novel methods of assessing dietary intake

Throughout this dissertation, analysis of dietary data was limited due to poor return of food diaries. Despite the use of nutritional biomarkers to assess the compliance to the milk intervention, there were insufficient return of food diaries to assess what was driving the change in fatty acid concentrations that were reflective of milk fat.

The issue of dietary recall is unfortunately not uncommon; research has identified the need to improve compliance and has started to adapt technology to address these limitations [423]. Such examples are: (1) image-assisted (i.e., micro-cameras) [424]; (2) image-based (i.e.,

mobile food records) [424]; and (3) web-based food recording [425]. There is a need to engage children in self-reporting dietary intake which could be done through different motivation and accuracy techniques using technology [385], which should be explored and considered in future trials.

7.4 Public Health Implications

This dissertation has public health implications. The interventions presented in this study were based on current Canadian health guidelines. The dietary guidelines (Canada's Food Guide to Healthy Eating) used in this study was developed in 2007. Currently, this version of the Canadian food guide is under review and an updated guide is scheduled to be launched within the coming year. Therefore, the results from this dissertation are timely and suggest that the current guidelines can help children with obesity, however, with support. In this case, the support was provided by registered dietitians. Moreover, food guides should not be simply handed out and briefly reviewed as results may not be as beneficial to children: the control group of this study resulted in significant increases in fat mass, waist circumference and trunk fat mass by 12-mo. These results suggest that diet and PA guidelines may be used as a backdrop to interventions but must be tailored to the needs of families.

In 2016, the World Health Organization released a report on childhood obesity and stated: "*There is an urgent need to act now to improve the health of this generation and the next*" and urges the use of family-based lifestyle in the management of childhood obesity [1]. In Canada, the Pan-Canadian Health Network, a network of individuals (e.g., academics, researchers, public servants, members of non-governmental organizations, and health professionals) representing all Provinces of Canada (except Quebec) has identified the need to address childhood obesity in Canada [426]. This network has published various Provincial initiatives that are currently in place to help Canadians achieve healthier lifestyles. Although Quebec is not a part of this network, Quebec supports the efforts of the Pan-Canadian Health Network and has developed programs to help Quebec citizens engage in healthier lifestyles. For instance, "*Québec en forme"*, a non-profit organization, aims to promote active living and healthy eating among Quebec youth (http://www.quebecenforme.org/en.aspx). The other Provinces have similar organizations that target children and youth and engage the community at

large [426], which is especially important given a child's environment, or community, plays an important role in achieving a healthy lifestyle.

7.5 Conclusion

In summary, this dissertation has reviewed the impact of a 1 year family-centered lifestyle intervention in pre-pubertal children with obesity on changes in: (1) adiposity; (2) bone health; and (3) eating behaviors. This thesis also explored the potential effects of changes these health outcomes if children increased their daily servings of milk and milk products above current Canadian dietary guidelines and participated in daily weight-bearing types of activities.

The results presented in this thesis suggest that children randomized to intervention groups resulted in favorable changes in body composition (i.e., change in BAZ in StnTx (-0.3±0.4) and ModTx (-0.7±0.6) by 12 months compared to baseline), and eating behaviors (i.e., ModTx reduced Food Approach eating behaviors at 6 months) whereas bone health was generally maintained among all groups. However, due to the study design of the MY LIFE Study, the manuscripts presented in this dissertation are not positioned to conclude if increasing the daily consumption of milk and milk product intakes and participating in daily weight-bearing types of activities has any added benefit on these health outcomes in children with obesity. Nonetheless, this body of work contributes to our knowledge of the use of family-centered lifestyle interventions for the management of childhood obesity, and suggests different assessment methods that should be considered in future trials.

Childhood obesity is a challenge to treat; it is a serious condition that affects too many children worldwide. Its etiology is complex; however, research has identified certain determinants and risk factors for developing childhood obesity. Treatment programs should start when children are young and include the family, and need to be tailored to meet the needs of the family so changes in health behaviors are achieved and sustained. This dissertation offers insight and discussion in the research area of childhood obesity, that will benefit all those who work in treating this unfortunate disease.

References

1. World Health Organization: **Report of the Commission on Ending Childhood Obesity.** 2016. Geneva: Switzerland [http://www.who.int/end-childhood-obesity/en/]. *Accessed*: June 16 2017.

2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al: **Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study 2013.** *Lancet* 2014, **384:**766-781.

3. Statistics Canada: **Canadian Health Measures Survey.** 2012- 2013, Government of Canada: Ottawa, Ontario, Canada [https://www.statcan.gc.ca/pub/82-625-x/2014001/article/14105-eng.htm]. *Accessed*: July 15, 2017.

4. Simmonds M, Llewellyn A, Owen CG, Woolacott N: **Predicting adult obesity from childhood obesity: A systematic review and meta-analysis.** *Obes Rev* 2016, **17:**95-107.

5. Davison KK, Birch LL: **Childhood overweight: A contextual model and recommendations for future research.** *Obes Rev* 2001, **2:**159-171.

6. Pena MM, Dixon B, Taveras EM: **Are you talking to ME? The importance of ethnicity and culture in childhood obesity prevention and management.** *Child Obes* 2012, **8:**23-27.

7. Ebbeling CB, Pawlak DB, Ludwig DS: **Childhood obesity: Public-health crisis, common sense cure.** *Lancet* 2002, **360:**473-482.

8. Young-Hyman D, Schlundt DG, Herman L, De Luca F, Counts D: **Evaluation of the insulin resistance syndrome in 5- to 10-year-old overweight/ obese African-American children.** *Diabetes Care* 2001, **24:**1359-1364.

9. Llewellyn A, Simmonds M, Owen CG, Woolacott N: **Childhood obesity as a predictor of morbidity in adulthood: A systematic review and meta-analysis.** *Obes Rev* 2016, **17:**56-67.

10. Griffiths LJ, Wolke D, Page AS, Horwood JP: **Obesity and bullying: Different effects for boys and girls.** *Arch Dis Child* 2006, **91:**121-125.

11. Latzer Y, Stein D: **A review of the psychological and familial perspectives of childhood obesity.** *J Eat Disord* 2013, **1:**7.

12. Cornette R: **The emotional impact of obesity on children.** *Worldviews Evid Based Nurs* 2008, **5:**136-141.

13. Reinehr T: **Lifestyle intervention in childhood obesity: Changes and challenges.** *Nat Rev Endocrinol* 2013, **9:**607-614.

14. Grimm ER, Steinle NI: **Genetics of eating behavior: Established and emerging concepts.** *Nutr Rev* 2011, **69:**52-60.

15. Webber L, Hill C, Saxton J, Van Jaarsveld CH, Wardle J: **Eating behaviour and weight in children.** *Int J Obes (Lond)* 2009, **33:**21-28.

16. Parkinson KN, Drewett RF, Le Couteur AS, Adamson AJ, Gateshead Milennium Study Core T: **Do maternal ratings of appetite in infants predict later Child Eating Behaviour Questionnaire scores and body mass index?** *Appetite* 2010, **54:**186-190.

17. Spence JC, Carson V, Casey L, Boule N: **Examining behavioural susceptibility to obesity among Canadian pre-school children: The role of eating behaviours.** *Int J Pediatr Obes* 2011, **6:**e501-507.

18. Viana V, Sinde S, Saxton JC: **Children's Eating Behaviour Questionnaire: Associations with BMI in Portuguese children.** *Br J Nutr* 2008, **100:**445-450.

19. dos Passos DR, Gigante DP, Maciel FV, Matijasevich A: **Children's eating behaviour: comparison between normal and overweight children from a school in Pelotas, Rio Grande do Sul, Brazil.** *Rev Paul Pediatr* 2015, **33:**42-49.

20. Santos JL, Ho-Urriola JA, Gonzalez A, Smalley SV, Dominguez-Vasquez P, Cataldo R, et al: **Association between eating behavior scores and obesity in Chilean children.** *Nutr J* 2011, **10:**108.

21. Lu L, Xun P, Wan Y, He K, Cai W: **Long-term association between dairy consumption and risk of childhood obesity: A systematic review and meta-analysis of prospective cohort studies.** *Eur J Clin Nutr* 2016, **70:**414-423.

22. Dror DK: **Dairy consumption and pre-school, school-age and adolescent obesity in developed countries: A systematic review and meta-analysis.** *Obes Rev* 2014, **15:**516-527.

23. Health Canada: **Canada's Food Guide to Healthy Eating.** 2011, Health Canada; Government of Canada: Ottawa, Ontario, Canada [https://www.canada.ca/en/health-canada/services/canada-food-guides.html]. *Accessed*: July 15, 2017.

24. Sioen I, Lust E, De Henauw S, Moreno LA, Jimenez-Pavon D: **Associations between body composition and bone health in children and adolescents: A systematic review.** *Calcif Tissue Int* 2016, **99:**557-577.

25. Kelley JC, Crabtree N, Zemel BS: **Bone density in the obese child: Clinical considerations and diagnostic challenges.** *Calcif Tissue Int* 2017, **100:**514-527.

26. Paulis WD, Silva S, Koes BW, van Middelkoop M: **Overweight and obesity are associated with musculoskeletal complaints as early as childhood: A systematic review.** *Obes Rev* 2014, **15:**52-67.

27. Cole ZA, Harvey NC, Kim M, Ntani G, Robinson SM, Inskip HM, et al: **Increased fat mass is associated with increased bone size but reduced volumetric density in pre pubertal children.** *Bone* 2012, **50:**562-567.

28. Ehehalt S, Binder G, Schurr N, Pfaff C, Ranke MB, Schweizer R: **The functional muscle-bone unit in obese children: Altered bone structure leads to normal strength strain index.** *Exp Clin Endocrinol Diabetes* 2011, **119:**321,326.

29. Chaplais E, Thivel D, Greene D, Dutheil F, Duche P, Naughton G, et al: **Bone-adiposity cross-talk: Implications for pediatric obesity. A narrative review of literature.** *J Bone Miner Metab* 2015, **33:**592-602.

30. Zemel MB: **The role of dairy foods in weight management.** *J Am Coll Nutr* 2005, **24:**537s-546s.

31. Bendtsen LQ, Lorenzen JK, Bendsen NT, Rasmussen C, Astrup A: **Effect of dairy proteins on appetite, energy expenditure, body weight, and composition: A review of the evidence from controlled clinical trials.** *Adv Nutr* 2013, **4:**418-438.

32. Specker B, Thiex NW, Sudhagoni RG: **Does exercise influence pediatric bone? A systematic review.** *Clin Orthop Relat Res* 2015, **473:**3658-3672.

33. Kelley GA, Kelley KS, Pate RR: **Effects of exercise on BMI z-score in overweight and obese children and adolescents: A systematic review with meta-analysis.** *BMC Pediatr* 2014, **14:**225.

34. Behringer M, Gruetzner S, McCourt M, Mester J: **Effects of weight-bearing activities on bone mineral content and density in children and adolescents: A meta-analysis.** *J Bone Miner Res* 2014, **29:**467-478.

35. Canadian Society for Exercise Physiology: **Canadian physical activity guidelines for children 5-11.** 2015. Ottawa, Ontario, Canada, [http://www.csep.ca/cmfiles/guidelines/csep_guidelines_handbook.pdf]. *Accessed*: August 3 2017.

36. Roberts KC, Shields M, deGroh M, Aziz A, Gilbert JA: **Overweight and obesity in children and adolescents: Results from the 2009 to 2011 Canadian Health Measures Survey.** *Health Rep* 2012, **3:**37-41.

37. Statistics Canada: **Canadian Community Health Survey.** 2004, Government of Canada: Ottawa, Ontario, Canada [http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey&SDDS=5049]. *Accessed*: August 9 2017.

38. World Health Organization: **Growth reference data for 5-19 years.** 2012, Geneva, Switzerland [http://www.who.int/growthref/en/]. *Accessed*: June 15, 2017.

39. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al: **CDC growth charts: United States.** *Advance data* 2000**:**1-27.

40. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH: **Establishing a standard definition for child overweight and obesity worldwide: International survey.** *BMJ* 2000, **320:**1240-1243.

41. Hoelscher DM, Kirk S, Ritchie L, Cunningham-Sabo L: **Position of the Academy of Nutrition and Dietetics: Interventions for the prevention and treatment of pediatric overweight and obesity.** *J Acad Nutr Diet* 2013, **113:**1375-1394.

42. Yanovski JA: **Pediatric obesity. An introduction.** *Appetite* 2015, **93:**3-12.

43. Whitaker RC, Pepe MS, Wright JA, Seidel KD, Dietz WH: **Early adiposity rebound and the risk of adult obesity.** *Pediatrics* 1998, **101:**E5.

44. Wellens RI, Roche AF, Khamis HJ, Jackson AS, Pollock ML, Siervogel RM: **Relationships between the body mass index and body composition.** *Obes Res* 1996, **4:**35-44.

45. McCarthy HD: **Measuring growth and obesity across childhood and adolescence.** *Proc Nutr Soc* 2014, **73:**210-217.

46. McCarthy HD, Cole TJ, Fry T, Jebb SA, Prentice AM: **Body fat reference curves for children.** *Int J Obes (Lond)* 2006, **30:**598-602.

47. Katzmarzyk PT: **Waist circumference percentiles for Canadian youth 11-18 y of age.** *Eur J Clin Nutr* 2004, **58:**1011-1015.

48. Kuhle S, Maguire B, Ata N, Hamilton D: **Percentile curves for anthropometric measures for Canadian children and youth.** *PLoS One* 2015, **10:**e0132891.

49. Mehta SK: **Waist circumference to height ratio in children and adolescents.** *Clin Pediatr (Phila)* 2015, **54:**652-658.

50. McCarthy HD, Ashwell M: **A study of central fatness using waist-to-height ratios in UK children and adolescents over two decades supports the simple message: 'Keep your waist circumference to less than half your height'.** *Int J Obes (Lond)* 2006, **30:**988-992.

51. Wells JCK, Fewtrell MS: **Measuring body composition.** *Arch Dis Child* 2006, **91:**612-617.

52. Damilakis J, Adams JE, Guglielmi G, Link TM: **Radiation exposure in x-ray-based imaging techniques used in osteoporosis.** *Eur Radiol* 2010, **20:**2707-2714.

53. Berger A: **Bone mineral density scans.** *BMJ* 2002, **325:**484-484.

54. Petak S, Barbu CG, Yu EW, Fielding R, Mulligan K, Sabowitz B, et al: **The official positions of the International Society for Clinical Densitometry: Body composition analysis reporting.** *J Clin Densitom* 2013, **16:**508-519.

55. Adams JE, Engelke K, Zemel BS, Ward KA: **Quantitative computer tomography in children and adolescents: The 2013 ISCD Pediatric Official Positions.** *J Clin Densitom* 2014, **17:**258-274.

56. Gurnani M, Birken C, Hamilton J: **Childhood obesity: Causes, consequences, and management.** *Pediatr Clin North Am* 2015, **62:**821-840.

57. Langley-Evans SC, Moran VH: **Childhood obesity: Risk factors, prevention and management.** *Matern Child Nutr* 2014, **10:**453-455.

58. Wardle J, Carnell S, Haworth CMA, Plomin R: **Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment.** *Am J Clin Nutr* 2008, **87:**398-404.

59. Bray GA: **The epidemic of obesity and changes in food intake: The fluoride hypothesis.** *Physiol Behav* 2004, **82:**115-121.

60. van Dijk SJ, Molloy PL, Varinli H, Morrison JL, Muhlhausler BS: **Epigenetics and human obesity.** *Int J Obes (Lond)* 2015, **39:**85-97.

61. Jéquier E, Tappy L: **Regulation of body weight in humans.** *Physiol Rev* 1999, **79:**451-480.

62. Spiegelman BM, Flier JS: **Obesity and the regulation of energy balance.** *Cell* 2001, **104:**531-543.

63. Schwartz MW, Morton GJ: **Obesity: Keeping hunger at bay.** *Nature* 2002, **418:**595-597.

64. Kringelbach ML, Stein A, van Hartevelt TJ: **The functional human neuroanatomy of food pleasure cycles.** *Physiol Behav* 2012, **106:**307-316.

65. Berthoud HR, Morrison C: **The brain, appetite, and obesity.** *Annu Rev Psychol* 2008, **59:**55-92.

66. Austin J, Marks D: **Hormonal regulators of appetite.** *Int J Pediatr Endocrinol* 2009, **2009:**141753.

67. Klok MD, Jakobsdottir S, Drent ML: **The role of leptin and ghrelin in the regulation of food intake and body weight in humans: A review.** *Obes Rev* 2007, **8:**21-34.

68. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K: **Ghrelin is a growth-hormone-releasing acylated peptide from stomach.** *Nature* 1999, **402:**656-660.

69. Zou CC, Liang L, Zhao ZY: **Factors associated with fasting plasma ghrelin levels in children and adolescents.** *World J Gastroenterol* 2008, **14:**790-794.

70. Blundell J, de Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, et al: **Appetite control: Methodological aspects of the evaluation of foods.** *Obes Rev* 2010, **11:**251-270.

71. Cammisotto P, Bendayan M: **A review on gastric leptin: The exocrine secretion of a gastric hormone.** *Anat Cell Biol* 2012, **45:**1-16.

72. Tremblay A, Bellisle F: **Nutrients, satiety, and control of energy intake.** *Appl Physiol Nutr Metab* 2015, **40:**971-979.

73. Soenen S, Westerterp-Plantenga MS: **Proteins and satiety: Implications for weight management.** *Curr Opin Clin Nutr Metab Care* 2008, **11:**747-751.

74. Pereira PC: **Milk nutritional composition and its role in human health.** *Nutrition* 2014, **30:**619-627.

75. Haug A, Høstmark AT, Harstad OM: **Bovine milk in human nutrition: A review.** *Lipids Health Dis* 2007, **6:**25-25.

76. Vien S, Luhovyy BL, Patel BP, Panahi S, El Khoury D, Mollard RC, et al: **Pre- and within-meal effects of fluid dairy products on appetite, food intake, glycemia, and regulatory hormones in children.** *Appl Physiol Nutr Metab* 2017, **42:**302-310.

77. Mehrabani S, Safavi SM, Mehrabani S, Asemi M, Feizi A, Bellissimo N, et al: **Effects of low-fat milk consumption at breakfast on satiety and short-term energy intake in 10- to 12-year-old obese boys.** *Eur J Nutr* 2016, **55:**1389-1396.

78. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B: **Slow and fast dietary proteins differently modulate postprandial protein accretion.** *Proc Natl Acad Sci U S A* 1997, **94:**14930-14935.

79. Bornet FR, Jardy-Gennetier AE, Jacquet N, Stowell J: **Glycaemic response to foods: Impact on satiety and long-term weight regulation.** *Appetite* 2007, **49:**535-553.

80. Wang P, Mariman E, Renes J, Keijer J: **The secretory function of adipocytes in the physiology of white adipose tissue.** *J Cell Physiol* 2008, **216:**3-13.

81. Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, et al: **Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor.** *N Engl J Med* 2007, **356:**237-247.

82. Sinha MK, Sturis J, Ohannesian J, Magosin S, Stephens T, Heiman ML, et al: **Ultradian oscillations of leptin secretion in humans.** *Biochem Biophys Res Commun* 1996, **228:**733-738.

83. Lasa A, Miranda J, Bullo M, Casas R, Salas-Salvado J, Larretxi I, et al: **Comparative effect of two Mediterranean diets versus a low-fat diet on glycaemic control in individuals with type 2 diabetes.** *Eur J Clin Nutr* 2014, **68:**767-772.

84. Ratliff J, Mutungi G, Puglisi MJ, Volek JS, Fernandez ML: **Carbohydrate restriction (with or without additional dietary cholesterol provided by eggs) reduces insulin resistance and plasma leptin without modifying appetite hormones in adult men.** *Nutr Res* 2009, **29:**262-268.

85. Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, et al: **A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations.** *Am J Clin Nutr* 2005, **82:**41-48.

86. Ibarra-Reynoso Ldel R, Pisarchyk L, Perez-Luque EL, Garay-Sevilla ME, Malacara JM: **Dietary restriction in obese children and its relation with eating behavior, fibroblast growth factor 21 and leptin: A prospective clinical intervention study.** *Nutr Metab (Lond)* 2015, **12:**31.

87. Arrigo T, Gitto E, Ferrau V, Munafo C, Alibrandi A, Marseglia GL, et al: **Effect of weight reduction on leptin, total ghrelin and obestatin concentrations in prepubertal children.** *J Biol Regul Homeost Agents* 2012, **26:**S95-103.

88. Kopp W, Blum WF, Ziegler A, Mathiak K, Lubbert H, Herpertz S, et al: **Serum leptin and body weight in females with anorexia and bulimia nervosa.** *Horm Metab Res* 1998, **30:**272-275.

89. Jimerson DC, Wolfe BE, Carroll DP, Keel PK: **Psychobiology of purging disorder: Reduction in circulating leptin levels in purging disorder in comparison with controls.** *Int J Eat Disord* 2010, **43:**584-588.

90. Tanofsky-Kraff M, Marcus MD, Yanovski SZ, Yanovski JA: **Loss of control eating disorder in children age 12 years and younger: Proposed research criteria.** *Eat Behav* 2008, **9:**360-365.

91. Miller R, Tanofsky-Kraff M, Shomaker LB, Field SE, Hannallah L, Reina SA, et al: **Serum leptin and loss of control eating in children and adolescents.** *Int J Obes (Lond)* 2014, **38:**397-403.

92. Wardle J, Guthrie C, Sanderson S, Rapoport L: **Development of the Children's Eating Behaviour Questionnaire.** *J Child Psychol Psychiatry* 2001, **42:**963-970.

93. Heindel JJ, Vandenberg LN: **Developmental origins of health and disease: A paradigm for understanding disease cause and prevention.** *Curr Opin Pediatr* 2015, **27:**248-253.

94. Santangeli L, Sattar N, Huda SS: **Impact of maternal obesity on perinatal and childhood outcomes.** *Best Pract Res Clin Obstet Gynaecol* 2015, **29:**438-448.

95. Oken E, Levitan EB, Gillman MW: **Maternal smoking during pregnancy and child overweight: Systematic review and meta-analysis.** *Int J Obes (Lond)* 2008, **32:**201-210.

96. Kramer MS, Matush L, Vanilovich I, Platt RW, Bogdanovich N, Sevkovskaya Z, et al: **A randomized breast-feeding promotion intervention did not reduce child obesity in Belarus.** *J Nutr* 2009, **139:**417s-421s.

97. Freedman DS, Khan LK, Serdula MK, Ogden CL, Dietz WH: **Racial and ethnic differences in secular trends for childhood BMI, weight, and height.** *Obesity (Silver Spring)* 2006, **14:**301-308.

98. Gupta N, Goel K, Shah P, Misra A: **Childhood obesity in developing countries: Epidemiology, determinants, and prevention.** *Endocr Rev* 2012, **33:**48-70.

99. Wang Y, Lim H: **The global childhood obesity epidemic and the association between socio-economic status and childhood obesity.** *Int Rev Psychiatry* 2012, **24:**176-188.

100. Cooke MJ, Wilk P, Paul KW, Gonneville SL: **Predictors of obesity among Metis children: Socio-economic, behavioural and cultural factors.** *Can J Public Health* 2013, **104:**e298-303.

101. Grafova IB: **Overweight children: Assessing the contribution of the built environment.** *Prev Med* 2008, **47:**304-308.

102. Wolch J, Jerrett M, Reynolds K, McConnell R, Chang R, Dahmann N, et al: **Childhood obesity and proximity to urban parks and recreational resources: A longitudinal cohort study.** *Health Place* 2011, **17:**207-214.

103. Johnson 3rd JA, Johnson AM: **Urban-rural differences in childhood and adolescent obesity in the United States: A systematic review and meta-analysis.** *Child Obes* 2015, **11:**233-241.

104. Moore LL, Gao D, Bradlee ML, Cupples LA, Sundarajan-Ramamurti A, Proctor MH, et al: **Does early physical activity predict body fat change throughout childhood?** *Prev Med* 2003, **37:**10-17.

105. Tremblay MS, Leblanc AG, Janssen I, Kho ME, Hicks A, Murumets K, et al: **Canadian sedentary behaviour guidelines for children and youth.** *Appl Physiol Nutr Metab* 2011, **36:**59-64; 65-71.

106. George K, Kristi K, Russell P: **Exercise and BMI z-score in overweight and obese children and adolescents: A systematic review and network meta-analysis of randomized trials.** *J Evid Based Med* 2016.

107. Schranz N, Tomkinson G, Olds T: **What is the effect of resistance training on the strength, body composition and psychosocial status of overweight and obese children and adolescents? A systematic review and meta-analysis.** *Sports Med* 2013, **43:**893-907.

108. Sallis JF, Prochaska JJ, Taylor WC: **A review of correlates of physical activity of children and adolescents.** *Med Sci Sports Exerc* 2000, **32:**963-975.

109. Van Der Horst K, Paw MJ, Twisk JW, Van Mechelen W: **A brief review on correlates of physical activity and sedentariness in youth.** *Med Sci Sports Exerc* 2007, **39:**1241-1250.

110. Hinkley T, Crawford D, Salmon J, Okely AD, Hesketh K: **Preschool children and physical activity: A review of correlates.** *Am J Prev Med* 2008, **34:**435-441.

111. Hinkley T, Salmon J, Okely AD, Hesketh K, Crawford D: **Correlates of preschool children's physical activity.** *Am J Prev Med* 2012, **43:**159-167.

112. Rey-López JP, Vicente-Rodríguez G, Biosca M, Moreno LA: **Sedentary behaviour and obesity development in children and adolescents.** *Nutr Metab Cardiovasc Dis* 2008, **18:**242-251.

113. Ekelund U, Brage S, Froberg K, Harro M, Anderssen SA, Sardinha LB, et al: **TV viewing and physical activity are independently associated with metabolic risk in children: The European Youth Heart Study.** *PLoS Med* 2006, **3:**e488.

114. Wahi G, Parkin PC, Beyene J, Uleryk EM, Birken CS: **Effectiveness of interventions aimed at reducing screen time in children: A systematic review and meta-analysis of randomized controlled trials.** *Arch Pediatr Adolesc Med* 2011, **165:**979-986.

115. Wu L, Sun S, He Y, Jiang B: **The effect of interventions targeting screen time reduction: A systematic review and meta-analysis.** *Medicine (Baltimore)* 2016, **95:**e4029.

116. DeMattia L, Lemont L, Meurer L: **Do interventions to limit sedentary behaviours change behaviour and reduce childhood obesity? A critical review of the literature.** *Obes Rev* 2007, **8:**69-81.

117. Butte NF, Ellis KJ: **Comment on: Obesity and the environment: Where do we go from here?** *Science* 2003, **301:**598; author reply 598.

118. Moreno LA, Rodriguez G: **Dietary risk factors for development of childhood obesity.** *Curr Opin Clin Nutr Metab Care* 2007, **10:**336-341.

119. Garriguet D: **Overview of Canadians' eating habits.** 2004, Statistics Canada; Ottawa, Ontario, Canada [http://publications.gc.ca/Collection/Statcan/82-620-M/82-620-MIE2006002.pdf]. *Accessed*: July 15, 2017.

120. Dubois L, Carter MA, Farmer A, Girard M, Burnier D, Tatone-Tokuda F, et al: **Higher intakes of energy and grain products at 4 years of age are associated with being overweight at 6 years of age.** *J Nutr* 2011, **141:**2024-2029.

121. Johnson L, Mander AP, Jones LR, Emmett PM, Jebb SA: **Energy-dense, low-fiber, high-fat dietary pattern is associated with increased fatness in childhood.** *Am J Clin Nutr* 2008, **87:**846-854.

122. Ambrosini GL, Emmett PM, Northstone K, Howe LD, Tilling K, Jebb SA: **Identification of a dietary pattern prospectively associated with increased adiposity during childhood and adolescence.** *Int J Obes (Lond)* 2012, **36:**1299-1305.

123. Shields M: **Overweight and obesity among children and youth.** *Health Rep* 2006, **17:**27-42.

124. Blanchette L, Brug J: **Determinants of fruit and vegetable consumption among 6-12-year-old children and effective interventions to increase consumption.** *J Hum Nutr Diet* 2005, **18:**431-443.

125. Deshmukh-Taskar PR, Nicklas TA, O'Neil CE, Keast DR, Radcliffe JD, Cho S: **The relationship of breakfast skipping and type of breakfast consumption with nutrient intake and weight status in children and adolescents: The National Health and Nutrition Examination Survey 1999-2006.** *J Am Diet Assoc* 2010, **110:**869-878.

126. Veugelers PJ, Fitzgerald AL: **Prevalence of and risk factors for childhood overweight and obesity.** *CMAJ* 2005, **173:**607-613.

127. Larson N, Story M: **A review of snacking patterns among children and adolescents: What are the implications of snacking for weight status?** *Child Obes* 2013, **9:**104-115.

128. Gilbert JA, Miller D, Olson S, St-Pierre S: **After-school snack intake among Canadian children and adolescents.** *Can J Public Health* 2012, **103:**e448-452.

129. Dubois L, Farmer A, Girard M, Peterson K: **Social factors and television use during meals and snacks is associated with higher BMI among pre-school children.** *Public Health Nutr* 2008, **11:**1267-1279.

130. Malik VS, Schulze MB, Hu FB: **Intake of sugar-sweetened beverages and weight gain: A systematic review.** *Am J Clin Nutr* 2006, **84:**274-288.

131. Danyliw AD, Vatanparast H, Nikpartow N, Whiting SJ: **Beverage patterns among Canadian children and relationship to overweight and obesity.** *Appl Physiol Nutr Metab* 2012, **37:**900-906.

132. O'Connor TM, Yang SJ, Nicklas TA: **Beverage intake among preschool children and its effect on weight status.** *Pediatrics* 2006, **118:**e1010-1018.

133. Bray GA, Nielsen SJ, Popkin BM: **Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity.** *Am J Clin Nutr* 2004, **79:**537-543.

134. Ebbeling CB, Sinclair KB, Pereira MA, Garcia-Lago E, Feldman HA, Ludwig DS: **Compensation for energy intake from fast food among overweight and lean adolescents.** *JAMA* 2004, **291:**2828-2833.

135. Braithwaite I, Stewart AW, Hancox RJ, Beasley R, Murphy R, Mitchell EA: **Fast-food consumption and body mass index in children and adolescents: An international cross-sectional study.** *BMJ Open* 2014, **4:**e005813.

136. Dror DK, Allen LH: **Dairy product intake in children and adolescents in developed countries: Trends, nutritional contribution, and a review of association with health outcomes.** *Nutr Rev* 2014, **72:**68-81.

137. Fisher J, Mitchell D, Smiciklas-Wright H, Birch L: **Maternal milk consumption predicts the tradeoff between milk and soft drinks in young girls' diets.** *J Nutr* 2001, **131:**246-250.

138. Moreno LA, Rodriguez G, Fleta J, Bueno-Lozano M, Lazaro A, Bueno G: **Trends of dietary habits in adolescents.** *Crit Rev Food Sci Nutr* 2010, **50:**106-112.

139. Gahagan S: **Development of eating behavior: Biology and context.** *J Dev Behav Pediatr* 2012, **33:**261-271.

140. Scaglioni S, Arrizza C, Vecchi F, Tedeschi S: **Determinants of children's eating behavior.** *Am J Clin Nutr* 2011, **94:**2006S-2011S.

141. Birch LL, Fisher JO: **Development of eating behaviors among children and adolescents.** *Pediatrics* 1998, **101:**539-549.

142. Llewellyn CH, van Jaarsveld CH, Johnson L, Carnell S, Wardle J: **Nature and nurture in infant appetite: Analysis of the Gemini twin birth cohort.** *Am J Clin Nutr* 2010, **91:**1172-1179.

143. Savage JS, Fisher JO, Birch LL: **Parental influence on eating behavior: Conception to adolescence.** *J Law Med Ethics* 2007, **35:**22-34.

144. Faith MS, Scanlon KS, Birch LL, Francis LA, Sherry B: **Parent-child feeding strategies and their relationships to child eating and weight status.** *Obes Res* 2004, **12:**1711-1722.

145. Fisher JO, Kral TVE: **Super-size me: Portion size effects on young children's eating.** *Physiol Behav* 2008, **94:**39-47.

146. Ello-Martin JA, Ledikwe JH, Rolls BJ: **The influence of food portion size and energy density on energy intake: Implications for weight management.** *Am J Clin Nutr* 2005, **82:**236s-241s.

147. Jansen PW, Roza SJ, Jaddoe VWV, Mackenbach JD, Raat H, Hofman A, et al: **Children's eating behavior, feeding practices of parents and weight problems in early childhood: Results from the population-based Generation R Study.** *Int J Behav Nutr Phys Act* 2012, **9:**130-130.

148. Freedman DS, Mei Z, Srinivasan SR, Berenson GS, Dietz WH: **Cardiovascular risk factors and excess adiposity among overweight children and adolescents: The Bogalusa Heart Study.** *J Pediatr* 2007, **150:**12-17.e12.

149. Flynn J: **The changing face of pediatric hypertension in the era of the childhood obesity epidemic.** *Pediatr Nephrol* 2013, **28:**1059-1066.

150. Cook S, Kavey REW: **Dyslipidemia and pediatric obesity.** *Pediatr Clin North Am* 2011, **58:**1363-1373.

151. Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, et al: **Prevalence of impaired glucose tolerance among children and adolescents with marked obesity.** *N Engl J Med* 2002, **346:**802-810.

152. Staiano AE, Broyles ST, Gupta AK, Katzmarzyk PT: **Ethnic and sex differences in visceral, subcutaneous, and total body fat in children and adolescents.** *Obesity (Silver Spring)* 2013, **21:**1251-1255.

153. Weiss R, Dufour S, Taksali SE, Tamborlane WV, Petersen KF, Bonadonna RC, et al: **Prediabetes in obese youth: A syndrome of impaired glucose tolerance, severe insulin resistance, and altered myocellular and abdominal fat partitioning.** *Lancet* 2003, **362:**951-957.

154. Amed S, Dean HJ, Panagiotopoulos C, Sellers EA, Hadjiyannakis S, Laubscher TA, et al: **Type 2 diabetes, medication-induced diabetes, and monogenic diabetes in Canadian children: A prospective national surveillance study.** *Diabetes Care* 2010, **33:**786-791.

155. Dabelea D, Bell RA, D'Agostino RB, Jr., Imperatore G, Johansen JM, Linder B, et al: **Incidence of diabetes in youth in the United States.** *JAMA* 2007, **297:**2716-2724.

156. AlKhater SA: **Paediatric non-alcoholic fatty liver disease: An overview.** *Obes Rev* 2015, **16:**393-405.

157. Redline S, Tishler PV, Schluchter M, Aylor J, Clark K, Graham G: **Risk factors for sleep-disordered breathing in children: Associations with obesity, race, and respiratory problems.** *Am J Respir Crit Care Med* 1999, **159:**1527-1532.

158. Kutlubay Z, Engin B, Bairamov O, Tuzun Y: **Acanthosis nigricans: A fold (intertriginous) dermatosis.** *Clin Dermatol* 2015, **33:**466-470.

159. Belenchia AM, Tosh AK, Hillman LS, Peterson CA: **Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: A randomized controlled trial.** *Am J Clin Nutr* 2013, **97:**774-781.

160. Saintonge S, Bang H, Gerber LM: **Implications of a new definition of vitamin D deficiency in a multiracial us adolescent population: The National Health and Nutrition Examination Survey III.** *Pediatrics* 2009, **123:**797-803.

161. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF: **Decreased bioavailability of vitamin D in obesity.** *Am J Clin Nutr* 2000, **72:**690-693.

162. Nead KG, Halterman JS, Kaczorowski JM, Auinger P, Weitzman M: **Overweight children and adolescents: A risk group for iron deficiency.** *Pediatrics* 2004, **114:**104-108.

163. Eddy KT, Tanofsky-Kraff M, Thompson-Brenner H, Herzog DB, Brown TA, Ludwig DS: **Eating disorder pathology among overweight treatment-seeking youth: Clinical correlates and cross-sectional risk modeling.** *Behav Res Ther* 2007, **45:**2360-2371.

164. Schwimmer JB, Burwinkle TM, Varni JW: **Health-related quality of life of severely obese children and adolescents.** *JAMA* 2003, **289:**1813-1819.

165. Dimitri P, Bishop N, Walsh JS, Eastell R: **Obesity is a risk factor for fracture in children but is protective against fracture in adults: A paradox.** *Bone* 2012, **50:**457-466.

166. McGraw B, McClenaghan BA, Williams HG, Dickerson J, Ward DS: **Gait and postural stability in obese and nonobese prepubertal boys.** *Arch Phys Med Rehabil* 2000, **81:**484-489.

167. Wosje KS, Khoury PR, Claytor RP, Copeland KA, Hornung RW, Daniels SR, et al: **Dietary patterns associated with fat and bone mass in young children.** *Am J Clin Nutr* 2010, **92:**294-303.

168. Beck TJ, Petit MA, Wu G, LeBoff MS, Cauley JA, Chen Z: **Does obesity really make the femur stronger? BMD, geometry, and fracture incidence in the Women's Health Initiative-Observational Study.** *J Bone Miner Res* 2009, **24:**1369-1379.

169. Leonrd MB, Shults J, Wilson BA, Terhakovec AM, Zemel BS: **Obesity during childhood and adolescence augments bone mass and bone dimension.** *Am J Clin Nutr* 2004, **80**.

170. Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, HA M: **Bone structure and volumetric BMD in overweight children: A longitudinal study.** *J Bone Miner Res* 2008, **23:**1946-1953.

171. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D: **Bone mineral density in prepubertal obese and control children: Relation to body weight, lean mass, and fat mass.** *J Bone Miner Metab* 2008, **26:**73-78.

172. Viljakainen HT, Pekkinen M, Saarnio E, Karp H, Lamberg-Allardt C, Makitie O: **Dual effect of adipose tissue on bone health during growth.** *Bone* 2011, **48:**212-217.

173. Russell M, Mendes N, Miller KK, Rosen CJ, Lee H, Klibanski A, et al: **Visceral fat is a negative predictor of bone density measures in obese adolescent girls.** *J Clin Endocrinol Metab* 2010, **95:**1247-1255.

174. Goulding A, Taylor RW, Grant AM, Murdoch L, Williams SM, Taylor BJ: **Relationship of total body fat mass to bone area in New Zealand five-year-olds.** *Calcif Tissue Int* 2008, **82:**293-299.

175. Clark EM, Ness AR, Tobias JH: **Adipose tissue stimulates bone growth in prepubertal children.** *J Clin Endocrinol Metab* 2006, **91:**2534-2541.

176. Janicka A, Wren TA, Sanchez MM, Dorey F, Kim PS, Mittelman SD, et al: **Fat mass is not beneficial to bone in adolescents and young adults.** *J Clin Endocrinol Metab* 2007, **92:**143-147.

177. Dimitri P, Jacques RM, Paggiosi M, King D, Walsh J, Taylor ZA, et al: **Leptin may play a role in bone microstructural alterations in obese children.** *J Clin Endocrinol Metab* 2015, **100:**594-602.

178. Farr JN, Amin S, LeBrasseur NK, Atkinson EJ, Achenbach SJ, McCready LK, et al: **Body composition during childhood and adolescence: Relations to bone strength and microstructure.** *J Clin Endocrinol Metab* 2014, **99:**4641-4648.

179. Turner CH, Robling AG: **Designing exercise regimens to increase bone strength.** *Exerc Sport Sci Rev* 2003, **31:**45-50.

180. Chevalley T, Bonjour JP, Ferrari S, Hans D, Rizzoli R: **Skeletal site selectivity in the effects of calcium supplementation on areal bone mineral density gain: A randomized, double-blind, placebo-controlled trial in prepubertal boys.** *J Clin Endocrinol Metab* 2005, **90:**3342-3349.

181. Cadogan J, Eastell R, Jones N, Barker ME: **Milk intake and bone mineral acquisition in adolescent girls: Randomised, controlled intervention trial.** *BMJ* 1997, **315:**1255-1260.

182. Spence LA, Cifelli CJ, Miller GD: **The role of dairy products in healthy weight and body composition in children and adolescents.** *Curr Nutr Food Sci* 2011, **7:**40-49.

183. Huncharek M, Muscat J, Kupelnick B: **Impact of dairy products and dietary calcium on bone-mineral content in children: Results of a meta-analysis.** *Bone* 2008, **43:**312-321.

184. Zibellini J, Seimon RV, Lee CM, Gibson AA, Hsu MS, Shapses SA, et al: **Does diet-induced weight loss lead to bone loss in overweight or obese adults? A systematic review and meta-analysis of clinical trials.** *J Bone Miner Res* 2015, **30:**2168-2178.

185. Campos RM, de Piano A, da Silva PL, Carnier J, Sanches PL, Corgosinho FC, et al: **The role of pro/anti-inflammatory adipokines on bone metabolism in NAFLD obese adolescents: Effects of long-term interdisciplinary therapy.** *Endocrine* 2012, **42:**146-156.

186. Campos RM, de Mello MT, Tock L, da Silva PL, Corgosinho FC, Carnier J, et al: **Interaction of bone mineral density, adipokines and hormones in obese adolescents girls submitted in an interdisciplinary therapy.** *J Pediatr Endocrinol Metab* 2013, **26:**663-668.

187. Rourke KM, Brehm BJ, Cassell C, Sethuraman G: **Effect of weight change on bone mass in female adolescents.** *J Am Diet Assoc* 2003, **103:**369-372.

188. Huang Y, Eapen E, Steele S, Grey V: **Establishment of reference intervals for bone markers in children and adolescents.** *Clin Biochem* 2011, **44:**771-778.

189. Yang L, Grey V: **Pediatric reference intervals for bone markers.** *Clin Biochem* 2006, **39:**561-568.

190. Redman LM, Rood J, Anton SD: **Calorie restriction and bone health in young, overweight individuals.** *Arch Intern Med* 2008, **168:**1859-1866.

191. Gajewska J, Weker H, Ambroszkiewicz J, Szamotulska K, Chelchowska M, Franek E, et al: **Alterations in markers of bone metabolism and adipokines following a 3-month lifestyle intervention induced weight loss in obese prepubertal children.** *Exp Clin Endocrinol Diabetes* 2013, **121:**498-504.

192. Crofton PM, Shrivastava A, Wade JC, Stephen R, Kelnar CJ, Lyon AJ, et al: **Bone and collagen markers in preterm infants: Relationship with growth and bone mineral content over the first 10 weeks of life.** *Pediatr Res* 1999, **46:**581-587.

193. van Coeverden SC, Netelenbos JC, de Ridder CM, Roos JC, Popp-Snijders C, Delemarre-van de Waal HA: **Bone metabolism markers and bone mass in healthy pubertal boys and girls.** *Clin Endocrinol (Oxf)* 2002, **57:**107-116.

194. Seibel MJ: **Biochemical markers of bone turnover part I: Biochemistry and variability.** *Clin Biochem Rev* 2005, **26:**97-122.

195. Banfi G, Daverio R: **In vitro stability of osteocalcin.** *Clin Chem* 1994, **40:**833-834.

196. Power MJ, O'Dwyer B, Breen E, Fottrell PF: **Osteocalcin concentrations in plasma prepared with different anticoagulants.** *Clin Chem* 1991, **37:**281-284.

197. Cioffi M, Molinari AM, Gazzerro P, Di Finizio B, Fratta M, Deufemia A, et al: **Serum osteocalcin in 1634 healthy children.** *Clin Chem* 1997, **43:**543-545.

198. Reinehr T, Roth CL: **A new link between skeleton, obesity and insulin resistance: Relationships between osteocalcin, leptin and insulin resistance in obese children before and after weight loss.** *Int J Obes* 2010, **34:**852-858.

199. Chubb SAP: **Measurement of C-terminal telopeptide of type I collagen (CTX) in serum.** *Clin Biochem* 2012, **45:**928-935.

200. Crofton PM, Evans N, Taylor MRH, Holland CV: **Serum CrossLaps: Pediatric reference intervals from birth to 19 years of age.** *Clin Chem* 2002, **48:**671-673.

201. Gajewska J, Klemarczyk W, Ambroszkiewicz J, Szamotulska K, Chelchowska M, Weker H: **Associations between IGF-I, IGF-binding proteins and bone turnover markers in prepubertal obese children.** *J Pediatr Endocrinol Metab* 2015, **28:**563-569.

202. Dimitri P, Wales JK, Bishop N: **Adipokines, bone-derived factors and bone turnover in obese children: Evidence for altered fat-bone signalling resulting in reduced bone mass.** *Bone* 2011, **48:**189-196.

203. Holick MF: **Vitamin D deficiency.** *N Engl J Med* 2007, **357:**266-281.

204. Canadian Paediatric Society: **Vitamin D supplementation: Recommendations for Canadian mothers and infants.** *Paediatr Child Health* 2007, **12:**583-589.

205. Ross AC, Taylor CL, Yaktine AL: **Dietary reference intakes for calcium and vitamin D.** Washington (DC): National Academies Press (US); 2011.

206. Melamed ML, Kumar J: **Low levels of 25-hydroxyvitamin D in the pediatric populations: Prevalence and clinical outcomes.** *Pediatric health* 2010, **4:**89-97.

207. Reinehr T, de Sousa G, Alexy U, Kersting M, Andler W: **Vitamin D status and parathyroid hormone in obese children before and after weight loss.** *Eur J Endocrinol* 2007, **157:**225-232.

208. Epstein LH, Wing RR, Penner BC, Kress MJ: **Effect of diet and controlled exercise on weight loss in obese children.** *J Pediatr* 1985, **107:**358-361.

209. Peirson L, Fitzpatrick-Lewis D, Morrison K, Warren R, Usman Ali M, Raina P: **Treatment of overweight and obesity in children and youth: A systematic review and meta-analysis.** *CMAJ* 2015, **3:**E35-46.

210. Edmunds L, Waters E, Elliott EJ: **Evidence based management of childhood obesity.** *BMJ* 2001, **323:**916-919.

211. Epstein LH, Valoski A, Wing RR, McCurley J: **Ten-year follow-up of behavioral, family-based treatment for obese children.** *JAMA* 1990, **264:**2519-2523.

212. Faith MS, Van Horn L, Appel LJ, Burke LE, Carson JA, Franch HA, et al: **Evaluating parents and adult caregivers as "agents of change" for treating obese children: Evidence for parent behavior change strategies and research gaps: A scientific statement from the American Heart Association.** *Circulation* 2012, **125:**1186-1207.

213. Epstein LH, Masek BJ, Marshall WR: **A nutritionally based school program for control of eating in obese children.** *Behavior Therapy* 1978, **9:**766-778.

214. Epstein LH, Wing RR, Koeske R, Andrasik F, Ossip DJ: **Child and parent weight loss in family-based behavior modification programs.** *Consult Clin Psychol* 1981, **49:**674-685.

215. Sung-Chan P, Sung YW, Zhao X, Brownson RC: **Family-based models for childhood-obesity intervention: A systematic review of randomized controlled trials.** *Obes Rev* 2013, **14:**265-278.

216. Rajjo T, Mohammed K, Alsawas M, Ahmed AT, Farah W, Asi N, et al: **Treatment of pediatric obesity: An umbrella systematic review.** *J Clin Endocrinol Metab* 2017, **102:**763-775.

217. Wilfley DE, Tibbs TL, Van Buren DJ, Reach KP, Walker MS, Epstein LH: **Lifestyle interventions in the treatment of childhood overweight: A meta-analytic review of randomized controlled trials.** *Health Psychol* 2007, **26:**521-532.

218. Janicke DM, Steele RG, Gayes LA, Lim CS, Clifford LM, Schneider EM, et al: **Systematic review and meta-analysis of comprehensive behavioral family lifestyle interventions addressing pediatric obesity.** *J Pediatr Psychol* 2014, **39:**809-825.

219. Oude Luttikhuis H, Baur L, Jansen H, Shrewsbury VA, O'Malley C, Stolk RP, et al: **Interventions for treating obesity in children.** *Cochrane Database Syst Rev* 2009**:**CD001872.

220. Foster BA, Farragher J, Parker P, Sosa ET: **Treatment interventions for early childhood obesity: A systematic review.** *Acad Pediatr* 2015, **15:**353-361.

221. McGovern L, Johnson JN, Paulo R, Hettinger A, Singhal V, Kamath C, et al: **Clinical review: Treatment of pediatric obesity: A systematic review and meta-analysis of randomized trials.** *J Clin Endocrinol Metab* 2008, **93:**4600-4605.

222. Goldschmidt AB, Best JR, Stein RI, Saelens BE, Epstein LH, Wilfley DE: **Predictors of child weight loss and maintenance among family-based treatment completers.** *J Consult Clin Psychol* 2014, **82:**1140-1150.

223. Braet C: **Patient characteristics as predictors of weight loss after an obesity treatment for children.** *Obesity (Silver Spring)* 2006, **14:**148-155.

224. Epstein LH, Koeske R, Wing RR, Valoski A: **The effect of family variables on child weight change.** *Health Psychol* 1986, **5:**1-11.

225. Epstein LH, Wisniewski L, Weng R: **Child and parent psychological problems influence child weight control.** *Obes Res* 1994, **2:**509-515.

226. Dougkas A, Reynolds CK, Givens ID, Elwood PC, Minihane AM: **Associations between dairy consumption and body weight: A review of the evidence and underlying mechanisms.** *Nutr Res Rev* 2011, **24:**72-95.

227. Kelishadi R, Zemel MB, Hashemipour M, Hosseini M, Mohammadifard N, Poursafa P: **Can a dairy-rich diet be effective in long-term weight control of young children?** *J Am Coll Nutr* 2009, **28:**601-610.

228. St-Onge MP, Goree LL, Gower B: **High-milk supplementation with healthy diet counseling does not affect weight loss but ameliorates insulin action compared with low-milk supplementation in overweight children.** *J Nutr* 2009, **139:**933-938.

229. Albala C, Ebbeling CB, Cifuentes M, Lera L, Bustos N, Ludwig DS: **Effects of replacing the habitual consumption of sugar-sweetened beverages with milk in Chilean children.** *Am J Clin Nutr* 2008, **88:**605-611.

230. Ghayour-Mobarhan M, Sahebkar A, Vakili R, Safarian M, Nematy M, Lotfian E, et al: **Investigation of the effect of high dairy diet on body mass index and body fat in overweight and obese children.** *Indian J Pediatr* 2009, **76:**1145-1150.

231. Hendrie GA, Golley RK: **Changing from regular-fat to low-fat dairy foods reduces saturated fat intake but not energy intake in 4-13-y-old children.** *Am J Clin Nutr* 2011, **93:**1117-1127.

232. Livingstone MB, Robson PJ, Wallace JM: **Issues in dietary intake assessment of children and adolescents.** *Br J Nutr* 2004, **92 Suppl 2:**S213-222.

233. Mansson HL: **Fatty acids in bovine milk fat.** *Food Nutr Res* 2008, **52**.

234. Djoussé L: **Is plasma pentadecanoic acid a reasonable biomarker of dairy consumption?** *J Am Heart Assoc* 2013, **2:**e000393.

235. Wolk A, Vessby B, Ljung H, Barrefors P: **Evaluation of a biological marker of dairy fat intake.** *Am J Clin Nutr* 1998, **68:**291-295.

236. Wolk A, Furuheim M, Vessby B: **Fatty acid composition of adipose tissue and serum lipids are valid biological markers of dairy fat intake in men.** *J Nutr* 2001, **131:**828-833.

237. Aragona J, Cassady J, Drabman RS: **Treating overweight children through parental training and contingency contracting.** *J Appl Behav Anal* 1975, **8:**269-278.

238. Epstein LH, Wing RR, Koeske R, Valoski A: **Effects of diet plus exercise on weight change in parents and children.** *J Consult Clin Psychol* 1984, **52:**429-437.

239. Janicke DM, Sallinen BJ, Perri MG, Lutes LD, Huerta M, Silverstein JH, et al: **Comparison of parent-only vs family-based interventions for overweight children in underserved rural settings: Outcomes from project STORY.** *Arch Pediatr Adolesc Med* 2008, **162:**1119-1125.

240. Jiang JX, Xia XL, Greiner T, Lian GL, Rosenqvist U: **A two year family based behaviour treatment for obese children.** *Arch Dis Child* 2005, **90:**1235-1238.

241. Kirschenbaum DS, Harris ES, Tomarken AJ: **Effects of parental involvement in behavioral weight loss therapy for preadolescents.** *Behavior Therapy* 1984, **15:**485-500.

242. Rodearmel SJ, Wyatt HR, Barry MJ, Dong F, Pan D, Israel RG, et al: **A family-based approach to preventing excessive weight gain.** *Obesity (Silver Spring)* 2006, **14:**1392-1401.

243. Wheeler ME, Hess KW: **Treatment of juvenile obesity by successive approximation control of eating.** *J Behav Ther Exp Psychiatry* 1976, **7:**235-241.

244. White MA, Martin PD, Newton RL, Walden HM, York-Crowe EE, Gordon ST, et al: **Mediators of weight loss in a family-based intervention presented over the internet.** *Obes Res* 2004, **12:**1050-1059.

245. Baker S, Barlow S, Cochran W, Fuchs G, Klish W, Krebs N, et al: **Overweight children and adolescents: A clinical report of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition.** *J Pediatr Gastroenterol Nutr* 2005, **40:**533-543.

246. Jolliffe CJ, Janssen I: **Vascular risks and management of obesity in children and adolescents.** *Vasc Health Risk Manag* 2006, **2:**171-187.

247. Griffiths LJ, Parsons TJ, Hill AJ: **Self-esteem and quality of life in obese children and adolescents: A systematic review.** *Int J Pediatr Obes* 2010, **5:**282-304.

248. Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM: **Overweight and obese children have low bone mass and area for their weight.** *Int J Obes Relat Metab Disord* 2000, **24:**627-632.

249. Ducher G: **Overweight children have poor bone strength relative to body weight, placing them at greater risk for forearm fractures.** *J Sports Sci Med* 2011**:**S6-S6.

250. Davidson PL, Goulding A, Chalmers DJ: **Biomechanical analysis of arm fracture in obese boys.** *J Paediatr Child Health* 2003, **39:**657-664.

251. Tremblay MS, Warburton D, Janssen I I, Paterson D, Latimer A, Rhodes R, et al: **New Canadian physical activity guidelines.** *Appl Physiol Nutr Metab* 2011, **36:**36-46.

252. Colley R, Garriguet D, Janssen I, Ian C, Cora L, Clarke J, et al: **Physical activity of Canadian children and youth: Accelerometer results from the 2007 to 2009 Canadian Health Measures Survey.** *Health Reports* 2011, **22:**15-23.

253. Van Loan M: **The role of dairy foods and dietary calcium in weight management.** *J Am Coll Nutr* 2009, **28 Suppl 1:**120S-129S.

254. Latzer Y, Edmunds L, Fenig S, Golan M, Gur E, Hochberg Z, et al: **Managing childhood overweight: Behavior, family, pharmacology, and bariatric surgery interventions.** *Obesity* 2009, **17:**411-423.

255. Mason HN, Crabtree V, Caudill P, Topp R: **Childhood obesity: A transtheoretical case management approach.** *J Pediatr Nurs* 2008, **23:**337-344.

256. Stewart L, Chapple J, Hughes AR, Poustie V, Reilly JJ: **The use of behavioural change techniques in the treatment of paediatric obesity: Qualitative evaluation of parental perspectives on treatment.** *J Hum Nutr Diet* 2008, **21:**464-473.

257. Kitzmann K, Beech B: **Family-based interventions for pediatric obesity: Methodological and conceptual challenges from family psychology.** *J Fam Psychol* 2006, **20:**175-189.

258. Towey M, Harrell R, Lee B: **Evaluation of "one body, one life": A community-based family intervention for the prevention of obesity in children.** *J Obes* 2011, **2011:**619-643.

259. Stice E, Shaw H, Marti CN: **A meta-analytic review of obesity prevention programs for children and adolescents: The skinny on interventions that work.** *Psychol Bull* 2006, **132:**667-691.

260. Waters E, de Silva-Sanigorski A, Hall B. J., Brown T, Campbell K. J., Gao Y, et al: **Interventions for preventing obesity in children.** *Cochrane Database Syst Rev* 2011, **12:**CD001871.

261. Lazar-Antman MA, Leet AI: **Effects of obesity on pediatric fracture care and management.** *J Bone Joint Surg Am* 2012, **94:**855-861.

262. Schoenau E, Neu CM, Mokov E, Wassmer G, F M: **Influence of puberty on muscle area and cortical bone area of the forearm in boys and girls.** *J Clin Endocrinol Metab* 2000, **85:**1095-1098.

263. Ducher G, Bass SL, Naughton G, Eser P, Telford RD, Daly RM: **Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: Implications for bone strength at the distal forearm.** *Am J Clin Nutr* 2009, **90:**1104-1111.

264. Schoenau E, Fricke O: **Mechanical influences on bone development in children.** *Eur J Endocrinol* 2008, **159:**S27-S31.

265. Alemzadeh R, Kichler J, Babar G, Calhoun M: **Hypovitaminosis D in obese children and adolescents: Relationship with adiposity, insulin sensitivity, ethnicity, and season.** *Metabolism* 2008, **57:**183-191.

266. Smotkin-Tangorra M, Purushothaman R, Gupta A, Nejati G, Anhalt H, Ten S: **Prevalence of vitamin D insufficiency in obese children and adolescents.** *J Pediatr Endocrinol Metab* 2007, **20:**817-823.

267. Olson ML, Maalouf NM, JD O, White PC, Hutchison MR: **Vitamin D deficiency in obese children and its relationship to glucose homeostasis.** *J Clin Endocrinol Metab* 2012, **97:**279-285.

268. Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML: **Prevalence and ssociations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001–2004.** *Pediatrics* 2009, **124:**e362-e370.

269. Chan GM, Hoffman K, McMurry M: **Effects of dairy products on bone and body composition in pubertal girls.** *J Pediatr* 1995, **126:**551-556.

270. Meisels SJ, Plunkett JW, Roloff DW, Pasick PL, Stiefel GS: **Growth and development of preterm infants with respiratory distress syndrome and bronchopulmonary dysplasia.** *Pediatrics* 1986, **77:**345-352.

271. Nowson CA, Green RM, Hopper JL, Sherwin AJ, Young D, Kaymakci B, et al: **A co-twin study of the effect of calcium supplementation on bone density during adolescence.** *Osteoporos Int* 1997, **7:**219-225.

272. Johnston CC, Jr., Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, et al: **Calcium supplementation and increases in bone mineral density in children.** *N Engl J Med* 1992, **327:**82-87.

273. Slemenda CW, Peacock M, Hui S, Zhou L, Johnston CC: **Reduced rates of skeletal remodeling are associated with increased bone mineral density during the development of peak skeletal mass.** *J Bone Miner Res* 1997, **12:**676-682.

274. Lee WT, Leung SS, Leung DM, Wang SH, Xu YC, Zeng WP, et al: **Bone mineral acquisition in low calcium intake children following the withdrawal of calcium supplement.** *Acta Paediatr* 1997, **86:**570-576.

275. Vatanparast H, Whiting SJ: **Calcium supplementation trials and bone mass development in children, adolescents, and young adults.** *Nutr Rev* 2006, **64:**204-209.

276. French SA, Fulkerson JA, Story M: **Increasing weight-bearing physical activity and calcium intake for bone mass growth in children and adolescents: A review of intervention trials.** *Prev Med* 2000, **31:**722-731.

277. Volek JS, Gomez AL, Scheett TP, Sharman MJ, French DN, Rubin MR, et al: **Increasing fluid milk favorably affects bone mineral density responses to resistance training in adolescent boys.** *J Am Diet Assoc* 2003, **103:**1353-1356.

278. Tremblay A, Gilbert J: **Human obesity: Is insufficient calcium/dairy intake part of the problem?** *J Am Coll Nutr* 2011, **30:**449S-453S.

279. Louie JC, Flood VM, Hector DJ, Rangan AM, Gill TP: **Dairy consumption and overweight and obesity: A systematic review of prospective cohort studies.** *Obes Rev* 2011, **12:**e582-592.

280. Berkey CS, Rockett HRH, Willett WC, Colditz GA: **Milk, dairy fat, dietary calcium, and weight gain: A longitudinal study of adolescents.** *Arch Pediatr Adolesc Med* 2005, **159:**543-550.

281. Barr SI: **Increased dairy product or calcium intake: Is body weight or composition affected in humans?** *J Nutr* 2003, **133:**245S-248S.

282. American Academy of Orthopaedic Surgeons: **Position statement: Children and musculoskeletal health.** 2011, American Academy of Orthopaedic Surgeons; Rosemont, Ill., USA. [https://www.aaos.org/uploadedFiles/PreProduction/About/Opinion_Statements/position/1170%20Children%20and%20Musculoskeletal%20Health.pdf]. *Accessed*: August 3, 2017.

283. Schulz K, Altman D, Moher D, The CONSORT Group: **CONSORT 2010 Statement: Updated guidelines for reporting parallel group randomised trials.** *BMC Medicine* 2010, **8:**18.

284. Lau DC, Douketis JD, Morrison KM, Hramiak IM, Sharma AM, Ur E: **2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [summary].** *CMAJ* 2007, **176:**S1-13.

285. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: **Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.** *Diabetologia* 1985, **28:**412-419.

286. Keskin M, Kurtoglu S, Kendirci M, Atabek ME, Yazici C: **Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents.** *Pediatrics* 2005, **115:**e500-503.

287. Rudolf MCJ, Walker J, Cole TJ: **What is the best way to measure waist circumference?** *Int J Pediatr Obes* 2007, **2:**58-61.

288. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J: **Development of a WHO growth reference for school-aged children and adolescents.** *Bull World Health Organ* 2007, **85:**660-667.

289. Marshall WA, Tanner JM: **Variations in pattern of pubertal changes in girls.** *Arch Dis Child* 1969, **44:**291-303.

290. Marshall WA, Tanner JM: **Variations in the pattern of pubertal changes in boys.** *Arch Dis Child* 1970, **45:**13-23.

291. Gordon CM, Bachrach LK, Carpenter TO, Crabtree N, El-Hajj Fuleihan G, Kutilek S, et al: **Dual energy x-ray absorptiometry interpretation and reporting in children and adolescents: The 2007 ISCD Pediatric Official Positions.** *J Clin Densitom* 2008, **11:**43-58.

292. Sherman M, Fan B, Wang L, Winer K, J S: **Evaluation of the android/gynoid regions for hologic pediatric whole body scans.** *J Clin Densitom* 2009, **12:**385.

293. Goulding A, Taylor RW, Jones IE, Manning PJ, Williams SM: **Spinal overload: A concern for obese children and adolescents?** *Osteoporos Int* 2002, **13:**835-840.

294. Goulding A, Jones IE, Taylor RW, Piggot JM, Taylor D: **Dynamic and static tests of balance and postural sway in boys: Effects of previous wrist bone fractures and high adiposity.** *Gait Posture* 2003, **17:**136-141.

295. Zemel B, Bass S, Binkley T, Ducher G, Macdonald H, McKay H, et al: **Peripheral quantitative computed tomography in children and adolescents: The 2007 ISCD Pediatric Official Positions.** *J Clin Densitom* 2008, **11:**59-74.

296. Braun MJ, Meta MD, Schneider P, Reiners C: **Clinical evaluation of a high-resolution new peripheral quantitative computerized tomography (pQCT) scanner for the bone densitometry at the lower limbs.** *Phys Med Biol* 1998, **43:**2279-2294.

297. Iuliano-Burns S, Saxon L, Naughton G, Gibbons K, Bass SL: **Regional specificity of exercise and calcium during skeletal growth in girls: A randomized controlled trial.** *J Bone Miner Res* 2003, **18:**156-162.

298. Carson V, Kuhle S, Spence JC, Veugelers PJ: **Parents' perception of neighbourhood environment as a determinant of screen time, physical activity and active transport.** *Can J Public Health* 2010, **101:**124-127.

299. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al: **International Physical Activity Questionnaire: 12-country reliability and validity.** *Med Sci Sports Exerc* 2003, **35:**1381-1395.

300. Etelson D, Brand DA, Patrick PA, Shirali A: **Childhood obesity: Do parents recognize this health risk?** *Obes Res* 2003, **11:**1362-1368.

301. Parmenter K, Wardle J: **Development of a general nutrition knowledge questionnaire for adults.** *Eur J Clin Nutr* 1999, **53:**298-308.

302. Rhodes RE, Naylor PJ, McKay HA: **Pilot study of a family physical activity planning intervention among parents and their children.** *J Behav Med* 2010, **33:**91-100.

303. Crocker PR, Bailey DA, Faulkner RA, Kowalski KC, McGrath R: **Measuring general levels of physical activity: Preliminary evidence for the Physical Activity Questionnaire for Older Children.** *Med Sci Sports Exerc* 1997, **29:**1344-1349.

304. Canadian Fitness and Lifestyle Research Institute: **Canada Fitness Survey.** 1981, Ottawa, Ontario, Canada *Accessed*:

305. Reinehr T, de Sousa G, Andler W: **Longitudinal analyses among overweight, insulin resistance, and cardiovascular risk factors in children.** *Obes Res* 2005, **13:**1824-1833.

306. Gozdzik A, Barta JL, Wu H, Wagner D, Cole DE, Vieth R, et al: **Low wintertime vitamin D levels in a sample of healthy young adults of diverse ancestry living in the Toronto area: Associations with vitamin D intake and skin pigmentation.** *BMC Public Health* 2008, **8:**336.

307. Shriver MD, Parra EJ: **Comparison of narrow-band reflectance spectroscopy and tristimulus colorimetry for measurements of skin and hair color in persons of different biological ancestry.** *Am J Phys Anthropol* 2000, **112:**17-27.

308. Fullerton A, Fischer T, Lahti A, Wilhelm KP, Takiwaki H, Serup J: **Guidelines for measurement of skin colour and erythema. A report from the Standardization Group of the European Society of Contact Dermatitis.** *Contact Dermatitis* 1996, **35:**1-10.

309. Chardon A, Cretois I, Hourseau C: **Skin colour typology and suntanning pathways.** *Int J Cosmet Sci* 1991, **13:**191-208.

310. Del Bino S, Sok J, Bessac E, Bernerd F: **Relationship between skin response to ultraviolet exposure and skin color type.** *Pigment Cell Res* 2006, **19:**606-614.

311. Reeder AI, Hammond VA, Gray AR: **Questionnaire items to assess skin color and erythemal sensitivity: Reliability, validity, and "the dark shift".** *Cancer Epidemiol Biomarkers Prev* 2010, **19:**1167-1173.

312. Sleddens E, Kremers S, Thijs C: **The Children's Eating Behaviour Questionnaire: Factorial validity and association with body mass index in Dutch children aged 6-7.** *Int J Behav Nutr Phys Act* 2008, **5:**49.

313. Carnell S, Wardle J: **Measuring behavioural susceptibility to obesity: Validation of the child eating behaviour questionnaire.** *Appetite* 2007, **48:**104-113.

314. Ajzen I: **The theory of planned behavior.** *Organ Behav Hum Decis Process* 1991, **50:**179-211.

315. Ajzen I: **Perceived behavioral control, self-efficacy, locus of control, and the theory of planned behavior.** *J Appl Soc Psychol* 2002, **32:**665-683.

316. Rhodes RE, Macdonald HM, McKay HA: **Predicting physical activity intention and behaviour among children in a longitudinal sample.** *Soc Sci Med* 2006, **62:**3146-3156.

317. Araujo-Soares V, McIntyre T, Sniehotta FF: **Predicting changes in physical activity among adolescents: The role of self-efficacy, intention, action planning and coping planning.** *Health Educ Res* 2009, **24:**128-139.

318. Ross MM, Kolbash S, Cohen GM, Skelton JA: **Multidisciplinary treatment of pediatric obesity: Nutrition evaluation and management.** *Nutr Clin Pract* 2010, **25:**327-334.

319. Yelling M, Lamb K, Swaine I: **Validity of a pictorial perceived exertion scale for effort estimation and effort production during stepping exercise in adolescent children.** *Eur Phys Educ Rev* 2002, **8:**157-175.

320. Belanger-Gravel A, Godin G, Vezina-Im LA, Amireault S, Poirier P: **The effect of theory-based interventions on physical activity participation among overweight/obese individuals: A systematic review.** *Obes Rev* 2011, **12:**430-439.

321. Reinehr T, Schaefer A, Winkel K, Finne E, Toschke AM, Kolip P: **An effective lifestyle intervention in overweight children: Findings from a randomized controlled trial on "Obeldicks light".** *Clin Nutr* 2010, **29:**331-336.

322. Resnicow K, Davis R, Rollnick S: **Motivational interviewing for pediatric obesity: Conceptual issues and evidence review.** *J Am Diet Assoc* 2006, **106:**2024-2033.

323. Shah W, Cannon C: **Craving Change Inc.** 2012 Calgary, Alberta, Canada [http://www.cravingchange.ca/]. *Accessed*: August 9 2017.

324. Katch VL: **Physical conditioning of children.** *J Adolesc Health Care* 1983, **3:**241-246.

325. Epstein LH, Paluch RA, Roemmich JN, Beecher MD: **Family-based obesity treatment, then and now: Twenty-five years of pediatric obesity treatment.** *Health Psychol* 2007, **26:**381-391.

326. Story MT, Neumark-Stzainer DR, Sherwood NE, Holt K, Sofka D, Trowbridge FL, et al: **Management of child and adolescent obesity: Attitudes, barriers, skills, and training needs among health care professionals.** *Pediatrics* 2002, **110:**210-214.

327. Morrissette PJ, Taylor D: **Family Counseling and Childhood Obesity: A Review of Approaches.** *Family J* 2002, **10:**19-26.

328. Barlow SE, Dietz WH: **Management of child and adolescent obesity: Summary and recommendations based on reports from pediatricians, pediatric nurse practitioners, and registered dietitians.** *Pediatrics* 2002, **110:**236-238.

329. Hirko KA, Kantor ED, Cohen SS, Blot WJ, Stampfer MJ, Signorello LB: **Body mass index in young adulthood, obesity trajectory, and premature mortality.** *Am J Epidemiol* 2015, **182:**441-450.

330. Canadian Task Force on Preventive Health Care, Parkin P, Connor Gorber S, Shaw E, Bell N, Jaramillo A, et al: **Recommendations for growth monitoring, and prevention and management of overweight and obesity in children and youth in primary care.** *CMAJ* 2015, **187:**411-421.

331. Cohen TR, Hazell TJ, Vanstone CA, Plourde H, Rodd CJ, Weiler HA: **A family-centered lifestyle intervention to improve body composition and bone mass in overweight and obese children 6 through 8 years: A randomized controlled trial study protocol.** *BMC Public Health* 2013, **13:**383.

332. Tanner JM: **Growth at Adolescence.** Second edn. Oxford, United Kingdom: Blackwell Scientific; 1962.

333. World Health Organization: **AnthroPlus for personal computers manual: software for assessing growth of the world's children and adolescents.** 2009, Geneva, Switzerland *Accessed*: June 15, 2017.

334. World Health Organization: **Obesity: Preventing and managing the global epidemic. Report of a WHO consultation.** 2000, Geneva, Switzerland [https://www.ncbi.nlm.nih.gov/pubmed/11234459]. *Accessed*: June 15, 2017.

335. Kelly T: **Practical and technical advantages of DXA visceral fat assessment compared with computed tomography.** 2012, Hologic Inc., Bedford, Massachusetts, USA [http://www.hologic.com/sites/default/files/white-papers/WP_00062_Visceral_Fat_06-12.pdf]. *Accessed*: August 9 2017.

336. Golley RK, Hendrie GA: **Evaluation of the relative concentration of serum fatty acids C14:0, C15:0 and C17:0 as markers of children's dairy fat intake.** *Ann Nutr Metab* 2014, **65:**310-316.

337. Lepage G, Roy CC: **Direct transesterification of all classes of lipids in a one-step reaction.** *J Lipid Res* 1986, **27:**114-120.

338. Hughes AR, Stewart L, Chapple J, McColl JH, Donaldson MD, Kelnar CJ, et al: **Randomized, controlled trial of a best-practice individualized behavioral program for treatment of childhood overweight: Scottish Childhood Overweight Treatment Trial (SCOTT).** *Pediatrics* 2008, **121:**e539-546.

339. Lichtman SW, Pisarska K, Berman ER, Pestone M, Dowling H, Offenbacher E, et al: **Discrepancy between self-reported and actual caloric intake and exercise in obese subjects.** *N Engl J Med* 1992, **327:**1893-1898.

340. Ellery CV, Weiler HA, Hazell TJ: **Physical activity assessment tools for use in overweight and obese children.** *Int J Obes (Lond)* 2014, **38:**1-10.

341. Julian-Almarcegui C, Gomez-Cabello A, Huybrechts I, Gonzalez-Aguero A, Kaufman JM, Casajus JA, et al: **Combined effects of interaction between physical activity and nutrition on bone health in children and adolescents: A systematic review.** *Nutr Rev* 2015, **73:**127-139.

342. Goodman CA, Hornberger TA, Robling AG: **Bone and skeletal muscle: Key players in mechanotransduction and potential overlapping mechanisms.** *Bone* 2015, **80:**24-36.

343. Farr JN, Dimitri P: **The impact of fat and obesity on bone microarchitecture and strength in children.** *Calcif Tissue Int* 2016.

344. Smith SM, Sumar B, Dixon KA: **Musculoskeletal pain in overweight and obese children.** *Int J Obes* 2014, **38:**11-15.

345. Timpson NJ, Sayers A, Davey-Smith G, Tobias JH: **How does body fat influence bone mass in childhood? A Mendelian randomization approach.** *J Bone Miner Res* 2009, **24:**522-533.

346. Rochefort GY, Rocher E, Aveline PC, Garnero P, Bab I, Chappard C, et al: **Osteocalcin-insulin relationship in obese children: A role for the skeleton in energy metabolism.** *Clin Endocrinol (Oxf)* 2011, **75:**265-270.

347. Cohen TR, Hazell TJ, Vanstone CA, Rodd C, Weiler HA: **A family-centered lifestyle intervention for obese six- to eight-year-old children: Results from a one-year randomized controlled trial conducted in Montreal, Canada.** *Can J Public Health* 2016, **107:**e453-e460.

348. Carter DR, Bouxsein ML, Marcus R: **New approaches for interpreting projected bone densitometry data.** *J Bone Miner Res* 1992, **7:**137-145.

349. Bass S, Delmas PD, Pearce G, Hendrich E, Tabensky A, Seeman E: **The differing tempo of growth in bone size, mass, and density in girls is region-specific.** *J Clin Invest* 1999, **104:**795-804.

350. Statistics Canada: **Directly measured physical activity of children and youth, 2012 and 2013.** 2015, Government of Canada: Ottawa, Ontario, Canada [http://www.statcan.gc.ca/pub/82-625-x/2015001/article/14136-eng.htm]. *Accessed*: July 7, 2017.

351. Abseyi N, Şıklar Z, Berberoğlu M, Hacıhamdioğlu B, Savaş Erdeve Ş, Öçal G: **Relationships between osteocalcin, glucose metabolism, and adiponectin in obese children: Is there crosstalk between bone tissue and glucose metabolism?** *J Clin Res Pediatr Endocrinol* 2012, **4:**182-188.

352. Calvo MS, Eyre DR, Gundberg CM: **Molecular basis and clinical application of biological markers of bone turnover.** *Endocr Rev* 1996, **17:**333-368.

353. Wang JW, Tang QY, Ruan HJ, Cai W: **Relation between serum osteocalcin levels and body composition in obese children.** *J Pediatr Gastroenterol Nutr* 2014, **58:**729-732.

354. Burrows T, Golley RK, Khambalia A, McNaughton SA, Magarey A, Rosenkranz RR, et al: **The quality of dietary intake methodology and reporting in child and adolescent obesity intervention trials: A systematic review.** *Obes Rev* 2012, **13:**1125-1138.

355. McCarthy EK, Chaoimh C, Murray DM, Hourihane JO, Kenny LC, Kiely M: **Eating behaviour and weight status at 2 years of age: Data from the Cork BASELINE Birth Cohort Study.** *Eur J Clin Nutr* 2015, **69:**1356-1359.

356. Loh DA, Moy FM, Zaharan NL, Mohamed Z: **Eating behaviour among multi-ethnic adolescents in a middle-income country as measured by the self-reported Children's Eating Behaviour Questionnaire.** *PLoS One* 2013, **8:**e82885.

357. Harrold JA, Halford JCG: **Appetite: Physiological and Neurobiological Aspects A2 - Caballero, Benjamin.** In *Encyclopedia of Human Nutrition (Third Edition).* Waltham: Academic Press; 2013: 100-107

358. Gutin B, Ramsey L, Barbeau P, Cannady W, Ferguson M, Litaker M, et al: **Plasma leptin concentrations in obese children: Changes during 4-mo periods with and without physical training.** *Am J Clin Nutr* 1999, **69:**388-394.

359. Siegrist M, Rank M, Wolfarth B, Langhof H, Haller B, Koenig W, et al: **Leptin, adiponectin, and short-term and long-term weight loss after a lifestyle intervention in obese children.** *Nutrition* 2013, **29:**851-857.

360. Moore LL, Bradlee ML, Gao D, Singer MR: **Low dairy intake in early childhood predicts excess body fat gain.** *Obesity (Silver Spring)* 2006, **14:**1010-1018.

361. Canadian Society for Exericse Physiology.: **Canadian physical activity guidelines for children 5-11.** 2015, Ottawa, ON.

362. United States Department of Agriculture.: **The 2015-2020 Dietary Guidelines for Americans.** Center for Nutrition Policy and Promotion.; Alexandria, VA , United States [https://health.gov/dietaryguidelines/2015/guidelines/]. *Accessed*: July 15, 2017.

363. de Boer MR, Waterlander WE, Kuijper LD, Steenhuis IH, Twisk JW: **Testing for baseline differences in randomized controlled trials: An unhealthy research behavior that is hard to eradicate.** *Int J Behav Nutr Phys Act* 2015, **12:**4.

364. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, et al: **CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials.** *BMJ*, **63:**e1-e37.

365. Peterson RL, Tran M, Koffel J, Stovitz SD: **Statistical testing of baseline differences in sports medicine RCTs: a systematic evaluation.** *BMJ Open Sport Exerc Med* 2017, **3**.

366. Sanchez U, Weisstaub G, Santos JL, Corvalan C, Uauy R: **GOCS cohort: Children's eating behavior scores and BMI.** *Eur J Clin Nutr* 2016, **70:**925-928.

367. Murer SB, Knopfli BH, Aeberli I, Jung A, Wildhaber J, Wildhaber-Brooks J, et al: **Baseline leptin and leptin reduction predict improvements in metabolic variables and long-term fat loss in obese children and adolescents: A prospective study of an inpatient weight-loss program.** *Am J Clin Nutr* 2011, **93:**695-702.

368. Reinehr T, Kleber M, de Sousa G, Andler W: **Leptin concentrations are a predictor of overweight reduction in a lifestyle intervention.** *Int J Pediatr Obes* 2009, **4:**215-223.

369. Cambuli VM, Musiu MC, Incani M, Paderi M, Serpe R, Marras V, et al: **Assessment of adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle intervention in overweight and obese children.** *J Clin Endocrinol Metab* 2008, **93:**3051-3057.

370. Onvani S, Haghighatdoost F, Surkan PJ, Azadbakht L: **Dairy products, satiety and food intake: A meta-analysis of clinical trials.** *Clinical Nutrition* 2017, **36:**389-398.

371. Larnkjaer A, Arnberg K, Michaelsen KF, Jensen SM, Molgaard C: **Effect of increased intake of skimmed milk, casein, whey or water on body composition and leptin in overweight adolescents: A randomized trial.** *Pediatric Obesity* 2015, **10:**461-467.

372. Magarey A, Watson J, Golley RK, Burrows T, Sutherland R, McNaughton SA, et al: **Assessing dietary intake in children and adolescents: Considerations and recommendations for obesity research.** *Int J Pediatr Obes* 2011, **6:**2-11.

373. Garg MK, Marwaha RK, Mahalle N, Tandon N: **Relationship of lean mass and obesity in Indian urban children and adolescents.** *Indian J Endocrinol Metab* 2016, **20:**779-783.

374. Major GC, Chaput JP, Ledoux M, St-Pierre S, Anderson GH, Zemel MB, et al: **Recent developments in calcium-related obesity research.** *Obes Rev* 2008, **9:**428-445.

375. Ho M, Garnett SP, Baur LA, Burrows T, Stewart L, Neve M, et al: **Impact of dietary and exercise interventions on weight change and metabolic outcomes in obese children and adolescents: A systematic review and meta-analysis of randomized trials.** *JAMA Pediatr* 2013, **167:**759-768.

376. Baptista F, Barrigas C, Vieira F, Santa-Clara H, Homens PM, Fragoso I, et al: **The role of lean body mass and physical activity in bone health in children.** *J Bone Miner Metab* 2012, **30:**100-108.

377. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC: **Regulation of adiposity by dietary calcium.** *FASEB J* 2000, **14:**1132-1138.

378. Shi H, Dirienzo D, Zemel MB: **Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice.** *FASEB J* 2001, **15:**291-293.

379. Zemel MB: **Role of calcium and dairy products in energy partitioning and weight management.** *Am J Clin Nutr* 2004, **79:**907S-912S.

380. Xue B, Greenberg AG, Kraemer FB, Zemel MB: **Mechanism of intracellular calcium ([Ca2+]i) inhibition of lipolysis in human adipocytes.** *FASEB J* 2001, **15:**2527-2529.

381. Cummings NK, James AP, Soares MJ: **The acute effects of different sources of dietary calcium on postprandial energy metabolism.** *Br J Nutr* 2006, **96:**138-144.

382. Gunther CW, Lyle RM, Legowski PA, James JM, McCabe LD, McCabe GP, et al: **Fat oxidation and its relation to serum parathyroid hormone in young women enrolled in a 1-y dairy calcium intervention.** *Am J Clin Nutr* 2005, **82:**1228-1234.

383. Teegarden D: **The influence of dairy product consumption on body composition.** *J Nutr* 2005, **135:**2749-2752.

384. Lund-Blix NA, Rønningen KS, Bøås H, Tapia G, Andersen LF: **Plasma phospholipid pentadecanoic acid, EPA, and DHA, and the frequency of dairy and fish product intake in young children.** *Food Nutr Res* 2016, **60:**10.3402.

385. Lu AS, Baranowski J, Islam N, Baranowski T: **How to engage children in self-administered dietary assessment programmes.** *J Hum Nutr Diet* 2014, **27:**5-9.

386. Zheng H, Clausen MR, Dalsgaard TK, Bertram HC: **Metabolomics to explore impact of dairy intake.** *Nutrients* 2015, **7:**4875-4896.

387. Bertram HC, Hoppe C, Petersen BO, Duus JO, Molgaard C, Michaelsen KF: **An NMR-based metabonomic investigation on effects of milk and meat protein diets given to 8-year-old boys.** *Br J Nutr* 2007, **97:**758-763.

388. Schoenau E: **From mechanostat theory to development of the "Functional Muscle-Bone-Unit".** *J Musculoskelet Neuronal Interact* 2005, **5:**232-238.

389. Shapses SA, Sukumar D: **Bone metabolism in obesity and weight loss.** *Annu Rev Nutr* 2012, **32:**287-309.

390. Gilsanz V, Chalfant J, Mo AO, Lee DC, Dorey FJ, Mittelman SD: **Reciprocal relations of subcutaneous and visceral fat to bone structure and strength.** *J Clin Endocrinol Metab* 2009, **94:**3387-3393.

391. Karlsson AK, Kullberg J, Stokland E, Allvin K, Gronowitz E, Svensson PA, et al: **Measurements of total and regional body composition in preschool children: A comparison of MRI, DXA, and anthropometric data.** *Obesity (Silver Spring)* 2013, **21:**1018-1024.

392. Utsal L, Tillmann V, Zilmer M, Maestu J, Purge P, Jurimae J, et al: **Elevated serum IL-6, IL-8, MCP-1, CRP, and IFN-gamma levels in 10- to 11-year-old boys with increased BMI.** *Horm Res Paediatr* 2012, **78:**31-39.

393. do Prado WL, de Piano A, Lazaretti-Castro M, de Mello MT, Stella SG, Tufik S, et al: **Relationship between bone mineral density, leptin and insulin concentration in Brazilian obese adolescents.** *J Bone Miner Metab* 2009, **27:**613-619.

394. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL: **Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes.** *Endocrinology* 1999, **140:**1630-1638.

395. Radetti G, Franceschi R, Adami S, Longhi S, Rossini M, Gatti D: **Higher circulating parathormone is associated with smaller and weaker bones in obese children.** *Calcif Tissue Int* 2014, **95:**1-7.

396. De Leonibus C, Marcovecchio ML, Chiavaroli V, de Giorgis T, Chiarelli F, Mohn A: **Timing of puberty and physical growth in obese children: A longitudinal study in boys and girls.** *Pediatr Obes* 2014, **9:**292-299.

397. Leonibus CD, Marcovecchio ML, Chiarelli F: **Update on statural growth and pubertal development in obese children.** *Pediatr Rep* 2012, **4:**e35.

398. Nagasaki K, Kikuchi T, Hiura M, Uchiyama M: **Obese Japanese children have low bone mineral density after puberty.** *J Bone Miner Metab* 2004, **22:**376-381.

399. Burt Solorzano CM, McCartney CR: **Obesity and the pubertal transition in girls and boys.** *Reproduction* 2010, **140:**399-410.

400. Kaplowitz PB: **Link between body fat and the timing of puberty.** *Pediatrics* 2008, **121 Suppl 3:**S208-217.

401. Martos-Moreno GÁ, Chowen JA, Argente J: **Metabolic signals in human puberty: Effects of over and undernutrition.** *Mol Cell Endocrinol* 2010, **324:**70-81.

402. Birch LL, McPhee L, Shoba BC, Steinberg L, Krehbiel R: **"Clean up your plate": Effects of child feeding practices on the conditioning of meal size.** *Learn Motiv* 1987, **18:**301-317.

403. Johnson SL, Birch LL: **Parents' and children's adiposity and eating style.** *Pediatrics* 1994, **94:**653-661.

404. Celi F, Bini V, Papi F, Contessa G, Santilli E, Falorni A: **Leptin serum levels are involved in the relapse after weight excess reduction in obese children and adolescents.** *Diabetes Nutr Metab* 2003, **16:**306-311.

405. Reinehr T, Kratzsch J, Kiess W, Andler W: **Circulating soluble leptin receptor, leptin, and insulin resistance before and after weight loss in obese children.** *Int J Obes (Lond)* 2005, **29:**1230-1235.

406. Reinehr T, de Sousa G, Roth CL: **Fasting glucagon-like peptide-1 and its relation to insulin in obese children before and after weight loss.** *J Pediatr Gastroenterol Nutr* 2007, **44:**608-612.

407. Gueugnon C, Mougin F, Nguyen NU, Bouhaddi M, Nicolet-Guenat M, Dumoulin G: **Ghrelin and PYY levels in adolescents with severe obesity: Effects of weight loss induced by long-term exercise training and modified food habits.** *Eur J Appl Physiol* 2012, **112:**1797-1805.

408. Horner K, Lee S: **Appetite-related peptides in childhood and adolescence: Role of ghrelin, PYY, and GLP-1.** *Appl Physiol Nutr Metab* 2015, **40:**1089-1099.

409. Maximova K, McGrath JJ, Barnett T, O'Loughlin J, Paradis G, Lambert M: **Do you see what I see? Weight status misperception and exposure to obesity among children and adolescents.** *Int J Obes (Lond)* 2008, **32:**1008-1015.

410. Edmunds LD: **Parents' perceptions of health professionals' responses when seeking help for their overweight children.** *Fam Pract* 2005, **22:**287-292.

411. Doolen J, Alpert PT, Miller SK: **Parental disconnect between perceived and actual weight status of children: A metasynthesis of the current research.** *J Am Acad Nurse Pract* 2009, **21:**160-166.

412. Rhee KE, De Lago CW, Arscott-Mills T, Mehta SD, Davis RK: **Factors associated with parental readiness to make changes for overweight children.** *Pediatrics* 2005, **116:**e94-101.

413. Kelly TL, Wilson KE, Heymsfield SB: **Dual energy x-ray absorptiometry body composition reference values from NHANES.** *PLoS ONE* 2009, **4:**e7038.

414. Fassnacht DB, Ali K, Silva C, Goncalves S, Machado PP: **Use of text messaging services to promote health behaviors in children.** *J Nutr Educ Behav* 2015, **47:**75-80.

415. De Cock N, Vangeel J, Lachat C, Beullens K, Vervoort L, Goossens L, et al: **Use of fitness and nutrition apps: Associations with body mass index, snacking, and drinking habits in adolescents.** *JMIR Mhealth Uhealth* 2017, **5:**e58.

416. Antwi F, Fazylova N, Garcon MC, Lopez L, Rubiano R, Slyer JT: **The effectiveness of web-based programs on the reduction of childhood obesity in school-aged children: A systematic review.** *JBI Libr Syst Rev* 2012, **10:**1-14.

417. McGoey T, Root Z, Bruner MW, Law B: **Evaluation of physical activity interventions in children via the reach, efficacy/effectiveness, adoption, implementation, and maintenance (RE-AIM) framework: A systematic review of randomized and non-randomized trials.** *Prev Med* 2016, **82:**8-19.

418. Canadian Society for Exercise Science: **Guidelines Glossary.** 2016. Ottawa, Ontario, Canada [http://www.csep.ca/guidelines/glossary-2016]. *Accessed*: May 12 2017.

419. Gao Z, Chen S: **Are field-based exergames useful in preventing childhood obesity? A systematic review.** *Obes Rev* 2014, **15:**676-691.

420. Lamboglia CM, da Silva VT, de Vasconcelos Filho JE, Pinheiro MH, Munguba MC, Silva Junior FV, et al: **Exergaming as a strategic tool in the fight against childhood obesity: A systematic review.** *J Obes* 2013, **2013:**438364.

421. Christison A, Khan HA: **Exergaming for health: A community-based pediatric weight management program using active video gaming.** *Clin Pediatr (Phila)* 2012, **51:**382-388.

422. Johnsen K, Ahn SJ, Moore J, Brown S, Robertson TP, Marable A, et al: **Mixed reality virtual pets to reduce childhood obesity.** *IEEE Trans Vis Comput Graph* 2014, **20:**523-530.

423. Thompson FE, Subar AF, Loria CM, Reedy JL, Baranowski T: **Need for technological innovation in dietary assessment.** *J Am Diet Assoc* 2010, **110:**48-51.

424. Boushey CJ, Spoden M, Zhu FM, Delp EJ, Kerr DA: **New mobile methods for dietary assessment: Review of image-assisted and image-based dietary assessment methods.** *Proc Nutr Soc* 2016**:**1-12.

425. Medin AC, Astrup H, Kasin BM, Andersen LF: **Evaluation of a web-based food record for children using direct unobtrusive lunch observations: A validation study.** *J Med Internet Res* 2015, **17:**e273.

426. Pan-Canadian Public Health Network: **Towards a Healthier Canada - 2015 Progress Report on Advancing the Federal / Provincial / Territorial Framework on Healthy Weights.** 2016. [http://phn-rsp.ca/index-eng.php]. *Accessed*: June 8 2017.

Appendix 1: Permission to use Figure 2.1

From: Kirsten Davison
Sent: April 12, 2017 12:19 PM
To: Tamara Cohen
Subject: Re: FW: Permission to use figure from Publication

Hello Tamara,
Thank you for your email. You are welcome to use our figure. Best of luck with your dissertation.

Kind Regards,
Kirsten Davison

On Wed, Apr 12, 2017 at 11:43 AM, Tamara Cohen <tamara.cohen@mail.mcgill.ca> wrote:

> Hello Dr. Davison,
>
> **I am e-mailing you for your permission to use your Figure from a 2001 Obes Rev publication titled: *Childhood overweight: a contextual model and recommendations for future research* in my PhD dissertation. I would like to cite your terrific model of Ecological predictors of childhood obesity and include the figure in my discussion.**
>
> Thank you,
> Tamara
>
> **Tamara R. Cohen, RD, MSc, PhD (C)**
> Candidate au Doctorat- Doctoral Candidate
> Université de McGill- McGill University
> Unité de recherche en nutrition clinique Mary Emily -
> Mary Emily Clinical Nutrition Research Unit
> 7 avenue Maple - Maple avenue
> Ste. Anne de Bellevue, Quebec H9X 2E3

--
Kirsten Davison, PhD
Donald and Sue Pritzker Associate Professor of Nutrition
Harvard T.H. Chan School of Public Health
ph: 617-432-1898 (Chan); 617-998-6613 (Landmark Building)