Nutritional, hormonal and genetic factors in the development of overeating tendency toward food addiction

By

Pardis Pedram

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ABSTRACT

Overeating is believed to be the primary factor responsible for the increasing prevalence of human obesity. A proportion of people develops a chronic obsessive/compulsive relationship to foods that is defined as food addiction (FA). The degree that FA contributes to obesity in the general population, and the key factors involved in FA, are unknown. The aims of the thesis were to assess and to find: 1) The prevalence of FA in the general population; 2) If clinical symptom counts of FA were significantly correlated with body composition measurements; 3) If food addicts were significantly more obese than controls, 4) If any macronutrient intake is associated with FA, 5) The link of hormones and neuropeptides that regulate appetite and metabolism with FA, 6) The differences of dietary nutrient intakes (micro- and macro-nutrients) between obese individuals with FA (FAO) or without FA (NFO), and 7) Discovery of novel FA associated candidate genes.

The current thesis consists of three phases. In phase I, I found that the prevalence of FA in the general NL population was 5.4% and women had double the prevalence relative to men. FA was significantly associated with obesity (vs normals). Additionally, FA was positively correlated with severity of obesity.

In phase II, compared to NFO, FAO individuals had lower levels of TSH, TNF-α, and amylin, but higher levels of prolactin. The total calorie intake, the dietary intake of fat and the percent calorie intake from fat and carbohydrates was higher in the FAO. FAO subjects consumed more sugar, minerals (including sodium, potassium, calcium and selenium), fat

and its components, omega 3 and 6 fatty acids, vitamin D and gamma-tocopherol than NFO.

The phase III by combining exome sequencing technology with genetic association analysis in 2 equally obese but with opposite extreme phenotype of FA, we discovered and validated two FA candidate genes: DRD2 and TIRAP. Our discoveries suggest that FA may represent a sub-group of obese individuals with unique nutritional, hormonal and genetic factors.

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List of Abbreviations

Abbreviation	Full name
%AF	Android fat percentage
%BF	Body fat percentage
%GF	Gynoid fat Percentage
%TF	Trunk fat Percentage
α-MSH	Alpha-melanocyte-stimulating hormone
AACE	American Association of Clinical Endocrinologists
ACTH	Adrenocorticotropic hormone
ADHD	Attention Deficit Hyperactivity Disorder
ADP	Air displacement plethysmography
AGRP	Agouti-related peptide
ANCOVA	Analysis of covariance
BDNF	Brain-derived neurotrophic factor,
BED	Binge Eating Disorder
BIA	Bioelectrical impedance analysis
BMI	Body mass index
C-peptide	Connecting peptide
CART	Cocaine and amphetamine regulated transcript
CCK	Cholecystokinin
CFI	Canada Foundation for Innovation
CHR	Chromosome

CIHR	Canadian Institutes of Health Research
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CODING	Complex Diseases in the Newfoundland Population: Environment and Genetics
CRF	Corticotropin releasing factor
Ctrl	Control
CT	Computerized tomography
DXA	Dual-energy X-ray absorptiometry
DSM-IV TR	Diagnostic and Statistical Manual IV, Text Revision
ENPPl	Ectonucleotide pyrophosphatase/phosphodiesterase I
ELISA	Enzyme-linked immunosorbent assay
FA	Food addiction
FAO	Food addicted obese
FFQ	Food frequency questionnaire
Freq	Minor allele frequencies
FSH	Follicle-stimulating hormone
FTO	Fat mass and obesity-associated gene
GABA	Gamma-aminobutyric acid
GAD2	Glutarnate decarboxylase 2
GHS-R	Growth hormone secretagogue receptor
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1

GWAS	Genome-wide association study
HDL	High-density lipoprotein
HOMA	Homeostatic model assessment
HOMA-β	Homeostatic model assessment-β-cell function
HOMA-IR	Homeostatic model assessment-insulin resistance
HREA	Health research ethics authority
HSC	High symptom count
HWE	Hardy-Weinberg Equilibrium
LDL	Low-density lipoprotein
LEP	Leptin
LEPR	Leptin receptor
LH	Luteinizing hormone
LSC	Low symptom counts
m-YFAS	Modified YFAS
MC4R	Melanocortin receptor 4
MCP	Monocyte chemotactic protein-1
mRNA	Messenger RNA
MCH	Melanin-concentrating hormone
MLGP	Multilocus genetic profile score
MRI	Magnetic resonance imaging
NAc	Nucleus Accumbens
NFO	Non-food addicted obese
NGS	Next-generation sequencing

NHANES	National Health and Nutrition Examination Survey
NL	Newfoundland and Labrador
NPY	Neuropeptide Y
NTRK2	Neurotrophic Receptor Tyrosine Kinase 2
OFC	Orbitofrontal Cortex
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor-1
PCSK1	Proprotein Convertase Subtilisin/Kexin type 1
POMC	Pro-opiomelanocortin
PP	Pancreatic polypeptide
POMC	Pro-OpioMelanoCortin
PYY	Peptide tyrosine tyosine (peptide YY)
SBE	Single base extension
SIM1	Single-Minded homolog 1
SD	Standard deviation
SLC6A14	solute carrier family 6 (amino acid transporter), member 14
SNP	Single-nucleotide polymorphism
T2D	Type 2 diabetes
TG	Triacylglycerol
TNF-α	Tumor necrosis factor alpha
TSH	Thyroid-stimulating hormone
VTA	ventral tegmental area

WHO	World Health Organization
YFAS	Yale Food Addiction Scale

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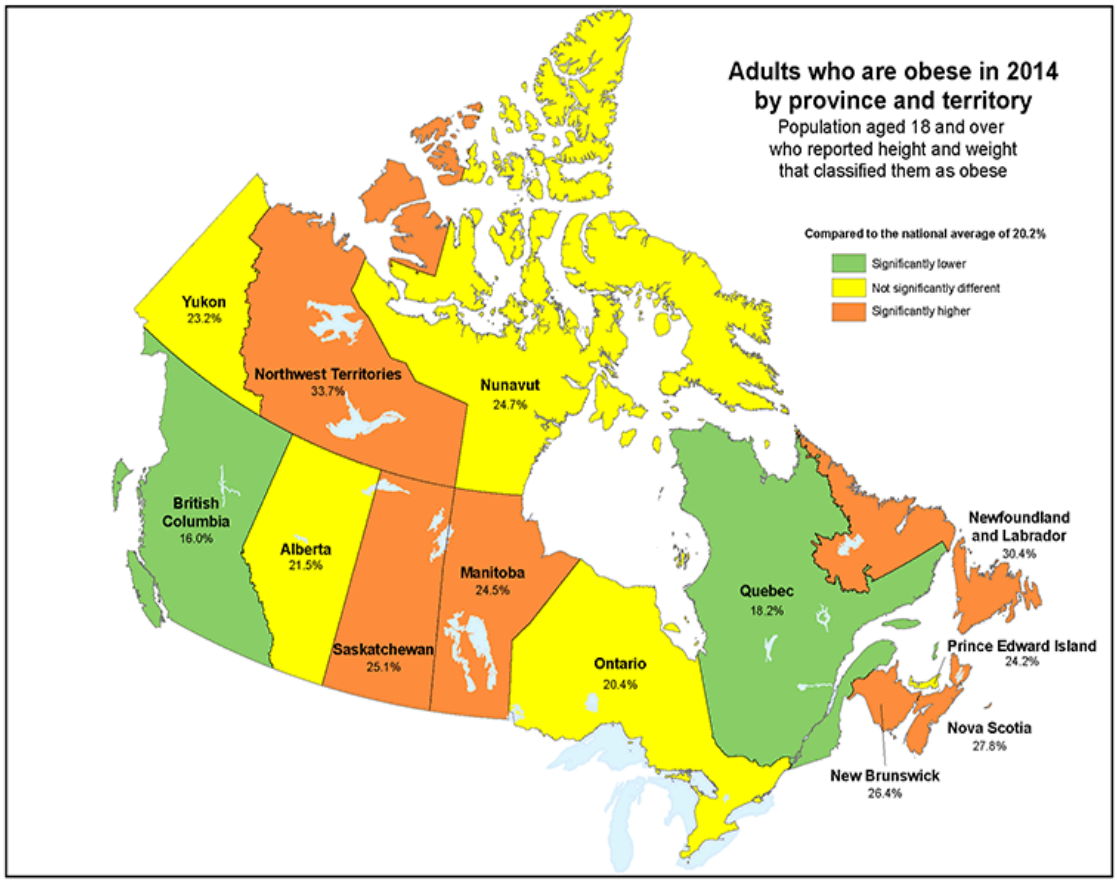
Chapter 1. Introduction

1.1 Prevalence and Health Consequences of Obesity

Overweight and obesity are the abnormal or excessive accumulation of adipose tissue generally resulting from a chronic positive energy imbalance (1, 2). Globally the prevalence of obesity has dramatically increased over the past three decades. Based on the most recent estimation of the World Health Organization (WHO), among the adult population (18 years of age or older) 1.9 billion people are overweight and 600 million are obese. In another word, 39% of world adult population is overweight and the global prevalence of obesity in adults is about 13% (2). In Canada, it has been estimated that 5.3 million adults are obese which means over one out of five Canadians are affected by obesity (3). Furthermore, it is projected that by 2019 about 50.4% of the adult population in Canada will be categorized as either overweight or obese (4). Based on a report published by Statistics Canada in 2014, Newfoundland and Labrador (NL) has the second highest obesity rate (30.4%) among the Canadian provinces, trailing only the Northwest Territories (3). The prevalence of obesity in NL is 10% higher than the national average indicating the existence of a substantial health problem in the province (Figure 1.1).

Excessive adiposity in the body is identified as a major factor that escalates the risk of many diseases such as type 2 diabetes (5, 6), cardiovascular disease (7, 8), stroke (9, 10), dyslipidemia (11), hypertension (12, 13), asthma (14), several type of cancers (15, 16) ,etc.. There are also evidence that demonstrates the fact that obesity causes a decrease in the quality of the life (17, 18) and overall life expectancy (19, 20). Obesity has been estimated as the second most preventable cause of death in the United States and globally as the fifth principal cause of death (2, 21).

In consequence of the high prevalence of obesity and its associated comorbidities, a significant fiscal burden is engaging on health systems. Medical costs linked to obesity were estimated to be $147 billion in 2008 (22). A 2010 report estimated that direct costs of overweight and obesity represented $6 billion – 4.1 % of Canada's total health care budget (23).



Source: Statistics Canada, Canadian Community Health Survey (CCHS), 2014.

Figure 1-1 Map of the prevalence of obesity in Canadian Adults according to body mass index (BMI ≥30.0 kg /m^2; ref 3)

1.2 Definition of obesity

Many methods can be used to categorize an individual as normal weight, overweight or obese. The most widely used method to determine obesity status is body mass index (BMI): dividing a person's weight in kilograms by the square of his/her height in meters (kg/m^2). According to the World Health Organization, a BMI > 25 kg/m^2 is classified as overweight, while a BMI > of 30 kg/m^2 is classified as obese (Table 1-1; ref 24). Simplicity, accessibility, and low cost are the main reasons that the majority of epidemiology studies employ the BMI (25). However, some disadvantages have been attributed to BMI including inaccuracy in representing body composition due to the inability to distinguish fat mass from fat-free mass (muscle and bone; refs 26-30). Furthermore, while sex differences in body fat percentage have been well documented, the BMI is unable to consider the sexual dimorphism of adiposity (31-33). In addition, there is no variation of accumulation of body fat with age or the inter-individual difference of body fat percentage among people within the same BMI category (30). Therefore, World Health Organization now admits that caution must be taken when utilizing BMI to classify obesity status because it may not correspond to the same degree of fatness in different individuals (2).

As a result, recent works have focused on identifying tools that are better able to predict disease risk. In terms of anthropometric measurements, waist circumference measurements and the ratio of waist to hip circumferences have been utilized to estimate central adiposity, a known contributor to cardiovascular, insulin resistance and other obesity-related diseases (34-36). Although these anthropometric classifications have proven useful in large-scale population studies, they are not without limitations. For instance, large interindividual

variation in visceral fat exists in individuals with the same waist circumference (37). Underwater weighing (hydro densitometry) has long been used as the traditional gold standard for quantifying body fat (38). Skinfold measures using caliper (39), air displacement plethysmography (ADP; ref 40), magnetic resonance imaging (MRI; ref 41), and bioelectrical impedance analysis (BIA; ref 42) have also been used to estimate body fat. However, now greater precision and simplicity are found using dual-energy X-ray absorptiometry (DXA). The DXA scanner uses two X-ray beams of different energies to distinguish between bone, muscle, and adipose tissue. Bone, lean mass, and fat all have different densities (bone > muscle > fat) which the scanner uses to tell the tissues apart from one another. Still, more accurate measures are evident when using MRIs (43) and CT scans (44), but due to the expensive operative costs, these are not usually employed in large-scale studies. Therefore DXA is considered as the gold standard for measuring body composition (45).

Another criteria for individual classification into different obesity status is recommended by Bray. These criteria can determine the individuals based on body fat percentage (%BF) according to age and sex specific (Table 1-2; refs 27, 46).

1.3 Etiology of obesity

The American Association of Clinical Endocrinologists (AACE) has recently identified obesity as a disease (5, 6). Obesity is a complex, multifactorial disease and the etiology has not been completely understood (47). Generally, obesity is the result of weight gain due to a chronic positive caloric balance: energy intake (caloric intake) is greater than energy

expenditure (basal metabolic rate, thermic effect of food/adaptive thermogenesis, and physical activity; ref 48). Although a lack of physical activity and/or the increased accessibility/consumption of calorie dense foods are the primary contributing factors to obesity development, however, genetics (49, 50), endocrine function (51, 52), behavioral patterns (53, 54) and environmental determinants (55) play a fundamental role in the development of obesity. The implications of this complexity impose significant challenges to researchers in order to understand the etiology of obesity

Table 1-1 BMI classification based on WHO*[24]

Classification	BMI (kg/m^2)	
	Principal cut-off points	Additional cut-off points
Underweight	< 18.50	< 18.50
Severe thinness	< 16.00	< 16.00
Moderate thinness	16.00 – 16.99	16.00 – 16.99
Mild thinness	17.00 – 18.49	17.00 – 18.49
Normal range	18.50 – 24.99	18.50 – 22.99
		23.00 – 24.99
Overweight	≥ 25.00	≥ 25.00
Pre-obese	25.00 – 29.99	25.00 – 27.49
		27.50 – 29.99
Obese	≥ 30.00	≥ 30.00
Obese Class I	30.00 – 34.99	30.00 – 32.49
		32.50 – 34.99
Obese Class II	35.00 – 39.99	35.00 – 37.49
		37.50 – 39.99
Obese Class III	≥ 40.00	≥ 40.00

*BMI, Body Mass Index; WHO, World Health Organization

Table 1-2 Obesity status based on Bray criteria (27)

	Women (%BF)*				Men (%BF)			
Age (years)	**Underweight**	**Normal weight**	**Overweight**	**Obese**	**Underweight**	**Normal weight**	**Overweight**	**Obese**
20-39	<21	21-32	33-38	39+	<8	8-20	21-25	26+
40-59	<23	23-34	35-40	41+	<11	11-22	23-28	29+
60-79	<25	25-37	38-42	43+	<13	13-24	25-30	31+

%BF, Body Fat Percentage

1.3.1 *Environmental Factors*

The rapid weight gain in the population over the last three decades is largely due to the environmental changes (56). Various environmental cues including the weather, culture, sedentary life style, industry marketing, portion size, the price of food, good tasting, and energy-dense foods have been thought to have a role in the increasing prevalence of obesity (57, 58). Food is also heavily advertised, and it has become acceptable to eat food everywhere (59). Rapid developments in technology have created a new world in which life has become much easier for most people. As a culture of pre-packaged and fast food, watching television, playing video games and surfing the Web has triumphed, a lifestyle of minimum physical activity is emerged (57). These factors result in a chronic excessive energy intake (calorie intake) and low energy expenditure and consequently to a chronic positive caloric balance.

1.3.2 *Genetics*

Genetic predisposition is a key factor responsible for the large individual difference in body weight, body fat and other obesity-related aspects (60, 61). In the 1960's the 'thrifty gene' hypothesis was proposed, whereby genes that predispose to obesity would have had a selective advantage in populations that frequently experienced starvation. People who possess these genes in today's obesogenic environment might be those that 'overreact' not just becoming slightly overweight, but extremely obese (62, 63). This can be seen in certain high-risk groups, such as Pima Indians and Pacific Islanders, and recent studies in the United States have shown that there is also a disproportionate level of obesity in African-

Americans and Hispanic-Americans compared with Caucasians. These differences cannot be explained by lifestyle, economic or environmental factors alone, indicating an important role of genetics (64). The discovery of ob gene and leptin contributed to promoting a significant progress in the knowledge and understanding on the genetic component of body weight regulation (65, 66). The role of leptin was first discovered in studies of severely obese ob/ob mice, which harbor mutations in the ob gene resulting in a complete lack of its protein product leptin which is derived from adipose tissue. Administration of recombinant leptin reduces the food intake and body weight of leptin-deficient ob/ob mice and corrects all their neuroendocrine and metabolic abnormalities (66-68)

When considering obesity, clearly heritability is not a fixed entity, as the proportion of the phenotype that can be explained by the genotype will be influenced by the varying exposure to obesogenic environmental factors in different individuals and families (68). Consequently estimates of the heritable variation contributing to obesity range from 30–60% in family studies to 60–80% in twin studies (69, 70).

Based on genetic and phenotypic characteristics, three types of obesity forms can be considered: monogenic non-syndromic obesity, monogenic syndromic obesity, and polygenic (common) obesity that is caused by the interaction between multiple genes and the environment (64, 71).

Monogenic forms of non-syndromic obesity result from an alteration of a single gene and are rare, affecting in total about 5 % of the population (68, 71). The mutations in genes that encode proteins with likely roles in appetite regulation are responsible for Mendelian

disorders in which obesity is the most obvious phenotype (64). There are more than 200 types of human obesity that are associated with homozygous forms of a single gene mutation (70, 71). Interestingly, all these mutations can be found in only 11 genes (70). There are six well-known genes with mutations in monogenic non-syndromic form of obesity explaining up to 10 % of cases with early-onset extreme obesity, affecting leptin gene (LEP), Leptin receptor (LEPR), Pro-OpioMelanoCortin (POMC), Proprotein Convertase Subtilisin/Kexin type 1(PCSK1), Neurotrophic Receptor Tyrosine Kinase 2 (NTRK2) and Single-Minded homolog 1 (SIM1; refs 68, 71, 72).

In monogenic syndromic obesity, at least 20 rare syndromes are caused by discrete genetic defects or chromosomal abnormalities (both autosomal and X-linked) characterized by obesity (64). Such as Prader-Willi syndrome (which involves a mutation or deletion of the paternally contributed chromosome 15qll-q13; ref 73), and Bardet Biedl syndrome (associated with mutations in at least twelve different loci; ref 74).

The genetic profile of polygenic or common form of obesity results from the effects of many altered genes (70). In theory, the genetic basis of polygenic obesity implies that the specific set of variants relevant for obesity vary considerably from one obese person to the next (75). In this model, many genes have a small influence on the adiposity status of a given individual (76). According to the latest installment of the Obesity Gene Map, more than 600 genes, markers, and chromosomal regions have been associated with obesity phenotypes (70). In contrast with monogenic obesity, in polygenic obesity each polymorphism leads to a variant that confers susceptibility, requiring additionally the presence of other variants and an obesogenic environment to determine the obese

phenotype (71). In the common form of obesity, there is no observable simple Mendelian inheritance pattern. There are a few exceptions to this, namely melanocortin 4 receptor (MC4R) and brain-derived neurotrophic factor (BDNF), with variants originally identified as causing rare monogenic obesity but now known to be frequent enough to account for a measurable proportion of common obesity cases (77).

The study of common obesity is far more complicated and challenging. However, the advent of new techniques facilitated this study by allowing the analysis of several loci at the same time (71). There are some approaches used in the detection and analysis of a candidate gene in body weight regulation: linkage studies, genome-wide association (GWA) studies, and candidate gene approach. Their objective is to determine whether an association between a genetic variation and an obesity-related trait do exist (71).

- Linkage studies: Positional cloning based on linkage results has identified a small number of possible candidates, including glutarnate decarboxylase 2 (GAD2, ref 78), ectonucleotide pyrophosphatase/phosphodiesterase I (ENPPl) (79), and solute carrier family 6 (amino acid transporter), member 14 (SLC6A14; refs 80, 81).

- Genome-wide association studies: To date, GWAS had identified more than 52 loci associated with obesity-related traits (71). The first loci identified through GWAS was the fat mass and obesity-associated (FTO) gene, and until now more than 50 genetic loci have been identified as being associated with at least one obesity-related trait (71, 82). A common variant in FTO was unequivocally associated with BMI and increased risk for obesity simultaneously by two groups. The effect size of FTO polymorphism

on BMI is modest, with homozygous individuals for the risk allele (in this case the A allele) weighing on average 3 kg more than those homozygous for the protective allele (in this case the T), with the difference representing approximately, 0.36 kg/m^2 (71, 83). Variation in FTO was estimated to account for approximately 1% of the total heritability of BMI. Although this is a relatively small effect on total adiposity, countless other groups have confirmed these initial findings (84-86). Additional candidates have since been identified through GWAS such as MC4R, NCPI, MAF, PTER, KCTDI5, MTCH2, NEGRI, SH2BI, and TMEMI8 (87-90). To date, more than 35 loci have been found associated with the increase of BMI (explaining ~1–3 % of the variance in BMI), while other loci correlate with abdominal obesity, establishing 13 loci associated with it, assessed by the waist-to-hip circumference ratio (71, 91).

- Candidate gene association studies: this approach can analyze genes involved in key metabolic pathways or those that have been shown to be important for obesity development in animal or human studies (65). This approach can be defined as the study of genetic influences on a complex trait by identifying variants, such as single nucleotide polymorphisms (SNPs), in or near a candidate gene that may have a role in the etiology of the disease (92). Selection of obesity candidate genes is typically based on prior knowledge of their known physiological role in pathways related to energy intake (INS, LEP, MC4R, NPY, POMC, AGRP, CARTPT, FTO or LEPR; refs 93, 94), energy expenditure (CLOCK, ADRB2, ADRB3, UCP1, UCP2 or UCP3; refs 95, 96), as well as or adipose tissue growth and development (PPARG2, CEBPA, IL6, FABP4, CD36, PNAPLA3 or PLPIN5; refs 65, 97, 98). In addition, candidate genes may also

be chosen on the basis of previous evidence of association with obesity-related traits in other populations. Candidate gene studies take advantage of both the increased statistical efficiency of association analysis of complex diseases and the biological understanding of the phenotype, tissues, genes and proteins that are likely to be involved in the disease (92).

- Exome sequencing studies: In complex trait genetics, exome sequencing studies bring to light rare coding variants that are undetected by microarray-based genome-wide association studies. Exome sequencing provides a complementary approach to comprehensively assessing the role of all coding variation, both common and rare. Therefore, the strong interest in exome sequencing stems from three factors: the potential to identify many genes underlying complex traits, straightforward functional annotation of coding variation, and cost being substantially lower (around 5 times) than whole-genome sequencing (99).

- The recent developments in the automated DNA sequencing instruments, which utilize modern advancements in engineering, chemistry, molecular biology and software development, has opened up new exciting opportunities. Currently, molecular diagnosis based on Sanger's sequencing is restricted to only a few genes as this technology is expensive, time-consuming, and labor-intensive. With the help of the next-generation sequencing (NGS) technology, a new molecular diagnosis method has been developed that makes it possible to perform the sequencing of the whole genomes or exomes, or several genes simultaneously. The NGS based innovative and cost-efficient genetic

testing methods portray a promising landscape for sensitive obesity multi-gene screening (71).

1.3.3 ***Unbalanced Diet***

Poor diet caused by micronutrient deficiencies and the consumption of insufficient calories has been always as a global burden and contributed to diet-related health issues (100, 101). Until about 50 years ago poor health caused by overweight and obesity was a negative consequence of a wealthy lifestyle. Now, however, the adverse effects of "over-nutrition" far exceed the problems caused by "under-nutrition" and create a double burden in low- and middle-income countries (101). The average energy supply has been estimated that increased from 2250 calories per person per day in 1961 to 2750 calories per person per day in 2007 (101). In general, during the last decades, the total food supply and consequently, total energy intake has increased (102, 103). One of the reasons is the shift of predominant dietary away from traditional foods prepared within the community towards ultra-processed and packaged food products. These type of food are most often nutritionally poor but dense in energy (101). Daily diet in North America, on average consists of about 15% protein, 35% fat, and 50% carbohydrates (104). Data from the National Health and Nutrition Examination Survey (NHANES) in the United State show parallel increases in the percentage of energy from carbohydrate sources over the last 30 years (101, 105).

The role of diet macronutrients (protein, carbohydrate and fat) in the development of obesity has been long debated (106). In many studies, the effects of different macronutrients intake level on both weight gain and weight loss have been assessed (101). The total fat,

carbohydrate and protein intake has not shown consistent associations with weight gain (102). Nevertheless, from the available data it appears that "a calorie is a calorie" regardless of its macronutrient source and there is a physiological support for this proposition, but over the time, changing the macronutrient composition of the diet may play a role in weight change (101).

- Fat: the role of dietary fat intake in weight loss or weight gain is controversy. The majority of the studies show a positive association between dietary fat intake and BMI in children, and adults (107-109). Several mechanisms may explain the relationship between high fat intake and greater body fat: 1) dietary fat is the most energy-dense macronutrient in the diet with providing approximately 38 kJ/g vs. 17 kJ/g for carbohydrate and protein which could lead to overconsumption of energy if food volume is unregulated; 2) fats could increase food consumption by lending greater flavour and palatability to food; and 3) carbohydrate produce a greater thermogenic effect than fat, suggesting that dietary fat may be utilized more efficiently and accumulate as body fat (107).

  In general, for preventing weight gain and obesity, the evidence supports that low-fat diets are an optimal choice. In addition, there is a positive association between weight loss and reduction of consumed percentage of the calorie from fat (110). In animal studies, as a rule, eating low-fat diet does not lead to become obese. When animals are exposed to different levels of dietary fat, there is a dose–response curve with a threshold of about 25% dietary fat which suggests that levels of dietary fat need to exceed 25%

in the diet before obesity develops (108). In human, a 10% reduction in dietary fat is predicted to produce a 4-5 kg loss in an individual with a BMI of 30 kg/m^2 (110).

- Carbohydrate: foods with a high energy percentage of carbohydrates can range from a low (for example, raw vegetables and fruits) to a high (for example, sugary candy) energy density (111). The evidence in the case of carbohydrate intake in relation to weight loss or weight gain is also controversy (111, 112). Several reports indicate a rapid weight loss after low-carbohydrate diet in the short period and this impact is even greater than a low-fat diet (113-116). However, this was not observed in all studies (112, 117). Thus, whether a reduction in carbohydrate intake offers any benefit beyond energy restriction alone is unclear (112). Several mechanisms may explain the relationship between low carbohydrate intake and weight loss: 1) the ketogenic effect of very low carbohydrate intake may facilitate weight loss through urinary excretion of ketones or even suppression appetite by circulating ketones, 2) the simplicity of the diet, besides the restriction of variety of the food choices and possibly a greater satiating effect of protein (111).

- Protein: the recommended dietary protein in Canada is 46 g/day for women and 56 g/day for men or 0.86 g/kg/day (106). These recommended dietary proteins are to prevent protein deficiency as a result of body nitrogen loss due to natural cell death or through normal metabolic processes such as ammonia detoxification and urea production (106). Weight loss intervention studies and randomized control trial support that weight loss and weight maintenance are more effective with an energy restricted diet high in protein than a diet with a lower protein composition (106, 118-121). Several

mechanisms may explain the relationship between high protein and intake weight loss: 1) increasing in lean body mass at the expense of fat mass (106, 122, 123), 2) high protein may exert a greater thermogenic effect compared to carbohydrate and fat and therefore may contribute to greater energy expenditure during rest (106, 124, 125). For the first time, a previous study in our lab showed that dietary protein is negatively associated with all obesity indexes (body composition measured by DXA, weight, BMI, waist circumference, and waist-to-hip ratio) in a free-living, non-dieting population. This association was independent of age, sex, physical activity level, total calorie intake, carbohydrate intake, smoking status, medication use and menopausal status at the population level. Furthermore, weight and BMI was significantly different between low and high protein consuming groups (106).

1.3.3.1 *Dietary intake assessment*

Several methods are available to assess individual's food and energy intake. The commonly used methods include: a single 24-hour recall, multiple 24-hour recalls or multiple diet records, food frequency questionnaires (FFQ), brief dietary screening tools (126, 127). Among them, FFQs are more popular and has been widely used for epidemiological purposes (128). Since Compared to other dietary assessment methods, the FFQs are easy to administer, has relatively low cost, and provides a rapid estimate of usual food intake (129, 130). In FFQs, selected food items are listed, and the intake frequencies and a usual portion or serving sizes (average quantity of foods per intake) are noted (128). The consumption of a food item is estimated by intake in grams per day by multiplying the standard serving size of each food by its intake frequency (128, 131). The number of food

items listed in a FFQs tends to vary widely due to the differences in the food supply and dietary habits from one population to another, (129, 130, 132). Therefore, there is no universally accepted FFQ that can be used for all populations (129). Many FFQs are modeled like Block, National Cancer Institute (NCI), Health Habits and History Questionnaire (HHHQ), and Willett (133-136). These formats differ most notably in their approach to ascertaining consumption frequency and quantity of listed foods (136). For dietary assessment in the target population, FFQs should be developed specifically for each study group and research purposes because diet may be influenced by ethnicity, culture, an individual's preference, economic status, etc. and must be validated in that specific population (136, 137).

The applicability and accuracy of FFQs are controversial and some limitations have been addressed in many studies. Part of the controversy is due to the fact that food frequency questionnaires needs to be tailored for the target population. In addition, this debate is also related to the inevitable biases due to the characteristic of questionnaire such as respondent-related reporting bias and recall bias (138, 139).

1.3.4 ***Overeating***

It has been well documented that chronic overconsumption of calories plays a fundamental role in the development of obesity (140). However, evidence suggests that overeating alone is not sufficient to explain the obesity epidemic. Thus, eating disorders may be due to an altered function in different systems and overeating may be a symptom rather than the cause of the obese phenotype (141). Overeating can occur in different conditions, for example in

response to stress. This reflex might reflect an attempt to self-soothe or self-medicate with "comfort" foods (142). Another type of overeating is habitual overeating. Environmental food-associated stimuli (easy to access, cheap and over sized food) can robustly enhance the desire to eat even in absence of food per se or in absence of physiological needs. Habits are formed through repeated reinforced action until the stimulus-response association lapses the goal of the behavior (142). However, habits can be changed to a compulsive behaviour when they persist despite a reduction in reinforce efficacy (142).

1.3.5 ***Food Addiction***

Overeating in some degree may occur in many individuals; however, a proportion of them may develop an obsessive/compulsive relationship to certain foods (143). These individuals chronically consume more food than they need to maintain health and show compulsive intake behaviors associated with loss of control of eating (144, 145).

Accumulating research evidence has documented neurobiological and behavioral similarities between compulsive overeating and psychoactive drug dependence, leading researchers to use the term of food addiction to describe this pattern of overeating (146-150). In animal models, foods high in sugar and fat are particularly associated with addiction-like eating behavior (151-153). Findings from human studies have suggested that the pattern of food intake in food addiction may parallel substance dependence and this phenomenon might be understood with the same neurobiological, behavioral and clinical framework as conventional drug dependence (154-156)

1.3.5.1 *Clinical and behavioral similarities between food addiction and substance dependence*

- Tolerance and withdrawal:

Generally, tolerance is characterized by a reduced responsiveness to a stimulus of a particular magnitude and is usually manifest by the need to use increasing doses to achieve the desired effect. Withdrawal symptoms are the groups of syndromes that occur upon the abrupt cessation of chronic stimulus. These phenomena are key characteristics of substance dependence (146, 157). The evidence supporting the probable tolerance effect in animal studies after intermittent sucrose that slowly increased sucrose consumption from 37 to 112 ml per day in rats (from 13 to 20ml in the first hour of access; refs 158, 159). The evidence of tolerance in the human comes largely from anecdotal clinical reports of individuals consuming more food in each binge as the disorder becomes more chronic (146). Tolerance in the human can be explained by a series of biologic responses designed to ready the body for metabolic work. These reactions start when the anticipation of food intake, and the preabsorptive signals generated by the body's first indication that food has been consumed. It helps the body to prepare for incoming nutrients, and food-related stimuli trigger a compensatory response of cephalic insulin secretion, which is similar to compensatory responses to drugs of abuse. The compensatory cephalic insulin secretion reduces the level of glucose in the blood stream which may result in the need for increased consumption of sugar to achieve desired effects (160).

Similar to substance dependence, a certain type of food, particularly high in sugar and fat can cause withdrawal symptoms when removed from the diet. These symptoms most clearly resemble the physical sign of distress seen in withdrawal (146, 151). Evidence from the animal research showed that removal of sugar from dependent animals results in a drop in body temperature and behavioral changes associated with withdrawal, such as aggression, anxiety, teeth chattering, forepaw tremor, and headshaking (146, 158). In human, evidence of sugar withdrawal mostly comes from clinical observation and self-reports. This symptom has been described as headaches, irritability, and flu-like symptoms among heavy sugar consumers who become abstinent (146).

- Loss of control:

  Loss of control is defined as continuing to use despite negative consequences, and an inability to cut down problematic use (161). For instance, some obese individuals continue to eat unhealthy food even in the face of severing negative consequences like diabetes and cardiovascular disease (162). In addition, food addiction and substance dependence share many of the same neural pathways, regarding both craving and loss of control (148).

- A great deal of time spent in activities necessary to obtain, use, or recover:

  It has been debated that this criterion for substance dependence cannot be applied for food addiction, due to the easy and inexpensive accessibility of calorie-dense, nutrient poor foods (160, 161, 163). However, it is possible for addiction to occur with something that is legal, socially acceptable to obtain and readily available, like nicotine. In the case of food

addiction, a person might drive across the town to a fast food restaurant to fulfill a craving even though they have other food available (160).

- A desire or repeated failed attempts to reduce or stop consumption:

  There is evidence that the repeated failure of such attempts is happened in approximately 83% of participants regaining their lost weight within 5 years (160).

- Continued use despite physical or psychologic problems:

  A study highlighted the difficulty of abstaining from a certain type of food even in the face of adverse consequences. In a large clinical trial, participants were asked to abstain from chocolate during the study. Surprisingly, of the 1200 participants, 139 persons were dropped from the study due to the difficulty of abstaining from chocolate (160, 164).

- Giving up other important activities

  Habitually, being overweight and obese is associated with less important life activities. In one hand, the lack of physical activity leads to be obese and on the other hand, obese individuals are usually giving up their activities because of the difficulty to move fast. As a possible part of weight bias experience, overweight/obese people even may experience less engagement in social activities, such as dating or marriage due to excessive food consumption and obesity (160, 165). A study on obese and non-obese young women has confirmed the hypothesis that eating food is more reinforcing than selected alternative activities (sedentary activities) to a greater extent for obese than for non-obese. In this study, subjects could choose to eat food or engage in sedentary activities (like playing

computer games) based on their responding in a computer-generated concurrent schedules task. However, further research is needed to explore the extent to which excess food consumption may begin to replace other important life activities (160, 166).

1.3.5.2 *Neurobiological similarities between food addiction and substance dependence*

Reward- associated learning plays a fundamental role in both food addiction and substance dependence (167). It is well known that certain foods, particularly those rich in sugars and fat, are potently rewarding. High-calorie foods can promote over-eating (eating that is uncoupled from energetic needs) and trigger learned associations between the stimulus and the reward (conditioning; ref 168). Both in food addiction and substance dependence have an activating effect on the dopaminergic mesolimbic reinforcement system ('reward system') (167). Animal studies demonstrated that sugar bingeing, like substance dependence, consistently stimulates dopamine release in the nucleus accumbens (NAc). In addition, compared to normal-weight rats fed a standard chow diet rats that become obese due to cafeteria-style diet show lower baseline levels of mesolimbic dopamine activity (147). In human studies, similar to substance dependents, food addicts show hypoactivation in the lateral orbitofrontal cortex (OFC), suggesting less inhibitory control in response to reward cues. However, after seeing a photo of a chocolate milkshake (anticipatory reward), a notable activation was seen in the amygdala and anterior cingulate cortex after seeing there was hypoactivation in the lateral OFC. (148). In addition both food addiction and substance dependence tolerance and/or a loss of control is related to hypoactivation of the lateral OFC (148).

1.3.5.3 *Assessment of food addiction*

Some researchers have argued that food addiction should be included as a substance use disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM; refs 140, 169), although others have been critical of the clinical validity or utility of the food addiction concept (144, 170). Yale Food Addiction Scale (YFAS) has been developed, and validated, as a tool for the diagnosis of food addiction (158, 171, 172). This questionnaire consists of 27 items that assess eating patterns over the past 12 months. The YFAS translates the Diagnostic and Statistical Manual IV, Text Revision (DSM-IV TR) substance dependence criteria in relation to eating behavior (including symptoms, such as tolerance and withdrawal symptoms, a vulnerability in social activities, difficulties cutting down or controlling substance use, etc.). The scale uses a combination of Likert scale and dichotomous scoring options. The criteria for food addiction are met when three or more symptoms are present within the past 12 months and clinically significant impairment or distress is present. The Likert scoring option is used for food addiction symptom counts (for instance, tolerance and withdrawal), ranging from 0 to 7 symptoms (158). Very few studies also have used the modified YFAS (m-YFAS) which consisted of nine core questions including one item for each symptom plus two items for clinical impairment and distress (173).

The YFAS criteria have been used to explore the prevalence of food addiction in eating disorder patients (174), obese subjects (175) and junior college students (176). In a previous study assessing obese patients with binge eating disorder (BED), the prevalence of food addiction was reported to be as high as 56.8% (174), suggesting an overlap between binge

eating and food addiction. The prevalence of food addiction in obese individuals seeking weight loss treatment was 15.2%, while in another study obese subjects not seeking weight loss, the prevalence of food addiction was 25% (175, 177). In a cohort of junior college students with a normal BMI range, 8.8% met the YFAS criteria of food addiction; however, the correlation between food addiction clinical symptom counts and BMI was negligible (176).

1.4 Approaches to Understanding the Etiology of Food Addiction

1.4.1 *Appetite-Regulating Hormones*

In humans, the regulation of food intake is based on an intricate feedback system controlled by hunger and satiety signals (178, 179). These signals are generated in the brain, peripheral tissue and/or organs through two complementary drives, including both homeostatic and hedonic pathways (179-181). The hedonic or reward-based regulation pathway is related to the mesolimbic dopamine pathway, which is stimulated in both drug abuse and the consumption of highly palatable foods (179). Evidence has shown that the release of dopamine coordinates food reward, which is impaired in food addicts (179, 182). In another word, hedonic appetite may be considered as a continuum, extending from the normal situation of individuals enjoying some foods more than others, through comfort eating which may occur when the mood is low, to the final extreme situation in which an individual appears to be addicted to food as to a drug of addiction (183). Contrastingly, the homeostatic pathway primarily regulates the energy balance between the brain and peripheries (for instance, digestive tract and adipose tissue; refs 178, 181, 184, 185). This

means that based on energy reservation and the psychological want for food, the brain increases or decreases food intake by interpreting the neuronal and hormonal signals received from peripheries (179, 185, 186). Therefore, in both pathways, a large number of neurotransmitters (dopamine, cannabinoids, opioids, gamma-aminobutyric acid (GABA) and serotonin), neuropeptides (α-MSH, β-endorphin, cortisol, melatonin, neurotensin, orexin A, oxytocin and substance P, *etc.*) and hormones (gut hormones, anterior pituitary hormones and adipokines) are involved, many of which can also be detectable in serum (181, 182, 185-195). Interestingly, many studies have linked these hormones and neuropeptides with the current obesity epidemic (186, 187, 189, 196, 197).

1.4.1.1 *Hedonic Aspects of Food intake*

Evidence in animals and humans support the theory that both consumption of highly palatable food and substance dependence share the same pathway within the limbic system to mediate motivated behavior (179, 198, 199). The majority of the studies have focused on mesolimbic dopamine pathways since most of the common drug of abuse increase dopamine signaling from nerve terminals originating in the ventral tegmental area (VTA) onto neurons in the nucleus accumbens (also called the ventral striatum). Dopaminergic transmission increased by both direct action on a dopaminergic neuron or indirectly through inhibition of GABAergic interneurons in the VTA (179, 198, 199). Furthermore, the neurotransmitter orexin is the peptide that is expressed by a population of lateral hypothalamic neurons and involve in mediating drug-induced activation of VTA dopamine neurons (179, 200, 201). Natural rewards such as food stimulate similar responses within the mesolimbic dopamine pathway. This release of dopamine is believed to coordinate

many aspects of an animal's attempts to obtain food rewards, including increased arousal, psychomotor activation, and conditioned learning (remembering food-associated stimuli). However, the mechanism by which food stimulates dopamine signaling is unclear (179). One possible mechanism is that orexin directly stimulating VTA dopamine neurons since orexin neurons may be activated during feeding (179).

1.4.1.2 *Homeostatic aspects of food intake*

Homeostatic control of feeding, unlike hedonic aspect, is primarily associated with regulation of energy balance. Therefore, it is focused on circulating hormones that rely on information about peripheral energy levels to the brain.

- ***Neuropeptides***

There is a strong evidence that hypothalamus plays an important role in feeding control (189, 202, 203). Orexigenic neuropeptides secreted by hypothalamus are neuropeptide Y (NPY), melanin-concentrating hormone (MCH), orexins, agouti-related peptide (AGRP), galanin, β-endorphin, and anorectic neuropeptides are cocaine and amphetamine regulated transcript (CART), Melanocortins (POMC), alpha-melanocyte-stimulating hormone (α-MSH), corticotropin releasing factor (CRF), serotonin and neurotensin (189). The main regions of hypothalamus involved in feeding and satiety are: 1) Arcuate (ARC): ARC acts as a feeding control center and integrates hormonal signals for energy homeostasis (189, 204). The ARC-median eminence area is one of the 'circumventricular' organs where the blood–brain barrier is specially modified to allow entry of peripheral peptides and proteins including insulin and leptin, both of which are considered to be signals of fat mass (189,

205). The ARC also contains a number of neurons that express NPY, AGRP, and POMC (189). 2) Paraventricular nucleus (PVN): the PVN is the main site of corticotropin releasing hormone (CRH) and thyrotropin releasing hormone (TRH) secretion (189). Furthermore, several neuronal pathways associated with energy balance converge in PVN such as orexins, α-MSH, major projections from NPY neurons of the ARC and the appetite stimulating peptide galanin. Therefore, PVN mostly plays a role in the integration of nutritional signals with the thyroid and hypothalamic-pituitary axis (189, 206). 4) Ventromedial nucleus of hypothalamus (VMH): VMH is mostly acting as a satiety center. Theses nucleus are a key target for leptin that acts as a feeding inhibitor on the hypothalamus and a stimulate for increasing energy expenditure. Lesions of either VMH or PVN produce syndromes of hyperphagia and obesity (189, 207). 5) Dorsomedial hypothalamic nucleus (DMH): DMH has extensive connections with the lateral hypothalamus besides other medial hypothalamic nuclei. Therefore this nucleus serves the function of integration and processing of information from these nuclei (189, 208). 6) Lateral hypothalamic area (LHA): LHA is the classical 'feeding center'. This area also contains glucose-sensitive neurons that are stimulated by hypoglycemia and consequently the marked hyperphagia which is normally induced by hypoglycemia (189, 209).

- ***Gut Hormones and Polypeptides***

The gastrointestinal tract is one of the largest endocrine organs in the human body that secrets more than 20 appetite-regulating hormones (210). Food intake and energy homeostasis are regulated by the brainstem and hypothalamus through a gut-brain communication pathway for neural and hormonal signals (211). Since gut hormones

influence appetite and play an integral role in glucose/lipid metabolism and insulin sensitivity through gut-brain communication (212, 213) investigating appetite-regulating hormones and food addiction may provide valuable insight into the underlying mechanisms responsible for the development of obesity. Most of the gut hormones are sensitive to gut nutrient content, and short-term feelings of hunger and satiety are believed to be mediated, in part, by coordinated changes in circulating gut hormone levels (210). Among these hormones amylin, also named islet amyloid polypeptide (IAPP), is a 37 AA peptide that is synthesized along with insulin in the β-cells of the endocrine pancreas (214, 215). Amylin is an important player in the control of energy balance. Amylin inhibits food intake by promoting meal-ending satiation, possibly through stimulation of its receptor in the area postrema (215-217). Amylin has multiple actions beside controlling food intake such as inhibiting secretion of glucagon and insulin, as well as that of lipase and amylase (196). Normally amylin and the hormone insulin are co-secreted from the β-cells in a molar ratio that remains constant, but this ratio can vary in different diseases such as obesity (214). Amylin residence time in the plasma is longer than insulin and similar to C-peptide, although amylin has a faster clearance rate than insulin by the kidneys (196). Evidence has shown that plasma concentration of amylin is significantly higher in both overweight/ obese adults and children than normal-weight individuals (189, 215, 218-220). Based on the recent studies in both animals and humans, there is a clear evidence that amylin is, at least, partly effective in obesity, however, the process is not clear yet (216).

The rest of anorexigenic gut hormones are pancreatic peptide YY (PYY), cholecystokinin (CCK), leptin, glucagon-like peptide-1(GLP-1), gastric inhibitory polypeptide (GIP),

pancreatic polypeptide (PP), C-peptide, and an only orexigenic gut hormone is ghrelin (189, 221). Ghrelin is a 28-amino acid peptide secreted from the stomach lining which binds to the growth hormone secretagogue receptor (GHS-R; ref 222). This hormone acts directly on the CNS via the arcuate nucleus of the hypothalamus (223). Ghrelin increases food intake and consequently body weight by stimulating the production of NPY and AGRP in the arcuate nucleus and antagonizes the leptin-induced inhibition of food intake (189, 224). In human, the ghrelin level increases during fasting and peaks just before consumption of a meal. The concentration decreases the following feeding and drops to a minimum value roughly one-hour post meal (189, 225-227). The results of the studies on the association between circulating ghrelin level and adiposity are controversy. In most of the cross-sectional studies, lean individuals have a higher level of circulating ghrelin with a negative association between adiposity and ghrelin (210, 228-231). However, other studies reported a positive or lack of association between ghrelin and increased adiposity (232, 233). Nevertheless, to the best of our knowledge, there is no study available regarding the differences in appetite-regulating gut hormonal level between being obese with and without food addiction.

- ***Pituitary polypeptide hormones***

Among pituitary polypeptide hormones, it has been well established that the hypothalamic-pituitary thyroid axis regulates body weight. Furthermore, evidence suggests that thyroid hormones may access the ARC and other regions of the hypothalamus to regulate appetite. It is well known that thyroid dysfunction has a significant impact on appetite and body weight. For instance, hypothyroidism causes a reduction in basal energy expenditure and

weight gain (234-238). In animal studies, an injection of TSH centrally shows a food intake reduction (234, 239). Several mechanisms have been postulated to mediate the orexigenic effect of thyroid hormones. Evidence has shown an increased hypothalamic NPY mRNA after peripheral administration of T3**,** suggesting that T3 may increase appetite via NPY. Furthermore, reduced hypothalamic POMC expression was reported after T3 administration (234, 240). In addition, evidence in recent human and animal studies also support that adipocytes and preadipocytes possess thyrotropin receptors (241, 242). In vitro and in vivo studies have demonstrated that thyrotropin via its receptors in fat tissues leads differentiation of preadipocytes into adipocytes, and expansion of adipose tissue (adipogenesis) (241, 243, 244). Several population-based studies also have shown a significant association of BMI with TSH levels (236, 244-246).

Another appetite-regulating hormone from the anterior pituitary gland is prolactin which is a peptide hormone produced by lactotropes of the anterior pituitary (247). Lactotrope function is primarily regulated by thyrotropin-releasing hormone (TRH). Furthermore, dopamine inhibits the synthesis and secretion of prolactin. There is also preliminary evidence that ghrelin stimulates prolactin secretion in healthy women, but the effect is less than TRH and is not additive to TRH (247). Prolactin is a multifunctional hormone and may have more functions than all other pituitary hormones combined (248). Animal studies provide the consistent results on the positive effect of increased food intake and body weight (248-250). Studies in rats have shown that injections of prolactin induced hyperplasia through PVN, VMH and paraventricular nucleus in the hypothalamus. The PVN was the most sensitive of these sites (251). In humans also long-term

hyperprolactinemia is often accompanied by weight gain (248). Data from a number of studies have suggested that the serum TSH and prolactin level may be a marker of alcohol, opium and cocaine dependence and craving (247, 252-255). However, to the best of our knowledge, there is no study available regarding the differences in TSH and prolactin level between being obese with and without food addiction.

There is also evidence that other anterior pituitary hormones like growth hormone (256, 257), luteinizing hormone (LH) and follicle-stimulating hormone (FSH; (258, 259), brain-derived neurotrophic factor (BDNF; refs 260-262), and adrenocorticotropic hormone (ACTH; refs 263, 264) control appetite, however, no study has been done on food addiction.

- ***Adipokines***

Adipose tissue is no longer considered an inert energy store, but is deemed an endocrine organ capable of synthesizing a plethora of adipokines (265). Adipokines play an important role in the regulation of appetite and eating behavior (266). Adipokines act centrally to regulate appetite and energy expenditure (267). The most famous adipokine secreted by adipose tissue is leptin. However, leptin also is produced in several cell types in other organs such as gastric cells, osteoblasts, and placenta. Despite the wide range of cells that leptin is synthesized, white adipose tissue is the most important source of the hormone and the leptin production correlates positively with adipose tissue mass (189, 268). The principal biologic effect of leptin on appetite is via the hypothalamus. VMH in the hypothalamus is mainly identified as a key target for leptin and acting as a satiety center

(189). Production of leptin from adipose tissue and consequently circulating leptin concentration is decreased when the energy stores are low. Therefore, the hypothalamic neurotransmitters that increase food intake like NPY and AGRP increases and the level of α-MSH and CART declines (189).

Another adipokine is tumor necrosis factor alpha (TNFα), as a cytokine with an anorexigenic effect. TNFα administered either centrally or systemically reduces food intake, and in the early stages of obesity, inflammation also occurs in the mediobasal hypothalamus (269). Furthermore, evidence has suggested that elevated leptin levels may contribute to the effect of TNFα to decrease food intake (270). However, a study on rats has suggested that, under high-fat feeding, increased hypothalamic levels of TNFα coincide with increased feeding and obesity (197). Despite the role of TNFα in food intake, there is a general agreement that TNFα level is elevated in obese adults (271, 272). However, other factors related to obesity may cause higher TNFα level as an inflammatory factor, including, cardiovascular disease, and diabetes mellitus (273). In adults also TNFα is positively correlated to adipocyte size (272). In addition, it was reported that the levels of circulating TNF-α have been altered in alcoholics, cocaine abusers, and opiate addicts. In addition, it has been suggested that TNF-α can be a potential diagnostic biomarker for drugs of abuse (274-278). In an animal model, TNF-α has been investigated as a potential therapeutic target to prevent drug abuse and to increase the chance of cessation (275). Despite the different roles of the discussed adipokines in appetite, obesity, and addiction, there is no study available on TNFα and leptin nor is there one on other adipokines like

adipsin, adiponectin, and resistin regarding the differences between being obese with and without food addiction.

In chapter 3 as a first of its kind, we explore the potential biomarkers that may differentiate being obese with and without food addiction by measuring and comparing various hormones and neuropeptides regulating appetite and metabolism. This information will help us to unravel how food addiction develops.

1.4.2 ***Dietary Intake***

As discussed before, evidence in animals has supported that foods with high fat are partially associated with addiction like behavior such as withdrawal syndrome like teeth chattering, tremor, head shaking and anxiety (152, 153, 279). Furthermore, in animals, there is evidence that high-fat diet either to reduce the expression of NPY in ARC or to stimulate NPY. The reason for this contradictory effect is not clear, but the type of fat ingested (poly saturated, monosaturated…etc.) might be important. For instance, saturated fat producing an upregulation of neuropeptide Y expression in contrast to polyunsaturated fat (280). In human studies, the abuse potential of carbohydrate beverage has been tested in a double blind, placebo-controlled trial study. The samples were 61 overweight carbohydrate craver Women. After being induced into sad mood participants were exposed double blind to taste matched carbohydrate and protein beverage and they were asked to choose the drink that made them feel better, interestingly they significantly preferred the carbohydrate beverage. They had a greater liking and greater reduction in dysphoria after administration of carbohydrate beverage (281). Overall, there appears to be significant support for the notion

that high intake of fats and sugars is closely linked to the diagnosis of food addiction. As discussed in the section 1.3.3, there is evidence on the role of diet macronutrients (protein, carbohydrate and fat) in the development of obesity. However, there is no study available on the potential differences of macro- and micro-nutrients between being obese with and without food addiction, which will be critical to unravel how food addiction develops. Therefore, in chapter 2 and 3, as a first of its kind, we explore the potential differences in dietary nutrient intakes among obese with and without food addiction.

1.4.3 ***Genetics***

Evidence from family, twin, and adoption studies across several drug classes (opioids, cocaine, cannabis, nicotine, alcohol) strongly implicates the role of a genetic factor in each step of addiction including vulnerability to initiation, continued use, and propensity to become dependent (282-284). There also exists a genetic overlap between drug and behavioral addictions like gambling (284). Many studies have discovered genes that increase the vulnerability in the development of substance dependence (285-291). For instance, twin studies have shown that the heritability of addictions ranges from 39% (hallucinogens) to 72% (cocaine) (292). Importantly, however, the predisposition to addiction may be due to genetic variants that are common to all addictions and/or to those specific to a particular addiction (285). However, to the best of our knowledge, there is very little information regarding genes associated with YFAS food addiction. A few studies like the genome-wide investigation (GWAS) of food addiction (293), evaluation of potential involvements in dopaminergic reward pathways in the brain (294) or mu-opioid receptor gene were reported (295). The GWAS of food addiction defined by modified

YFAS was done on 9,314 women of European ancestry. They did not find a candidate SNP or the gene for drug addiction to be significantly associated with food addiction after correction for multiple testing (293). In the relationship of the genes related to dopaminergic reward pathways with food addiction, a multilocus genetic profile score [MLGP] as an index of elevated dopamine signaling based on six known dopamine-related polymorphisms (located in or close to ANKK, DRD2, SLC6A3 and COMT genes) was used to study food addiction by investigating whether this score distinguished those with YFAS-diagnosed food addiction from controls. The investigators reported that the MLGP score was significantly higher in the food-addiction group than in the controls, suggesting the involvement of dopamine signaling pathway (294). In the study of the relationship of mu-opioid receptor gene (OPRM1) with food addiction, a functional A118G single nucleotide polymorphism was tested. Results confirmed that the food-addiction group had significantly higher levels of hedonic responsiveness to food suggests that this bio-behavioral trait may foster a proneness to overeating, to episodes of binge eating and ultimately to a compulsive and addictive pattern of food intake (295). However, a comprehensive understanding of the genomic landscape of food addiction is lacking. In chapter 4 for the fist time, we performed a two-stage study using an exome sequencing technology as a screening stage followed by a verification study using the most significantly associated genes discovered to validate candidate genes related to food addiction.

1.5 Rationale

Given the complexity of obesity and the evidence on the role of food addiction in the increasing prevalence of human obesity, this thesis attempted to answer many fundamental questions to explore whether and to what degree, food addiction contributes to the common form of human obesity. Furthermore, this thesis sought to further understand how food addiction is involved in the development of human obesity from the aspects of physiology, endocrinology, nutrition and genetics.

Study on food addiction is on the early stage. The prevalence of food addiction in the general population was not available and this was an essential first step towards evaluating the potential contribution of food addiction to human obesity; therefore, as a first step, in Chapter 2 we asnwered this question using a large number of subjects from the CODING (Complex Diseases in the Newfoundland Population: Environment and Genetics) study that could represent the general population in the province of Newfoundland and Labrador. CODING study as a population based study also provided us a legitimate number of females and males to examine the difference of the prevalence of food addiction in the two sex groups. Because there is an evidence of gender difference in the other types of eating disorders, however no study is available in food addiction.

Furthermore, to find a strong evidence that food addiction contributes to the rising prevalence of obesity in the general population we performed our study based on both BMI and body fat percentage determined by DXA, the latter is an accurate measurement of body fat and body composition. The available variety of obesity-related measurements (weight,

height, BMI, waist and hip circumference, body and trunk fat percentage) in the CODING study also provided us an opportunity to strongly prove the association of food addiction with common form of human obesity by assessing the correlation of clinical symptom counts of food addiction with these measurements. In addition, the study in Chapter 2 was our first step in understanding the dietary patterns particularly macronutrients consumption in food addicted individual recruited from the general population. Therefore, the objectives of my thesis in Chapter 2 was to assess: 1) the prevalence of food addiction in the Newfoundland population; 2) if the clinical symptom counts of food addiction are significantly correlated with the severity of obesity in the general population; 3) if individuals diagnosed as food addicted are significantly more obese than their non-food addicted counterparts; and 4) if food addicted subjects consumed more or less of any of the three macronutrients (i.e., fat, protein and carbohydrates).

The study in Chapter 3 and 4, as a first in this field, was to unerstand the probable etiology of food addiction from the aspects of hormonal levels, nutritional intakes, and genetics. Therefore, we employed a unique study design with two equally obese groups (matched with age, sex, and physical activity) but different in only one aspect which was a phenotype of food addition (diagnosed as food addicts or with high food addiction symptom count). This unique study design enabled us to distinguish the factors which are related to only food addiction from other confounding factors. As mentioned previously, evidence from both humans and animals support the similarities between food addiction and substance dependence. To find out whether the diffrences between the two groups of euqally obese

patients are related to food adiction, we sought for any clue that was common with other types of addictions.

As mentioned in the section 1.4.1, numerous hormones are likely involved in regulating appetite and food intake, however there is no study available regarding what hormones are related to food addiction. In addition, in section 1.4.2, a few evidence was mentioned in both animals and human that demonstrates which types of food can triger addiction. In Chapter 2, we started to reveal the nutritional charactersitics (macro-nutients intake) of food addcits in the general population, but still no reported evidence on the potential differences of macro- and micro-nutrients between being obese with and without food addiction, which will be critical to unravel how food addiction develops. Therefore, in Chapter 3 we attempted to explore potential biomarkers that may differentiate being obese with and without food addiction by measuring and comparing various hormones and neuropeptides that are regulating appetite and metabolism and dietary nutrient intakes between the groups.

As I mentioned previously in section 1.4.3, many studies have discovered genes related to other types of addiction, however, there is little information available regarding genes associated with an addictive tendency toward food. Therefore, our final goal in this thesis (Chapter 4) was to discover genes related to food addiction. To achieve this goal, we designed a two-stage study, a combination of exome sequencing method to screen the entire exome and a candidate gene association approach to verify food addiction candidate genes. Exome sequencing was employed as the first step because this technique enables the unbiased discovery of coding variations for subsequent association testing for complex traits. More importantly, exome sequencing is providing a well-defined and interpretable

target for mutations in the locus. These mutations create variants at these loci that can be identified as being associated with the trait which in our study was food addiction. Since this technique is very expensive, therefore, we designed our unique population as I mentioned previously to achieve our goal in a most effective way. Therefore, we proposed three sets of equally number of subjects (8 in each group) matched with age, sex, physical activity and to be non-smoker. Since one of the major challenges in finding food addiction genes in obese patients is to separate the genes causing obesity by food addiction from genes that cause obesity by other mechanisms, therefore both groups were obese and the key difference was food addiction clinical symptom counts. The third group was a healthy control group. The reason we chose food addiction clinical symptom counts unlike Chapter 3 was firstly based on the findings in Chapter 2 that provided us the evidence that the clinical symptom count of food addiction was highly associated with the severity of obesity. In addition, in Chapter 4 we aimed to investigate a quantitative relationship between addiction genes and addictive tendencies towards food; the food addiction diagnosis is a binary and "Yes" or "No" qualitative method would not meet this objective. Therefore, we decided to use the YFAS clinical symptom counts as a semi-quantitative genetic marker in the analysis. The large number of subjects in CODING study gave us this chance to verify our results in a candidate gene association approach.

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2

Chapter 2. Food Addiction: Its Prevalence and Significant Association with Obesity in the General Population

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2.1 Introduction

Overweight and obesity are the abnormal or excessive accumulation of adipose tissue generally resulting from a chronic positive energy imbalance (1, 2). Recently it has been shown that globally approximately 1.0 billion adults are overweight, and a further 475 million are obese (3). In the United States, the prevalence of obesity among adults increased by 1.1% between 2007 and 2009. If this trend continues, by 2050 close to 100% of Americans will be overweight or obese (4). Obesity and overweight are the fifth leading cause of global death (2) and the second most preventable cause of death in the United States (5). Obesity is a complex multifactorial disease but the causes are not yet completely known (6). Weight gain is usually the result of a complex interaction between an individual's biology and environmental factors which lead to energy surplus (7). In westernized society, one of the main causes of a chronic energy surplus is a reduced physical activity level owing to a sedentary lifestyle. Another equally important cause of energy surplus is overeating (8, 9). Overeating in some degree may occur in many individuals; however, a proportion may develop an obsessive/compulsive relationship to certain foods (10). These individuals chronically consume more food than they need to maintain health and show compulsive intake behaviors associated with loss of control of eating (9, 11).

Accumulating research evidence has documented neurobiological and behavioral similarities between compulsive overeating and psychoactive drug dependence, leading researchers to use the term of food addiction to describe this pattern of overeating (12-16). In animal models, foods high in sugar and fat are particularly associated with addiction-

like eating behavior (17-19). In human studies, it has also been suggested that the pattern of food intake in food addiction may parallel substance dependence and that this phenomenon might be understood with the same neurobiological, behavioral and clinical framework as conventional drug dependence (20-22). Some researchers have argued that food addiction should be included as a substance use disorder in the Diagnostic and Statistical Manual of mental Disorders (DSM; ref 23, 24), although others have been critical of the clinical validity or utility of the food addiction concept (9, 25). Recently, the Yale Food Addiction Scale (YFAS) has been developed, and validated, as a tool for the diagnosis of food addiction (26-28). The YFAS criteria have been used to explore the prevalence of food addiction in eating disorder patients (29), obese subjects (30) and junior college students (21). There is a growing interest in the role of food addiction in the increasing prevalence of human obesity which has reached an epidemic degree globally (14). However, the exploration of food addiction in humans is at an early stage and many fundamental questions are yet to be answered (25, 26).

First, the prevalence of food addiction in the general population has not yet been assessed and this is an essential first step towards evaluating the potential contribution of food addiction to human obesity. Only a few human studies are currently available and they were performed on specific cohorts like eating disorder patients (29), small stratified groups such as obese adults seeking weight loss (31) or junior college students (21). However, no data are currently available regarding the role of food addiction in the general population and there seems to be a high proportion of food addiction in obese with binge eating and obese seeking weight loss. However, the association of food addiction with BMI in junior college

students was negligibly weak. Therefore, a second equally important question to be answered is whether food addiction is significantly correlated with the severity of obesity in the general population. A third question concerns the intake of macronutrient in food addiction, because data suggest that each macronutrient may play a different role (32).

Hence the current study was designed to assess: 1) the prevalence of food addiction in the Newfoundland population; 2) if the clinical symptom counts of food addiction are significantly correlated with the severity of obesity in the general population; 3) if individuals classified as food addicted are significantly more obese than their non-food addicted counterparts; and 4) if food addicted subjects consumed more or less of any of the three macronutrients (i.e., fat, protein and carbohydrates).

2.2 Materials and Methods

Ethics Statement

This study was approved by the Health Research Ethics Authority (HREA), Memorial University of Newfoundland, Canada. All participants provided written informed consent.

Study Sample

A total of 652 participants (415 females, 237 males) were recruited from the Canadian province of Newfoundland and Labrador (NL) via advertisements, posted flyers, and word of mouth. The inclusion criteria were: 1) age >19 years, 2) born in NL with a family who lived in NL for at least three generations, 3) healthy without serious metabolic, cardiovascular or endocrine diseases, 4) not pregnant at the time of the study.

Anthropometric Measurements

Body weight, height, waist and hip circumference were measured after a 12 hours fasting period. Subjects were weighed to the nearest 0.1 (kg) in a standard hospital gown on a platform manual scale balance (Health O Meter, Bridgeview, IL). A fixed stadiometer was used to measure height to the nearest 0.1 (cm). Hip circumference was measured with the flexible measuring tape to the nearest 0.1 (cm) at the level of largest circumference between the waist and thighs while the participant was in a standing position. The same procedure was used to measure waist circumference at the level of the umbilicus, midway between the lowest rib and iliac crest. BMI was calculated by dividing participants' weight in kilograms by the square of his/her height in meter (kg/m2). The subjects were classified as underweight/normal (BMI $\leq$ 24.99) and overweight/obese (BMI $\geq$ 25.00) based on BMI according to World Health Organization criteria (33).

Body Composition Assessment

Whole body composition measurements including fat mass and lean body mass were measured using Dual-energy X-ray absorptiometry (DXA; Lunar Prodigy; GE Medical Systems, Madison, WI, USA). The measurements were performed in a supine position after 12 hours fasting. Total percent body fat (BF%) and percent trunk fat (TF%) were determined (34). The subjects were also classified as under/normal weight and overweight/obese based on BF% according to the criteria recommended by Bray (35).

Food Addiction Assessment

The diagnosis of food addiction was based on the Yale Food Addiction Scale (YFAS, ref 26). This questionnaire consists of 27 items that assess eating patterns over the past 12 months. The YFAS translates the Diagnostic and Statistical Manual IV TR (DSM-IV TR) substance dependence criteria in relation to eating behavior (including symptoms such as tolerance and withdrawal symptoms, vulnerability in social activities, difficulties cutting down or controlling substance use, etc.) by applying the DSM-IV TR. The scale uses a combination of Likert scale and dichotomous scoring options. The criteria for food addiction are met when three or more symptoms are present within the past 12 months and clinically significant impairment or distress is present. The Likert scoring option is used for food addiction symptom counts (e.g. tolerance and withdrawal) ranging from 0 to 7 symptoms (26, 29).

Macronutrient intake and Physical Activity Assessment

Macronutrient intake (protein, fat and carbohydrate) during the past 12 months was assessed using the Willett Food Frequency Questionnaire (FFQ; ref 36). Participants indicated their average use of a list of common food items, over the last 12 months. The amount of each selected food was converted to a mean daily intake value. The average daily intake for each food item consumed was entered into NutriBase Clinical Nutrition Manager (software version 9.0; CyberSoft Inc, Arizona). The total intake for each macronutrient per day was computed by the software for each subject (37). This questionnaire has been validated numerous times in different populations and has been validated in Newfoundland population (38-40).

The Baecke physical activity questionnaire was used to assess physical activity. This questionnaire assesses physical activity using three indices including work, sport and leisure (41).

Statistical Analysis

Statistical analyses were performed using the R project for statistical computing version 2.15.2 (R Development Core Team). Data are presented as mean ± standard deviations (SD), maximum and minimum. Student t-test analyses were used to investigate the differences in measured variables between females and males. The prevalence of food addiction was assessed in both the total cohort and different adiposity subgroups according to BMI and BF% by sexes. Relative risk ratios defined as the prevalence ratio were calculated to assess differences in the risk of food addiction between sexes and between participants of different obesity status.

Student t-tests and Mann-Whitney-U tests (a non-parametric test) were employed to compare the anthropometric data related to obesity measures and macronutrients intake between food addiction and non-food addiction groups. Furthermore, to take possible confounding factors into consideration, an ANCOVA was conducted to compare differences between food addicted and non-food addicted groups on obesity measurements with age, sex, smoking status, medication use, and physical activity entered as covariates. Spearman partial correlation coefficients controlling for age, sex, smoking, medication use and physical activity were calculated to investigate the association between food addiction and the severity of obesity. For all analyses, the alpha level was set at 0.05.

2.3 Results

Physical Parameters and Prevalence of Food Addiction

Demographic and physical characteristics of the participants are presented in Table 2.1. The prevalence of food addiction according to the YFAS criteria was 5.4% in the entire population (in women and men it was 6.7% and 3.0%, respectively; Table 2-2). When participants were classified as under/normal weight or overweight/obese based on BMI, the prevalence of food addiction was 1.6% and 7.7% in these two groups respectively. When subjects were classified as under/normal weight or overweight/ obese based on BF% the prevalence of food addiction was 2.9% and 6.8%, respectively. The percentage of food addiction significantly increased with increasing obesity status regardless of how adiposity was defined (RR = 0.21, $p < 0.001$ and RR = 0.42, $p = 0.03$, respectively). When the sample was split based on gender, this trend remained significant only in females whose adiposity was classified using BMI (RR = 0.13, $p < 0.001$). The prevalence of food addiction was higher in women than in men (RR = 2.28, $p = 0.046$). Additionally, when using BMI adiposity classifications, but not the BF% adiposity classifications, overweight/ obese women had higher prevalence of food addiction as compared to overweight/ obese men (RR = 3.50, $p = 0.002$).

When food addicted subjects were classified by weight status based on BMI, 11.4% were under/normal weight, 88.6% were overweight/obese. When food addicted subjects were classified into adiposity group based on BF%, 20% were under/normal weight, 80% were overweight/obese (Table 2-3).

Correlations between clinical symptom counts of food addiction and obesity

Spearman partial correlation coefficients controlling for sex and age were used to assess the relationship between the symptom counts of food addiction and obesity measurements in the entire sample and in the non-food addicted subjects. All obesity related measurements (specifically markers related to central obesity) had strong positive correlations with YFAS symptom counts in both groups (Table 2-4). Furthermore, when we controlled for potential confounding factors including smoking, medication use and physical activity, the correlations remained significant.

Table 2-1 Characteristics of Study Participants*

	Entire cohort Mean ± SD (Min - Max)	Female Mean ± SD (Min - Max)	Male Mean ± SD (Min - Max)
Number	652	415	237
Age (year) †	44.3 ± 12.9 (20 - 90)	45.1 ± 12.9 (20 - 90)	42.9 ± 12.8 (20 - 75)
Height (cm) †	168.4 ± 9.1 (147.7 - 196.6)	163.3 ± 5.9 (147.7 - 187.6)	177.3 ± 6.6 (156.8 - 196.6)
Weight (kg) †	78.1 ± 18.3 (46.6 - 149.8)	71.5 ± 15.7 (46.6 - 149.8)	89.8 ± 16.6 (57.1 - 149.5)
BMI (kg/m^2) †	27.4 ± 5.4 (17.05 - 54.2)	26.8 ± 5.7 (17.05 - 54.2)	28.5 ± 4.6 (19.10 - 42.5)
BF% †	33.3 ± 10.2 (5.3 - 60.2)	37.3 ± 8.8 (16.2 - 60.2)	26.1 ± 8.2 (5.3 - 42.6)
TF%†	36.3 ± 10.6 (5.3 - 61.8)	38.9 ± 10.1 (15.5 - 61.8)	31.7 ± 9.9 (5.3 - 50.7)
Waist (cm) †	94.9 ± 14.9 (52 - 168)	91.7 ± 15.02 (52 - 168)	100.6 ± 12.8 (65 - 131)
Hip (cm)	100.5 ± 12.1 (74 - 155)	100.2 ± 13.2 (74 - 155)	100.9 ± 9.9 (79 - 134)
Waist / Hip †	0.9 ± 0.08 (0.68 - 1.62)	0.9 ± 0.07 (0.68 - 1.62)	1.0 ± 0.05 (0.75 - 1.14)

* Mean ± standard deviation (SD), (Maximum – Minimum), BMI – Body mass index, BF% – percent body fat, TF% – percent trunk fat.

† Significant difference between women and men (Independent t-test, $p < 0.05$).

Table 2-2 Prevalence of food addiction according to sex and obesity status*

		Entire population (%)	Female (%)	Male (%)	*Relative Risk*
Entire Cohort		5.4	6.7	3.0	*2.28*†
Obesity status (BMI)	Under/Normal weight	1.6	1.5	1.9	*0.81*
	Overweight/Obese	7.7	11.4	3.3	*3.50*†
	Relative Risk	*0.21*‡	*0.13*‡	*0.58*	
Obesity status (%BF)	Under/Normal weight	2.9	3.7	1.3	*2.96*
	Overweight/Obese	6.8	8.7	3.8	*2.28*
	Relative Risk	*0.42*‡	*0.43*	*0.33*	

* Prevalence of food addiction (%), BMI – Body mass index and BF% – percent body fat.
Obesity status (Under/Normal weight and Overweight/Obese) was defined by BMI (33)and %BF according to the Bray (35) and world health organization (WHO) criteria, respectively.

† Relative risk between females and males (Fisher's exact test, $p < 0.05$).

‡ Relative risk between under/normal and overweight/obese groups (Fisher's exact test, $p < 0.05$).

Table 2-3 The proportion of food addiction according to obesity status*

		Under/Normal weight% (n)	Overweight/Obese% (n)
Food addition	BMI	11.4% (4)	88.6% (31)
	BF%	20.0% (7)	80.0% (28)
Entire Cohort	BMI	38.2% (249)	61.8% (403)
	BF%	37.1% (242)	62.9% (410)

* Proportion of food addiction (%), number of food addicts (n), BMI – Body mass index and BF% – percent body fat.
Obesity status (Under/Normal weight and Overweight/Obese) was defined by BMI (33)and %BF according to the Bray (35) and world health organization (WHO) criteria, respectively.

Table 2-4 Correlation between food addiction clinical symptom counts with obesity measurements*

		BMI (kg/m^2)	Weight (Kg)	Hip (cm)	Waist (cm)	Waist/ hip	Height (cm)	BF%	TF%
Entire									
Cohort	r	0.36	0.35	0.36	0.35	0.15	0.0091	0.31	0.32
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.82	<0.001	<0.001
NFA									
	r	0.32	0.30	0.31	0.30	0.12	0.007	0.27	0.28
	p	<0.001	<0.001	<0.001	<0.001	0.003	0.86	<0.001	<0.001

* NFA – non-food addiction, BMI – body mass index, BF% – percent body fat and TF% – percent trunk fat. Significance level for Spearman partial correlation (r), controlling for age and sex, were set to $p < 0.05$.

Comparison of obesity measurements and macronutrient intake between food addiction and non- food addiction groups

Both student t-test and Mann- Whiney U test showed significant differences in all obesity measurements between food addiction and non-food addiction groups ($p < 0.001$) (Table 2-5). To consider the other confounding factors, we conducted an ANCOVA controlling for sex, age, medication use, physical activity and smoking. All the differences remained significant. Food addicted subjects on average weighed 11.72 kg more and carried 4.61 more BMI than non- food addicted subjects. Additionally, food addicted subjects had 8.2% greater body fat and 8.5% more trunk fat.

Macronutrient intake was compared for the food addiction and non-food addiction group (Table 2-5). Overall, the amount of macronutrients consumed, expressed as gram per kilogram of body weight, was not significantly different between the food addicted and non-food addicted participants. However, the percent calorie intake from protein ($p = 0.04$ from Mann-Whitney-U test and $p = 0.03$ from ANCOVA) and the percent calorie intake from fat ($p = 0.04$ from Mann-Whitney-U test, $p = 0.11$ from ANCOVA) was significantly higher in food addicted as compared to non-food addiction participants.

Table 2-5 Obesity measurements and macronutrient intake characteristics of food addiction and non-food addiction*

	FA (Mean ± SD)	NFA (Mean ± SD)	Mean difference	P t test	P Mann-Whitney-U test
BMI (kg/m^2)	31.8 ± 6.6	27.2 ± 5.2	4.6	<0.001	<0.001
Weight (kg)	89.2 ± 21.5	77.5 ± 17.9	11.7	0.003	<0.001
Waist (cm)	105.5 ± 15.3	94.4 ± 14.6	11.2	<0.001	<0.001
Height (cm)	167.2 ± 9.4	168.5 ± 9.1	-1.3	0.42	0.25
Hip (cm)	110.7 ± 14.7	99.9 ± 11.7	10.8	<0.001	<0.001
BF%	41.04 ± 9.3	32.8 ± 10.05	8.2	<0.001	<0.001
TF%	44.3 ± 9.4	35.8 ± 10.5	8.5	<0.001	<0.001
Fat (g/kg) †	0.8 ± 0.4	0.8 ± 0.7	0.005	0.95	0.59
Carbohydrates (g/kg) †	3.5 ± 1.8	3.9 ± 2.7	0.4	0.25	0.23
Protein (g/kg) †	1.2 ± 0.5	1.2 ± 0.9	-0.06	0.47	0.89
Fat (%) ‡	26.6 ± 7.5	24.3 ± 7.2	2.3	0.08	0.04
Carbohydrate (%) ‡	52.2 ± 7.4	54.3 ± 8.5	2.1	0.11	0.07
Protein (%) ‡	19.0 ± 3.8	17.9 ± 3.9	1.1	0.10	0.04

* Mean ± standard deviation (SD), FA – food addiction, NFA – non-food addiction, BMI – body mass index, BF% – percent, body fat and TF% – percent trunk fat.

Independent t-test and Mann-Whitney-U test significance level was set to $p < 0.05$.

† Macronutrient intake (g) per unit body weight (kg).

‡ Macronutrient intake (% total calorie intake).

2.4 Discussion

In general, regardless of the various genetic predispositions and environmental influences, overeating is the primary factor responsible for the increasing prevalence of human obesity (14, 24). To the best of our knowledge this is the first study reporting the contribution of food addiction to the prevalence of human obesity in the general population (21, 29, 30). One important finding is an estimation of the prevalence of food addiction in the general Newfoundland population was at 5.4% (6.7% in women and 3.0% in men). In a previous study assessing obese patients with BED, the prevalence of food addiction was reported to be as high as 56.8% (29), suggesting an overlap between binge eating and food addiction. The prevalence of food addiction in obese individuals seeking weight loss treatment was 25%, while in another study obese subjects not seeking weight loss, the prevalence of food addiction was 15.2% (30, 31). In a cohort of junior college students with a normal BMI range, 8.8% met the YFAS criteria of food addiction; however the correlation between food addiction clinical symptom counts and BMI was negligible (21, 42). Our results indicated that 80-88.6% of food addicted individuals were overweight/obese based on Bray or BMI criteria providing strong evidence that food addiction has contributed to the rising prevalence of obesity in the general population. Of note, food addicted individuals were also observed in the underweight and normal weight cohort, however in a lower number. The current findings suggest that obesity featured with food addiction may represent an important subgroup of the obese with a distinctive aetiology. The identification of this subgroup will open a novel avenue to assess the aetiology of obesity and thus aid in finding new effective methods to treat and prevent obesity.

The subjects in the present study were recruited from the general Newfoundland population. The prevalence of overweight/obesity in the current study is similar to data reported from Health Canada on the province of Newfoundland (62.1%; ref 43). The prevalence of food addiction revealed in our study on the Newfoundland population may, to some degree, represent the prevalence in other Canadian provinces. Moreover, our findings also suggest a potential difference between men and women in regards to food addiction, as overweight/obese women classified using BMI had a significantly higher rate of food addiction as compared to men. This is similar to the case with eating disorders in which women also are significantly more likely to suffer from an eating disorder than men (44, 45). Nevertheless, larger studies in other populations are warranted to confirm the findings from our investigation.

The third major finding from the current study is the significant correlation between food addiction and the severity of obesity in the general Newfoundland population. This finding appears to be robust as we were able to demonstrate this significant correlation throughout a number of analyses controlling for many confounding factors. Firstly, the clinical symptom counts of food addiction was significantly correlated not only with BMI, but also with virtually all obesity related measurements including body weight, waist and hip circumferences, body fat and trunk fat percentage determined by DXA, an accurate measurement of body composition. This close correlation was seen in the non-food addicted group as well. We suggest that these robust and multiple correlations demonstrated a true association of food addiction with human obesity. Additionally, it was shown that obesity related variables were significantly different between food addicted and non-food

addicted subjects. Participants who met criteria for food addiction on average weighed 11.7 kg (25.79 lbs) more, had 4.6 higher BMI and possessed a 8.2% and 8.5% greater total body fat and trunk fat, respectively, as compared to non-food addicted subjects. These data provide the first direct evidence that food addiction is strongly associated with obesity in the general population. Importantly, the individuals who met the criteria for food addiction only represent between one fifth to one sixth of the total proportion of obese individuals in Newfoundland (25-30%; ref 43). This suggests that food addiction is likely an important factor in the development of human obesity but not the sole contributor.

Another important goal of our study was to examine differences in dietary patterns particularly macronutrients consumption between food addicted and non-food addicted subjects. Interestingly, the food-addicted subjects' diet consisted of a higher percentage of calories from fat and protein, possibly suggesting that these types of foods are more likely to be associated with compulsive overeating. Given the significance of these findings will be important to verify these findings in other populations.

In the present study the YFAS was used as a diagnostic tool to classify participants with food addiction, as this set of measure and the criteria on which it is based have been validated (26-28). Rather than directly asking if the subjects were addicted to food, the questionnaire assessed food addiction based on DSM-IV-TR criteria (42). Furthermore, using this set of criteria helped to distinguish subjects who regularly indulged in hyper palatable foods from those who have lost control over their eating behaviour (26).

One limitation of the present study was that the number of female participants was larger than the number of males. Given the sex difference in the prevalence of food addiction found in the present study, it is possible that the actual prevalence in the general population may be lower than 5.4% if the study had consisted of equal numbers of women and men. Future studies using cohorts with an equal number of females and males in the population are warranted.

In summary, our study has revealed for the first time that: 1) the prevalence of food addiction in the general Newfoundland population was 5.4%; 2) women are at high risk of food addiction than men; 3) food addiction contributes to human obesity and is significantly associated with the severity of obesity/amount of body fat from normal to obese individuals in the general population. Our findings provide strong evidence that food addiction may represent a distinct aetiology of human obesity in the general population.

Author Contributions

Pardis Pedram is the first author: coordinating data collection, analyzing the data and interpreting the results, as well as the preparation of the manuscript. Guang Sun had the general scientific responsibility in the study design, data interpretation and manuscript revision.

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3

Chapter 3. Hormonal and Dietary Characteristics in Obese Human Subjects with and without Food Addiction

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3.1 Introduction

Obesity is a multifaceted condition (1) and represents a pandemic that needs urgent attention (2). In Canada, over one in four adults are obese (3), and the province of Newfoundland has one of the highest rates of obesity in the country (after the Northwest Territories and Nunavut; ref 3, 4). Obesity is caused by multiple factors, including genetics, endocrine function, behavioral patterns and environmental determinants (5). It has been well documented that chronic overconsumption of calories plays a fundamental role in the development of obesity (6). In a previous study on the general Newfoundland population, our laboratory discovered that chronic compulsive overeating, defined as "food addiction" by the Yale Food Addiction Scale (YFAS; refs 7, 8), significantly contributes to human obesity (9). Additionally, the clinical symptom counts of food addiction defined by the YFAS is highly associated with the severity of obesity (9). Addiction is considered a psychological disorder with a definite neuro-endocrine basis; however, food addiction is still not defined as an independent disorder in Diagnostic and Statistical Manual (DSM) V (10, 11). Similar to drug addiction, food addicts lose control over food consumption despite the negative consequences relevant to obesity (12, 13). This suggests that they suffer from repeated failed attempts to reduce their food intake, and they are unable to abstain from certain types of food or to reduce consumption (12).

In humans, the regulation of food intake is based on an intricate feedback system controlled by hunger and satiety signals (5, 14, 15). These signals are generated in the brain, peripheral tissue and/or organs through two complementary drives, including both homeostatic and hedonic pathways (5, 15-17). The hedonic or reward-based regulation pathway is related

to the mesolimbic dopamine pathway, which is stimulated in both drug abuse and the consumption of highly palatable foods (15). Evidence has shown that the release of dopamine coordinates food reward, which is impaired in food addicts (15, 18). Contrastingly, the homeostatic pathway primarily regulates the energy balance between the brain and peripheries (for instance, digestive tract and adipose tissue; refs 14, 17, 19, 20). This means that based on energy reservation and the psychological want for food, the brain increases or decreases food intake by interpreting the neuronal and hormonal signals received form peripheries (15, 20, 21). Therefore, in both pathways, a large number of neurotransmitters (dopamine, cannabinoids, opioids, gamma-aminobutyric acid (GABA) and serotonin), neuropeptides (α-MSH, β-endorphin, cortisol, melatonin, neurotensin, orexin A, oxytocin and substance P, etc.) and hormones (gut hormones, anterior pituitary hormones and adipokines) are involved, many of which can also be detectable in serum (18, 20, 22-31). Interestingly, many studies have linked these hormones and neuropeptides with the current obesity epidemic (22, 23, 25, 32, 33). Moreover, in our previous aforementioned study on the general Newfoundland population, we have reported that food addicts consumed a higher percentage of calories from fat and protein (9). However, to the best of our knowledge, there is no study available regarding the differences in appetite regulating hormonal level between being obese with and without food addiction.

Furthermore, macronutrients have been reported to play an imperative role in obesity, addiction-like behaviour and metabolic consequences (34-36). However, there is no study available on the hormonal characteristics and potential differences of macro- and micro-nutrients between being obese with and without food addiction, which will be critical to

unravel how food addiction develops. Hence, the aim of the current study is to explore potential biomarkers that may differentiate being obese with and without food addiction by measuring and comparing various hormones and neuropeptides regulating appetite and metabolism and dietary nutrient intakes in both groups.

3.2 Experimental Section

Ethics Statement

This study was approved by the Health Research Ethics Authority (HREA), Memorial University of Newfoundland, St. John's, Canada, with Project Identification Code #10.33, (latest date of approval: 21 January 2014). All participants provided written and informed consent.

Study Sample

The food addiction study consists of 737 subjects recruited from the general Newfoundland and Labrador (NL) population. Among them, 36 subjects met the criteria of food addiction by the Yale Food Addiction Scale. Subjects with a body mass index (BMI) of 25 kg/m^2 or less were excluded [WHO criteria: greater than 25 is classified as overweight; over 30 is classified as obese (37)]. After exclusion, 29 subjects were left for analysis. Correspondingly, 29 non-food-addicted overweight/obese (NFO) subjects were selected and matched for age, sex, BMI and physical activity. All of the subjects were part of the population CODING (Complex Diseases in the Newfoundland population: Environment and Genetics) study (38, 39) and were recruited from the Canadian province of

Newfoundland and Labrador using advertisements, posted flyers and word of mouth. The inclusion criteria were: (1) age >19 years; (2) born in NL with family who lived in NL for at least three generations; (3) healthy without serious metabolic, cardiovascular or endocrine diseases; and (4) not pregnant at the time of the study.

Anthropometric Measurements

Body weight and height were measured after a 12-h fasting period. Subjects were weighed to the nearest 0.1 (kg) in a standard hospital gown on a platform manual scale balance (Health O Meter, Bridgeview, IL, USA). A fixed stadiometer was used to measure height to the nearest 0.1 (cm). BMI was calculated by dividing participants' weight in kilograms by the square of his/her height in meter (kg/m^2). The subjects were classified as overweight/obese (BMI $\geq$ 25.00) based on BMI according to the World Health Organization criteria (37).

Body Composition Assessment

Whole body composition measurements including fat mass and lean body mass were measured using dual-energy X-ray absorptiometry (DXA; Lunar Prodigy; GE Medical Systems, Madison, WI, USA). The measurements were performed in a supine position after 12 h fasting, and the total percent body fat (BF%) and percent trunk fat (TF%) were determined (40).

Food Addiction Assessment

The diagnosis of food addiction was based on the Yale Food Addiction Scale (YFAS; refs 7, 41). This questionnaire consists of 27 items that assess eating patterns over the past 12 months. The YFAS translates the Diagnostic and Statistical Manual IV, Text Revision (DSM-IV TR) substance dependence criteria in relation to eating behavior (including symptoms, such as tolerance and withdrawal symptoms, vulnerability in social activities, difficulties cutting down or controlling substance use, *etc.*) by applying the DSM-IV TR. The scale uses a combination of Likert scale and dichotomous scoring options. The criteria for food addiction are met when three or more symptoms are present within the past 12 months and clinically significant impairment or distress is present. The Likert scoring option is used for food addiction symptom counts (for instance, tolerance and withdrawal), ranging from 0 to 7 symptoms (13, 42).

Dietary Intakes Assessment

Macronutrients (protein, fat and carbohydrate) and 71 micronutrients intake during the past 12 months were assessed using the Willett Food Frequency Questionnaire (FFQ; ref 43). Participants indicated their average use of a list of common food items, over the last 12 months. The amount of each selected food was converted to a mean daily intake value. The average daily intake for each food item consumed was entered into NutriBase Clinical Nutrition Manager (software version 9.0; CyberSoftInc, Phoenix , AZ, USA), and daily intake of macro- and micro-nutrient intakes were computed (41, 44, 45).

Serum Metabolism Regulating Hormones and Neuropeptides Measurement

The concentration of a total of 34 hormones and neuropeptides were measured by magnetic bead-based quantitative immunoassay using the MAGPIX system (Millipore, Austin, TX, USA) or using enzyme-linked immunosorbent assays (ELISA; ALISEI QS, Radim, Italy) (using morning fasting serum). Gut hormones [amylin (total), ghrelin (active), leptin, total glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), pancreatic polypeptide (PP), pancreatic peptide YY (PYY), connecting peptide (C-peptide) and glucagon), pituitary polypeptide hormones (prolactin, brain-derived neurotrophic factor (BDNF), adrenocorticotropic hormone (ACTH), ciliary neurotrophic factor (CNTF), follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH) and thyroid-stimulating hormone (TSH)), adipokines (adiponectin, lipocalin 2, resistin, adipsin, plasminogen activator inhibitor-1 (PAI-1) and TNF-α) and neuropeptides (alpha-melanocyte-stimulating hormone (α-MSH), β-endorphin, cortisol, melatonin, neurotensin, orexin A, oxytocin, substance P, monocyte chemotactic protein-1 (MCP-1) and Agouti-related peptide (AgRP)] were measured in duplicate using the magnetic bead-based quantitative immunoassay with the MAGPIX system. The system was calibrated prior to each assay with the MAGPIX calibration kit, and performance was verified with the MAGPIX performance verification kit. Milliplex Analyst software was used for the analyses of data. Moreover, the concentration of fasting neuropeptide Y (NPY) was measured with the ELISA method (Millipore Corporation Pharmaceuticals, Billerica, MA, USA). All measured hormonal and neuropeptide levels were above the manufactural sensitivity. Moreover, there was no/negligible cross-reactivity between the antibodies for an analyte and any of the other analytes in these panels.

Serum Lipids, Glucose and Insulin Measurement

Concentrations of serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triacylglycerols (TG) and glucose were analyzed using Synchron reagents with an Lx20 analyzer (Beckman Coulter Inc., Fremont , CA, USA). Low-density lipoprotein (LDL) cholesterol was calculated by the following: total cholesterol-HDL-TG/2.2. Serum insulin was evaluated using an immunoassay analyzer (Immulite; DPC, Los Angeles, CA, USA). Additionally, the serum insulin level was measured using an immunoassay analyzer (Immulite; DPC, Los Angeles, CA, USA; ref 46, 47).

Physical Activity Assessment and Other Covariates

The Baecke physical activity questionnaire was used to assess physical activity. This questionnaire assesses physical activity using three indices, including work, sport and leisure. All participants completed forms to screen medical history, demographics (gender, age and family origin), disease status, cigarette usage and medication use (48, 49).

Statistical Analysis

All statistical analyses were completed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviations (SD). Student's *t*-test analyses were employed to investigate the differences in measured variables between food addicted and non-food-addicted obesity. For all analyses, statistical tests were two-sided and the alpha level was set at 0.05.

3.3 Results

Physical Characteristics and Fasting Serum Lipids, Glucose and Insulin Level

Demographic, fasting serum lipids, glucose and insulin level and physical characteristics of the participants are presented in Table 3-1 (adiposity is based on BMI). There were no significant differences for the aforementioned variables between the food-addicted overweight/obese (FAO) and non-food addicted-overweight/obese (NFO) groups.

The Comparison of Metabolism Regulating Hormones and Neuropeptides in Food-Addicted and Non-Food-Addicted Overweight/Obese

Serum hormonal levels were compared between the food addiction overweight/obese and non-food addiction overweight/obese groups (Table 3-2). The FAO group had a significantly lower level of amylin, TNF-α and TSH and a higher level of prolactin, as compared to the NFO group ($p < 0.05$).

Table 3-1 Characteristics of the study participants *.

Variables		NFO (Mean ± SD)	FAO (Mean ± SD)
Number		29	29
Age (year)		42 ± 8.9	42.5 ± 9.4
Sex	F	24	24
	M	5	5
BMI (kg/m^2)		32 ± 4.42	32.5 ± 6
BF%		42.32 ± 6.4	42.7 ± 7.8
TF%		45.1 ± 5.3	46.2 ± 7.1
Physical activity		7.1 ± 1.3	7.3 ± 1.1
Glucose (mmol/L)		5.2 ± 1	5.3 ± 0.8
Cholesterol (mmol/L)		5.3 ± 1	4.9 ± 1.3
TG (mmol/L)		1.4 ± 0.9	1.3 ± 0.7
HDL (mmol/L)		1.3 ± 0.3	1.4 ± 0.3
LDL (mmol/L)		2.9 ± 1.1	3.3 ± 1.0
Albumin (g/L)		39.5 ± 3.3	39.1 ± 2.9
Insulin (pmol/L)		90.4 ± 101.9	95.9 ± 139.9

* Mean ± standard deviation (SD); BMI, body mass index; BF%, percent body fat; TF%, percent trunk fat; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; FAO, food-addicted overweight/obese; NFO, non-food-addicted overweight/obese was defined by BMI according to World Health Organization (WHO) criteria (37) .

Table 3-2 Hormonal and neuropeptide characteristics in food-addicted and non-food-addicted overweight/obese *.

Hormones		FAO Mean ± SD (14–29)	NFO Mean ± SD (14–29)	*p* **
Neuropeptides	NPY (pg/mL)	8.81 ± 3.74	5.71 ± 3.82	0.65
	α-MSH (pg/mL)	148.06±84.16	147.2±89.12	0.88
	β-Endorphin (pg/mL)	377.86±90.82	396.54±108.3	0.48
	Cortisol (pg/mL)	230,056±100,323	232,807.9±138,900	0.09
	Melatonin (pg/mL)	3,320.9±1,377.7	3,652.75±1,652.43	0.65
	MCP1 (pg/mL)	294.43±88.2	282.56±90.11	0.83
	Neurotensin (pg/mL)	379.6±103.05	379.32±100.7	0.84
	Oxytocin (pg/mL)	119.5±49.13	120.22±57.86	0.78
	Orexin A (pg/mL)	969.6±438.2	974.5±347.5	0.28
	AGRP (pg/mL)	16.11±6.94	16.18±8.26	0.88
	Substance P (pg/mL)	39.16±12.51	39.7±15.06	0.53
Gut hormones	Amylin (pg/mL)	24.9±11.3	32.05±18.75	0.04
	GLP-1 (pg/mL)	19.91±22.54	21.4±22.1	0.10
	Ghrelin (pg/mL)	25.4±15.8	25.91±17	0.9
	Leptin (pg/mL)	20,795.4±12,173.3	18,206.72±10,765.9	0.50
	GIP (pg/mL)	17±16.31	17.05±12	0.90
	Glucagon (pg/mL)	22.61±10.5	45.1±52.02	0.77
	PP (pg/mL)	49.3±79.4	46.85±53.4	0.50
	PYY (pg/mL)	68.33±122.3	93±109.3	0.45
	C-peptide (pg/mL)	1,373.7±740.15	1269±506.74	0.50
Pituitary polypeptide hormones	Prolactin (pg/mL)	2,335.1±1,197.8	1,938.3±745.5	0.02
	ACTH (pg/mL)	3.05±2.56	5.55±6.93	0.12
	BDNF (pg/mL)	2,219.3±658.73	2,138.38±931.52	0.17
	LH (mlU/mL)	6.2±7.3	6.21±8.6	0.93
	FSH (mlU/mL)	13.27±18.75	9.58±14.12	0.35
	GH (pg/mL)	505.76±635.84	810.83±1,019.56	0.07
	TSH (μlU/mL)	0.23±0.32	1.1±2.14	0.01
	CNTF (pg/mL)	148.1±324.22	1,647.4±6,280.6	0.06
Adipokines	TNF-α (pg/mL)	4.21±1.23	4.5±2.2	0.02
	Adiponectin (pg/mL)	65,700.8±68,327.1	71,437.3±56,215.3	0.71
	Lipocalin (pg/mL)	357±151.7	462.2±153	0.71
	Adipsin (pg/mL)	7,167.66±2,888.25	8,009.9±2,733	0.86
	PAL1 (pg/mL)	261.3±88.8	261.31±88.84	0.80
	Resistin (pg/mL)	82±43.4	109±55.8	0.33

* Mean ± standard deviation (SD); FAO, food-addicted overweight/obese; NFO, non-food-addicted overweight/obese; NPY, neuropeptide Y; α-MSH, alpha-melanocyte-stimulating hormone; MCP1, monocyte

chemotactic protein-1; AGRP, agouti-related peptide; GLP-1, glucagon-like peptide-1; GIP, gastric inhibitory polypeptide; PP, pancreatic polypeptide; PYY, pancreatic peptide YY; ACTH, adrenocorticotropic hormone; BDNF, brain-derived neurotrophic factor; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; TSH, thyroid-stimulating hormone; CNTF, ciliary neurotrophic factor; PAL1, plasminogen activator inhibitor-1.

****** The independent *t*-test was set to $p < 0.05$. Some significant results would be no longer significant if multiple corrections were performed.

Comparison of Macronutrients and Micronutrients Intake between Food Addiction and Non-Food Addiction Overweight/Obese Groups

Total calorie intake and macronutrients consumed expressed in absolute grams and in gram per kg of body weight, BMI, %BF and %TF are shown in Table 3-3. Total calorie intake per kg of body weight was significantly higher in the FAO group. The amount of carbohydrate intake per kg of body weight, fat consumed (per kg body weight, per BMI, per percentage of trunk fat) and the percent calorie intake from fat were significantly higher in food-addicted obesity as compared to non-food-addicted obese subjects ($p < 0.05$).

In addition, micronutrient intakes expressed as gram per kg body weight were compared between the two groups (Table 3-4). In general, FAO consumed significantly higher amounts of dietary sugar, mineral substances, including sodium, potassium, calcium and selenium, fat, saturated fat, trans fat, monounsaturated fat, omega 3, omega 6, vitamin D and gamma-tocopherol than the NFO group.

Table 3-3 Macronutrient intake characteristics in food addiction and non-food addiction overweight/obese groups *.

Macronutrients		FAO (*n* = 29)	NFO (*n* = 29)	*p*
		Mean±SD	Mean±SD	
Calorie intake	Per person	2,077.4±687.6	1,714.0±612	0.7
	per kg body weight	24.4±10.9	19.5±6.6	0.02
	per BMI	66.2±26.5	54.1±19.5	0.3
	per BF%	50±16.4	42.0±19.4	0.7
	per TF%	45.6±14.8	38.6±15.5	0.8
Fat (g)	Per person	63.6±26.3	45±15.6	0.054
	per kg body weight	0.7±0.4	0.5±0.2	0.004
	per BMI	2±0.9	1.4±0.5	0.01
	per BF%	1.5±0.7	1.1±0.5	0.1
	per TF%	1.4±0.6	1±0.4	0.04
	percent calorie	27.1±7.5	23.4±4	0.005
Carbohydrate (g)	Per person	273±103	246.3±93	0.6
	per kg body weight	3.2±1.6	2.8±1	0.03
	per BMI	8.7±3.9	7.8±3	0.2
	per BF%	6.5±2.4	6.01±2.8	0.6
	per TF%	6±2.2	5.5±2.3	1
	percent calorie	51.2±7.1	56.3±5.2	0.3
Protein (g)	Per person	99±29	79.2±30.8	0.8
	per kg body weight	1.1±0.4	0.9±0.3	0.2
	per BMI	3.1±1.1	2.5±1	0.3
	per BF%	2.4±0.7	1.9±0.9	0.8
	per TF%	2.2±0.6	1.8±0.7	0.9
	percent calorie	19.3±3.9	18.2±2.6	0.2

* Mean ± standard deviation (SD); FAO, food-addicted overweight/obese; NFO, non-food-addicted overweight/obese; BMI, body mass index; BF%, percent body fat; TF%, percent trunk fat.

The independent *t*-test was set to $p < 0.05$.

Table 3-4 Significant differences of selected micronutrient intakes between food addicts (FAO) and non-food addicts (NFA) of overweight/obese groups *.

Micronutrient Intake	**FAO (*n* = 29)**	**NFO (*n* = 29)**	***p***
	Mean±SD	**Mean±SD**	
Sugar (g/kg)	1.4±0.8	0.2±0.5	0.03
Saturated fat (g/kg)	0.3±0.1	0.2±0.1	0.01
Trans fat (mg/kg)	1.0±0.0	0.1±0.0	0.01
Monounsaturated fat (g/kg)	0.3±0.1	0.2±0.1	0.01
Poly-saturated fat (g/kg)	0.1±0.1	0.1±0.0	0.0
Omega 3 (mg/kg)	7.0±0.0	5.0±0.0	0.01
Omega 6 (g/kg)	0.1±0.0	0.03±0.0	0.0
Vitamin B1 (mg/kg)	0.02±0.01	0.02±0.0	0.04
Vitamin D (IU/kg)	2.5±2.1	1.9±1.0	0.04
Dihydrophylloquinone (mcg/kg)	0.3±0.0	0.2±0.0	0.03
Gamma tocopherol (mg/kg)	0.3±0.0	0.0±0.0	0.04
Sodium (mg/kg)	26.1±12.0	19.4±6.3	0.01
Calcium (mg/kg)	13.0±7.1	10.0±4.0	0.02
Potassium (mg/kg)	50.8±21.3	41.2±16.8	0.04
Selenium (mg/kg)	1.4±0.6	1.1±0.3	0.02

* Mean ± standard deviation (SD); FAO, food-addicted overweight/obese; NFO, non-food-addicted overweight/obese. The independent *t*-test was set to $p < 0.05$.

3.4 Discussion

In general, endocrine factors have an important role as appetite regulating signals. A large number of hormones play a role in feeding regulation (15-17, 25). The abnormality in the aforementioned hormonal secretions can lead to overeating and, consequently, obesity (16, 25). Interestingly, similarities in hormonal changes have been found between obesity and substance abuse addiction (10, 18). According to the etiology, obesity is a complex disease and can be caused by many genetic and environmental factors. As we previously reported, food addiction may be an important factor leading to obesity as a unique etiology (41). To the best of our knowledge, this study is the first to attempt to prove the idea that obesity with a definite food addiction may manifest distinguished dietary intake and hormonal characteristics.

The first finding in the current study was the significantly lower serum level of TSH and the higher level of prolactin in obese food addicts as compared to obese non-food addicts. Several population-based studies have shown a significant association of BMI with TSH and prolactin levels (50-54) but it is not clear why and which sub-group of obese is associated with the abnormality of these hormones. Findings from our current study indicate that the combined abnormality of TSH and prolactin might be one of the hormonal characteristics in obesity with food addiction rather than in the general common form of obesity. Data from a number of studies have suggested that the serum TSH level may be a marker of alcohol, opium and cocaine dependence and craving (55-57). A significant negative correlation between TSH level and alcohol craving has been reported in alcohol-dependent subjects (55), and a significantly lower level of TSH has been found in opium

users as compared to healthy controls (58). However, we are the first lab that discovered low TSH is associated with obese food addicts. Taken together with our present findings, a lower level of circulating TSH is not only associated with alcohol, opium and cocaine dependence, but also with food addiction. The significant association of prolactin in obese food addicts and the data from other studies on alcoholics, heroin and cocaine addicts with elevated basal prolactin (55, 59-62) strongly suggests the involvement of circulating prolactin with food addiction, as well.

Another significant finding in the current study is the significant lower level of serum TNF-α in the obese food addiction group as compared to the obese non-food addiction group. TNF-α level is usually higher in the obese people compared to healthy controls (63). TNF-α is known as an anorexigenic cytokine, which reduces food intake. It is thought that the impaired actions of TNF-α may lead to obesity (33). It was reported that the levels of circulating TNF-α have been altered in alcoholics, cocaine abusers and opiate addicts. In addition, it has been suggested that TNF-α can be a potential diagnostic biomarker for drugs of abuse (64-69). In an animal model, TNF-α has been investigated as a potential therapeutic target to prevent drug abuse and to increase the chance of cessation. (65). The current findings of the association of low TNF-α with food addiction is very interesting and unique. There is more likely a specific manifestation in obese food addicts contrary to the increased level of TNF-α in obese people.

In the current study, we also measured serum neuropeptides regulating appetite. Neuropeptides are predominately synthesized and secreted in the central nervous system; however, levels of some neuropeptides can be detected in the peripheral circulation system

(23, 24, 27-31, 70). Abnormalities of neuropeptide levels have also been found in individuals with other addictions and obesity (71-75); however, in this study, no significant differences in the level of any of the measured neuropeptides were found between food addicted and non-food addicted obese subjects.

The third important finding in the current study was the significantly lower level of serum amylin in obese food addicts compared to the obese non-food addicts. This seems to be the first report regarding the link of amylin with food addiction or any other types of addictions. It is not clear at this stage if this low level of circulating amylin is a reflection of food addiction status or simply is just a secondary change owing to other factors. In a randomized crossover study on 10 healthy males consuming one meal high in carbohydrate or fat, it has been shown that amylin is affected by the macronutrient compositions of a meal, as the amylin level was greater after a high carbohydrate meal compared to a high fat meal (76). In this study, dietary fat intake was higher in obese food addicts, which may be at least partially responsible for the low level of serum amylin.

In our previous study, we found that all food addicts, regardless of obesity status, consumed a higher percentage of calories from fat (41); the same result was also found in an obese food addicts cohort. The high intake of dietary fat was further supported by the finding showing that obese food addicts consumed higher total calories per kilogram of body weight, higher carbohydrates per kilogram of body weight and dietary fat per kilogram of body weight (and per BMI and per percentage of trunk fat). For the first time, we also explored the potential differences of 71 micronutrients intake between food-addicted and non-food-addicted obese subjects. Corresponding to our previous discovery, we found that

obese food addicts consumed a significantly higher amount of fat subcomponents: saturated, monosaturated, poly-saturated and trans fat, omega 3 and 6, vitamin D, gamma tocopherol and dihydrophylloquinone [the main source in commercially-baked snacks and fried food (77)] compared with obese non-food addicts. In addition, obese food addicts consumed higher amounts of sodium and sugar. Therefore, taken together, the data suggest that obese food addicts may consume more hyper-palatable foods that are known to have high amounts of fat, sugar and salt (sodium).

In the present study, the Yale Food Addiction Scale (YFAS) and Willett Food Frequency Questionnaire (FFQ) were used as tools for the diagnosis of food addiction and measuring nutrient intake over the past 12 months. These sets of measures and the criteria on which they are based have been validated in different populations (7, 43, 78-81). The YFAS is the only tool available for the diagnosis of food addiction. Using this set of criteria can help to distinguish subjects who regularly indulge in hyper-palatable foods from those who have lost control over their eating behaviour (7, 41). However, since the aforementioned questionnaires are self-reported, there tends to be self-reporting bias.

It needs to indicate that food addiction is a complex disease, and numerous factors are involved in the etiology. Psychological conditions, like anxiety and depression, which may cause the fluctuation of TSH, prolactin and TNF-α, were not assessed in the current study (82-89). A related study showed that in alcohol-dependent patients, it has been shown that the hypothalamic-pituitary thyroid axis may have the ability to lead to anxious or depressed mood, which may further affect the TSH level (55).

In the current study, the active form of ghrelin was measured. However, the specific inhibitor was not added during sample collection, and therefore, it cannot be excluded that part of the ghrelin may have been degraded. Since all of the samples after blood drawing were placed immediately on ice during the entire process of all experiment, we believe that any degradation would be little, because enzymes that degrade ghrelin would have little activity at this ice-cold temperature.

The correction for multiple comparisons has not been made, since this study is a pioneering study and numerous markers were measured. Moreover, the sample size is relatively small in both groups. However, each of the individuals were well matched in both groups for gender, age, BMI and physical activity level, which would reduce the heterogeneity of subjects and increase the statistical power to detect possible difference in most variables between the two groups. Nonetheless, larger cohorts in different populations are warranted to replicate our findings.

3.5 Conclusions

To the best of our knowledge, this is the first study that has discovered significant differences in multiple aspects, including hormonal levels and nutritional intakes, between obese food addicts and obese non-food addicts. The findings provide valuable evidence to promote further understanding of the mechanism of food addiction and its role in the development of human obesity.

Author Contributions

Pardis Pedram is the first author: coordinating data collection, measuring the hormonal levels, analyzing the data and interpreting the results, as well as the preparation of the manuscript. Guang Sun had the general scientific responsibility in the study design, data interpretation and manuscript revision.

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Chapter 4. Two novel candidate genes identified in adults from the Newfoundland population with addictive tendencies towards Food

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4.1 Introduction

Over the past 3 decades, the global prevalence of obesity, defined by a body mass index (BMI) of 30 or higher, has increased substantially. By 2019, it is estimated that 55.4% of the Canadian adult population will be categorized as overweight or obese (1). Obesity is a multifactorial disease and the etiology is not completely understood (2). It has been well documented, however, that genetics (3, 4), endocrine function (5, 6), behavioral patterns (7, 8) and environmental determinants (9) play fundamental roles in the development of obesity. Genetic predisposition might be a key factor responsible for the large individual difference in body weight, body fat and other obesity-related aspects (10, 11). Estimates of the heritable variation contributing to obesity range from 30–60% in family studies to 60–80% in twin studies (12, 13). In a previous study, we discovered that chronic compulsive overeating, defined as food addiction by the Yale Food Addiction Scale (YFAS), significantly contributes to the common form of human obesity (14). Additionally, the clinical symptom count of food addiction defined by the YFAS is highly associated with the severity of obesity (14). Furthermore, one study has reported that the prevalence of food addiction in obese individuals seeking weight loss treatment was 15.2%, while in another study, the prevalence of food addiction in obese subjects not seeking weight loss was 25% (15, 16). These studies suggest that food addiction with co-morbid obesity may represent an important subgroup of the obese population with a distinctive etiology.

Accumulating evidence has demonstrated the neurobiological and behavioral similarities between food addiction and substance dependence in both human and animal studies (17-21). In animal models, foods high in sugar and fat are particularly associated with

addiction-like behavior (22-24). In human studies, it has also been suggested that the pattern of food intake in food addiction may parallel substance dependence and this phenomenon might share the same neurobiological, behavioral and clinical framework as conventional drug dependence (25-27). Data from family, twin, and adoption studies across several drug classes (opioids, cocaine, cannabis, nicotine, alcohol) strongly implicates the role of genetic factor involved in each aspect of addiction development including vulnerability to initiation, continued use, and propensity to become dependent (28-30). There also exists a genetic overlap between drug and behavioral addictions like gambling (30). Studies have discovered many genes that increase the vulnerability in the development of substance dependence (31-37). For instance, twin studies have shown that the heritability of addictions ranges from 39% (hallucinogens) to 72% (cocaine; ref 38). Importantly, however, the predisposition to addiction may be caused by genetic variants that are common to all addictions and by those specific to a particular addiction (31). However, to the best of our knowledge, there is little information available regarding genes associated with an addictive tendency toward food measured by YFAS. A few studies were reported including a genome-wide investigation of food addiction (39), an evaluation of potential involvements in dopaminergic reward pathways in the brain (40) or mu-opioid receptor gene (41). Exome sequencing is a powerful genome-wide screening method and is an effective way to discover disease causing mutations and genes. (42). Candidate gene-based association study is the most common method for discovering the link between complex disease and genes that are suspected to be associated with phenotypes of interest (43).

In the current study, which was the first of its kind in the field, we employed a two-stage approach: an exome sequencing technology as a screening stage followed by a genetic association study to discover and verify genes related to food addiction.

Ethics Statement

This study was approved by the Health Research Ethics Authority (HREA), Memorial University of Newfoundland, St. John's, Canada, with project identification code: #10.33, (latest date of approval: February 10, 2016). All participants provided written and informed consent.

4.2 Method

Study Sample

The current study was designed and performed in two stages. Stage I, included a genome-wide screening study using a whole-exome sequencing method on selected samples, and stage II was a verification study using a candidate gene association method on the genes related to addiction found in stage I, in the entire study population.

Stage I: A total of 752 subjects in the food addiction study were used for the selection of patients for the exome sequencing study. All the subjects were part of the CODING (Complex Diseases in the Newfoundland population: Environment and Genetics) study and were recruited from the Canadian province of Newfoundland and Labrador (NL) via advertisements, posted flyers, and word of mouth. The inclusion criteria were: 1) age >19 years, 2) born in NL with a family who lived in NL for at least three generations, 3) healthy

without serious metabolic, cardiovascular or endocrine diseases, 4) not pregnant at the time of the study (14, 44). Among the 752 subjects thirty-four subjects (25 females and 9 males) had a very high symptom counts (> or = 5) calculated by the Yale Food Addiction Scale. Subjects with a BMI of 25 kg/m2 or less were excluded [WHO criteria: greater than 25 is classified as overweight; over 30 is classified as obese (45)]. After exclusion, 8 overweight/obese females with high symptom counts (FAO), 8 overweight/obese females and 8 healthy normal weight females with low/zero symptom counts (NFO and control respectively) were selected. All subjects in the three groups were matched for age and physical activity. None of the subjects was smokers or alcoholics, nor substance addicted (or taking any medication).

Stage II: This stage contained the entire 752 subjects.

Anthropometric Measurements

Body weight and height were measured after a 12-hour fasting period. Subjects were weighed to the nearest 0.1 (kg) in a standard hospital gown on a platform manual scale balance (Health O Meter, Bridgeview, IL). A fixed stadiometer was used to measure height to the nearest 0.1 (cm). BMI was calculated by dividing participants' weight in kilograms by the square of his/her height in meter (kg/m^2). Subjects were classified as overweight/obese (BMI ≥25.00) and normal weight (BMI=18.5-24.99) based on BMI according to World Health Organization criteria (45).

Food Addiction Assessment

Food addiction diagnosis and food addiction symptom counts were calculated according to the YFAS (14, 46). This questionnaire consists of 25 items that assess eating patterns over the past 12 months. The YFAS translates the Diagnostic and Statistical Manual IV-TR (DSM-IV-TR) substance dependence criteria in relation to eating behavior (including symptoms such as tolerance and withdrawal symptoms, a vulnerability in social activities, difficulties cutting down or controlling substance use, etc.). The scale uses a combination of Likert scale and dichotomous scoring options. The Likert scoring option is used for food addiction symptom counts (for instance tolerance and withdrawal) ranging from 0 to 7 symptoms. The criteria for food addiction were met when three or more symptoms were present within the past 12 months and clinically significant impairment or distress was present. (46, 47).

Genomic DNA Isolation, Whole Exome Sequencing and Genotyping

Genomic DNA was isolated from ~5 ml of whole blood using the Wizard Genomic DNA Purification kit (Promega, Madison, WI) according to the manufacturer's protocol (48). In stage I, the samples were kept on the ice and they were sent to BGI Americas, for exomes sequencing. The sequencing was done using Latest Illumina HiSeq 4000 system with 100bp and 150bp paired-end sequencing with mapping rate above 98% (49). The coverage of target region and flanking region in our samples were above 99.6% overall.

In stage II, genotyping of the listed SNPs in Table 6 were completed in Génome Québec Innovation Centre using Sequenom iPLEX Gold genotyping technology. The genotyping reaction is based on a multiplex PCR followed by a template-directed single base extension

(SBE) using a probe. The products were then separated and detected by mass spectrometry (MALDI-TOF MS). The assay conversion rate was between 85% depending on the projects. The call rate was about 92% and the error rate was less than 0.1 % (50).

Physical Activity Assessment

The Baecke physical activity questionnaire was used to assess physical activity. This questionnaire assesses physical activity using three indices including work, sport, and leisure. (51). This questionnaire has been validated numerous times in different populations and has well applied in our studies in the Newfoundland population (44, 52-54). Physical activity level was used as a confounding factor in subjects' selection among the three groups in stage I study.

Statistical Analysis

In both stages the subjects were categorized by BMI and food addiction symptom counts into three groups: obese subjects with high food addiction symptom counts ≥5 (FAO), obese subjects with low/zero food addiction symptom counts (NFO) and normal weight subjects with low/zero food addiction symptom counts which was the healthy control group (Ctrl). In the exome sequencing study, we had limited subjects (8 in each group), the aim was to investigate a quantitative relationship between addiction genes and addictive tendencies towards food; the YFAS food addiction diagnosis is a binary and "Yes" or "No" qualitative method would not meet this objective. Therefore, we decided to use the YFAS clinical symptom counts as a semi-quantitative genetic marker in the analysis. Physical

characteristics are reported as mean±SD, maximum and minimum values using SPSS, version 20.0 (SPSS Inc, Chicago).

Stage I: The primary exome sequencing data included 270263 Single Nucleotide Polymorphisms (SNPs). The quality of data was checked using PLINK 1.9 (55, 56). The inclusion criteria were: 1) SNPs with call rates above 95%; 2) SNPs with minor allele frequencies 5% or greater; and 3) SNPs deviating from Hardy-Weinberg Equilibrium (HWE) at p-values <0.01 obtained from Pearson's chi-squared test. Following the aforementioned steps, linkage disequilibrium based SNPs pruning was performed. After all quality control steps, 12288 subsets of the tagging SNPs remained available for the analysis. To compare the differences between the three groups of FAO, NFO and Ctrl, genetic association analysis and odds ratio calculation were performed using a chi-squared test. For all analyses, the alpha level was set at 0.05. The correction for multiple comparisons was not performed, because the exome sequencing study was a pilot study and the sample size was relatively small in the three groups. However, each of the individuals was well matched between three groups for gender, age, and physical activity level that would reduce the heterogeneity of subjects and increase the chance to detect a potential difference among the three groups. The 100 SNPs with the most significant differences among the 3 groups were evaluated in the vast literature review to find out their relevant functions, the genes that each SNP falls in and furthermore the gene functions. All the discovered genes were categorized into 5 subgroups based on gene functions i.e. addiction, psychological disorders, energy metabolism and obesity or obesity-related diseases, cancer and the genes with unknown function or with other diseases out of the

previously listed diseases. The addiction-related genes were selected. These genes are associated with alcohol, nicotine, substance dependence or behavioral addictions, like gambling. Psychological disorder related genes were all associated with major depression (57, 58), bipolar disorder (59, 60), Alzheimer disease (61), Parkinson disease (62), schizophrenia (63), seizure (64), anorexia nervosa (65), Attention Deficit Hyperactivity Disorder (ADHD; refs 66, 67), and autism (68, 69). The reason these psychological disorders were selected was the positive association of these diseases with addiction, addiction mechanisms or addiction-like behavior (these data are presented in supplementary).

Stage II: A chi-square test was applied to compare the allele frequencies between the three groups of FAO, NFO, and Ctrl. Logistic regression under an additive model of inheritance adjusting for potential confounders (age, gender, and smoking) was used to find the association of the SNPs with high symptom counts within the three groups. We also divided the entire population to the subjects with high (HSC) and low symptom counts (LSC). The cut-off point for the high symptom counts was set based on 3 and above. In addition, the cut-off point for the high symptom count was raised to 4 and above to increase the chance of vulnerability of the subject to food addiction and we repeated all the analysis.

As there were three genotypic groups for each SNP, indicator coding as the reference group was tested in two ways of dominant and recessive, since a genetic marker consists of a single biallelic locus with alleles a and A (i.e., a SNP). Unordered possible genotypes are then a/a, a/A and A/A. A common recessive model indicates that two copies of allele A are required for a γ-fold increase in disease risk, and a common dominant model indicates that

either one or two copies of allele A are required for a γ-fold increase in disease risk (70). The significance level was set at alpha level =0.05. All the analyses were done using SPSS, version 20.0 (SPSS Inc, Chicago).

4.3 Results

Physical characteristics of subjects in stages I and II

Demographic and physical characteristics of the participants in the three groups based on adiposity and food addiction symptom counts are presented in Table 1 (adiposity was based on BMI).

Table 4-1 Characteristics of study participants in the two stages*

Groups		Number	Gender	Age(Y/O) (Min-Max)	Weight (kg) (Min-Max)	BMI(kg/m2) (Min-Max)	symptom count (Min-Max)
Stage I	FAO	8	F	37.4±4.3 (31-44)	90.8±9.1 (78-81)	33.6±3.1 (28.8-40)	5-7
	NFO	8	F	40.5±6.6 (30-49)	92.6±3.3 (88-97)	34.2±1.2 (32.4-36.2)	0-1
	Ctrl	8	F	44.5±11.1 (30-61)	59.1±4.9 (50-65)	21.6±1.8 (18.7-24)	0-1
Stage II	FAO	103	F:69 M:34	43.5±10.8 (19.8-73.8)	93.4±19.3 (63-149.8)	32.6±5.2 (25-54.2)	3-7
	NFO	359	F:170 M:189	45.5±12.2 (19-77.4)	87.3±15.8 (57-139.8)	29.7±4.1 (22.7-53.6)	0-2
	Ctrl	264	F:208 M:56	44±14.8 (19-90)	62.8±8.1 (38.6-95.8)	22.4±1.6 (16.8-25)	0-2

* Y/O– years old, Min– minimum, Max– Maximum, BMI – Body mass index, FAO –overweight/obese subjects with high Food addiction symptom, NFO –overweight/obese subjects with low/zero food addiction symptoms, Ctrl– Normal weight subjects with low/zero food addiction symptoms were defined by BMI according to world health organization (WHO) criteria (45)
**Mean±Standard deviation

Stage I: Exome Sequencing Study

I. SNPs with significant difference of allele frequencies in genes associated with addiction among the three groups

I.1. Comparison between FAO and NFO

Among the 100 SNPs with the most significant allele frequency differences between two groups of FAO and NFO, 7 SNPs located in 7 different genes were discovered that are associated with addiction (Table 2). All the SNPs had significantly higher minor allele frequencies in FAO than NFO. In the case of minor allele frequency equal to zero in any group, all the SNPs except rs2167068 were functional based on the information obtained from F-SNP database (http://compbio.cs.queensu.ca/F-SNP/). Among these addiction-related genes, TIRAP (71), ITPR2 (72) and MYPN (73) are positively associated with smoking. MMADHC (74), ERAP1 (75, 76), GRID1 (77, 78) and ITPR2 (79) are related to alcohol dependence. ITPR2 is also associated with gambling (80). NTM is closely linked to a related gene family member, opioid binding protein/cell adhesion molecule-like (OPCML), on chromosome 11 (81).

Table 4-2 SNPs with significantly different frequencies that are associated with addiction-related genes between FAO and NFO *

CHR	SNPs	Alleles	Minor Allele	Freq FAO	Freq NFO	P	OR	Gene
11	rs625413	T/C	T	0.4	0.1	0.01	11.7	TIRAP
2	rs10932467	G/T	G	0.3	0	0.02	0	MMADHC
5	rs26653	C/G	C	0.3	0	0.02	NA	ERAP1
11	rs2739287	T/C	T	0.5	0.1	0.02	7	NTM
10	rs3814183	A/G	A	0.4	0.06	0.03	9	MYPN
10	rs2607863	T/C	T	0.3	0	0.03	NA	GRID1
12	rs2167068	T/C	T	0.3	0	0.03	NA	ITPR2

* CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_FAO- minor allele frequencies in obese subjects with high symptom counts, Freq_NFO- minor allele frequencies in obese subjects with low/zero symptom counts, OR-odds ratio.

**Independent t-test, $p<0.05$

I.2. Comparison between FAO and Ctrl

Among the 100 SNPs with the most significant allele frequency differences between FAO and control, nine SNPs on 8 different genes were found and these genes had previously been associated with addiction (ndence or nicotine dependence.

Table 4-3). All the SNPs mentioned above had significantly higher minor allele frequencies in FAO than NFO. In the case of minor allele frequency equal to zero in any group, all the SNPs except rs28429551 were functional based on the information obtained from F-SNP database (http://compbio.cs.queensu.ca/F-SNP/). The addiction-related genes, including GPSM1 (82, 83), ZCCHC14 (84), TNN (85) , PPARD (86), CAV1 (87), CACNA1C (88) and SIM1 (89) were associated with alcohol dependence, drug dependence or nicotine dependence.

Table 4-3 SNPs with significantly different frequencies associated with addiction-related genes between FAO and Ctrl

CHR	SNP	Alleles	Minor Allele	Freq FAO	Freq Ctrl	P	OR	Gene
9	rs28429551	T/A	T	0.5	0	0.002	NA	GPSM1
16	rs1050847	C/T	C	0.5	0.06	0.007	15	ZCCHC14
	rs2042395	G/A	G	0.5	0.06	0.007	15	
11	rs2739287	T/C	T	0.5	0.07	0.01	13	NTM
1	rs4651335	C/G	C	0	0.3	0.01	0	TNN
6	rs2016520	C/T	C	0.3	0	0.01	NA	PPARD
7	rs1997623	A/C	A	0.3	0	0.01	NA	CAV1
12	rs7136355	T/C	T	0.3	0	0.02	NA	CACNA1C
6	rs397662	C/T	C	0.3	0	0.02	NA	SIM1

* CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_FAO- minor allele frequencies in obese subject with high symptom counts, Freq_Ctrl- minor allele frequencies in normal weight subject with low/zero symptom counts, OR-odds ratio
** Independent t-test, $p<0.05$

I.3. Comparison between NFO and Ctrl

In comparison between the two groups of obese and normal weight subjects both with low/zero symptom counts, only 35 SNPs had significant differences in allele frequencies. Among them, minor allele G of rs2511521 located in DRD2 (90, 91) had higher frequencies in the control group (Table 4).

Table 4-4 SNP with significantly different frequencies associated with addiction-related genes between NFO and healthy Ctrl*

CHR	SNP	Alleles	Minor allele	Freq_NFO	Freq_Ctrl	P	OR	Gene
11	rs2511521	G/A	G	0.1	0.5	0.02	0.1	DRD2

*CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_NFO minor allele frequencies in obese subject with low/zero symptom counts, Freq_Ctrl- minor allele frequencies in normal weight subject with low/zero symptom counts, OR-odds ratio
**Independent t-test, $p<0.05$

I.4. Comparison between FAO and NFO+Ctrl

When the subjects with low/zero symptom counts from NFO and Ctrl groups were combined (NFO+Ctrl), the allele frequencies were compared within FAO. Eight SNPs located in 8 addiction-related genes has been found (Table 5). All the SNPs had significantly higher minor allele frequencies in FAO. Among them, the frequency of minor allele T in rs2739287 located in NTM was significantly higher in FAO compared to NFO and Ctrl, remained strongly significant when the subjects in FAO were compared to the subjects in the combined NFO plus Ctrl group. In addition, 3 SNPs including rs2042395 (G), rs397662 (C) and rs7136355 (T) located in 3 addiction-related genes including

ZCCHC14, SIM1, and CACNA1C respectively, that had significant differences in allele frequencies between FAO and NFO, remained significant when FAO was compared to NFO plus Ctrl. In addition to the SNPs discussed above, rs2272409 (C), rs4728329 (A), rs4833463(A), and rs3934648 (T) located in ALK (92, 93), AKR1B10 (94), UGT8 (95, 96) and PTPN7 (78) respectively, showed higher allele frequencies in FAO. Based on the information obtained from F-SNP, both rs4728329 and rs4833463 with the minor allele frequency equal to zero in subjects with low/zero symptom counts were functional.

Table 4-5 SNPs with significantly different frequencies associated with addiction-related genes between NFO and NFO+Ctrl*

CHR	SNP	Alleles	Minor alleles	Freq_FAO	Freq_NC	P	OR	Gene
11	rs2739287	T/C	T	0.5	0.1	0.003	9	NTM
16	rs2042395	G/A	G	0.5	0.1	0.01	7	ZCCHC14
2	rs2272409	C/T	C	0.4	0.1	0.01	7	ALK
6	rs397662	C/T	C	0.3	0.04	0.01	12.3	SIM1
7	rs4728329	A/G	A	0.2	0	0.01	NA	AKR1B10
12	rs7136355	T/C	T	0.3	0.03	0.01	11.6	CACNA1C
4	rs4833463	A/G	A	0.2	0	0.01	NA	UGT8
1	rs3934648	T/C	T	0.4	0.1	0.02	5.8	PTPN7

CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_FAO- minor allele frequencies in obese subject with high symptom counts, Freq_NC- minor allele frequencies in obese and normal weight subject with low/zero symptom counts, OR-odds ratio

** Independent t-test, $p<0.05$

Stage II: The genetic association study

Genotype frequencies of SNPs located in addiction-related genes

Table 4-6 presents the comparison of genotypic frequencies of the 19 SNPs located in addiction-related genes among FAO, NFO, and healthy Ctrl groups.

Table 4-6 Comparisons of allelic and the genotypic frequencies of the SNPs located in addiction genes

SNPs	CHR	Groups	Genotype		
			TT	**TC**	**CC**
rs1050847	16	Ctrl	85(%32.2)	124(%47)	53(%20.1)
		NFO	106(%29.5)	189(%52.6)	64(%17.8)
		FAO	35(%34)	50(%48.5)	17(%16.5)
			TT	TG	GG
rs10932467	2	Ctrl	165(%62.5)	79(%29.9)	9(%3.4)
		NFO	225(%62.7)	116(%32.3)	12(%3.3)
		FAO	69(%67)	29(%28.2)	2(%1.9)
			CC	AC	AA
rs1997623	7	Ctrl	195(%73.9)	55(%20.8)	12(%4.5)
		NFO	248(%69.1)	101(%28.1)	8(%2.2)
		FAO	70(%68)	29(%28.2)	3(%2.9)
			TT	TC	CC
rs2016520	6	Ctrl	163(%61.7)	87(%33)	11(%4.2)
		NFO	225(%62.7)	119(%33.1)	15(%4.2)
		FAO	61(%59.2)	37(%35.9)	4(%3.9)
			AA	AG	GG
rs2042395	16	Ctrl	148(%56.1)	94(%35.6)	20(%7.6)
		NFO	217(%60.4)	125(%34.8)	17(%4.7)
		FAO	62(%60.2)	32(%31.1)	8(%7.8)
			CC	CT	TT
rs2167068	12	Ctrl	185(%70.1)	69(%26.1)	8(%3)
		NFO	276(%76.9)	74(%20.6)	9(%2.5)
		FAO	77(%74.8)	23(%22.3)	2(%1.9)
			AA	AG	GG
rs2511521	11	Ctrl	146(%55.3)	102(%38.6)	14(%5.3)
		NFO	223(%62.1)	123(%34.3)	13(%3.6)
		FAO	69(%67)	25(%24.3)	8(%7.8)

SNPs	CHR	Groups	Genotype		
			TT	**TC**	**CC**
rs2607863	10		CC	CT	TT
		Ctrl	229(%86.7)	31(%11.7)	2(%0.8)
		NFO	308(%85.8)	47(%13.1)	4(%1.1)
		FAO	85(%82.5)	17(%16.5)	0
rs26653	5		CC	CG	GG
		Ctrl	143(%54.2)	96(%36.4)	22(%8.3)
		NFO	195(%54.3)	129(%35.9)	35(%9.7)
		FAO	58(%56.3)	36(%35)	8(%7.8)
rs2739287	11		TT	TC	CC
		Ctrl	7(%2.7)	68(%25.8)	187(%70.8)
		NFO	2(%0.6)	93(%25.9)	264(%73.5)
		FAO	2(%1.9)	32(%31.1)	68(%66)
rs28429551	9		AA	AT	TT
		Ctrl	18(%6.8)	88(%33.3)	155(%58.7)
		NFO	17(%4.7)	138(%38.4)	200(%55.7)
		FAO	9(%8.7)	38(%36.9)	55(%53.4)
rs3814183	10		GG	AG	AA
		Ctrl	190(%72)	65(%24.6)	6(%2.3)
		NFO	245(%68.2)	106(%29.5)	8 (%2.2)
		FAO	76(%73.8)	24(%23.3)	2(%1.9)
rs3934648	1		CC	CT	TT
		Ctrl	152(%57.6)	98(%37.1)	12(%4.5)
		NFO	206(%57.4)	135(%37.6)	16(%4.5)
		FAO	63(%61.2)	35(%34)	4(%3.9)
rs397662	6		TT	TC	CC
		Ctrl	176 (%66.7)	70(%26.5)	4(%1.5)
		NFO	251(%69.9)	86(%24)	4(%1.1)
		FAO	75(%72.8)	22(%21.4)	1(%1)
rs4651335	1		CC	CG	GG
		Ctrl	181(%68.6)	77(%29.2)	4(%1.5)
		NFO	267(%74.4)	85(%23.7)	7(%1.9)
		FAO	70(%68.8)	29(%28.2)	3(%2.9)
rs4728329	7		GG	AG	AA
		Ctrl	221(%83.7)	39(%14.8)	2(%0.8)
		NFO	282(%78.6)	72(%20.1)	5(%1.4)
		FAO	85(%82.5)	17(%16.5)	0
rs4833463	4		AG	GG	

SNPs	CHR	Groups	Genotype		
			TT	**TC**	**CC**
		Ctrl	14(%5.3)	248(%93.9)	
		NFO	19(%5.3)	340(%94.7)	
		FAO	6(%5.8)	96(%93.2)	
			CC	CT	TT
rs625413	11	Ctrl	153(%58)	90(%34.1)	19(%7.2)
		NFO	231(%64.3)	110(%30.6)	18(%5.0)
		FAO	50(%48.5)	42(%40.8)	10(%9.7)
			CC	CT	TT
rs7136355	12	Ctrl	185(%70.1)	71(%26.9)	6(%2.3)
		NFO	237(%66)	109(%30.4)	12(%3.3)
		FAO	70(%67.8)	31(%30.1)	1(%1.0)

* CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, FAO–overweight/obese subjects with high food addiction symptom, NFO–overweight/obese subjects with low/zero food addiction symptoms, Ctrl– Normal weight subjects with low/zero food addiction symptoms was defined by BMI according to world health organization (WHO) criteria (45).

The association between SNPs in addiction-related gene and food addiction

Table 7 shows the significant results. rs2511521 located in DRD2 gene and rs625413 located in TIRAP gene were significantly associated with increased risk of food addiction. In model 1, the indicator coding as genotype reference was carrying the minor allele using a recessive model. The major allele A of rs2511521 in NFO subjects is associated with an increased risk of food addiction [OR=3.1(95% CI 1.1-8.2)]. In addition, minor allele T of rs625413 in NFO subjects is associated with an increased risk of food addiction [OR=2.5(95% CI 1.1-5.8)]. In model 2 the indicator coding as genotype reference was carrying the minor allele using a dominant model. The results remained significant for rs625413 [OR=1.8(95% CI 1.2-2.9)] in NFO.

All subjects were classified based solely on symptom counts to the subjects with high (HSC) and low symptom counts (LSC). The cut-off point for the high symptom counts was set based on 3 and above. Logistic regression under an additive model of inheritance adjusting for sex and age was performed based on the new classification as well (Table 8). All analyses were repeated after the cut-off point for the high symptom count was raised to 4 and above. In both classifications, minor allele T of rs625413 in subjects with LSC had increased the risk to food addiction.

In addition, all the analyses were completed between food addicts and non-food addicts, and female food addicts and all female non-food addicts (adjusting for major confounding factors of age and sex). No significant differences were found in the two models (1 and 2) of analysis.

Table 4-7 Significant association between SNPs and food addiction

Model of analysis	SNPs	Groups	Minor allele	Alleles	p	OR (95% CI)
Model 1	rs2511521 FAO	NFO	GG	AG	0.2	1.8(0.7-4.6)
				AA	0.03	3.1(1.1-8.2)
		Ctrl	GG	AG	0.7	1.2(0.5-3.0)
				AA	0.1	2.3(0.9-6.0)
	rs625413 FAO	NFO	TT	CT	0.04	2.5(1.1-5.8)
				CC	0.4	1.4(0.6-3.4)
		Ctrl	TT	CT	0.2	1.6(0.7-3.8)
				CC	0.8	1.1(0.5-2.6)
Model 2	rs2511521 FAO	NFO	GG+AG	AA	0.2	0.7(0.5-1.2)
		Ctrl	GG+AG	AA	0.048	0.6(0.4-1.0)
	rs625413 FAO	NFO	TT+CT	CC	0.007	1.8(1.2-2.9)
		Ctrl	TT+CT	CC	0.09	1.5(0.9-2.4)

* SNPs- Single Nucleotide Polymorphisms, OR- Odds Ratio, CI- Confidence Interval, FAO – overweight/obese subjects with high Food addiction symptom, NFO –overweight/obese subjects with low/zero food addiction symptoms, Ctrl– Normal weight subjects with low/zero food addiction symptoms was defined by BMI according to world health organization (WHO) criteria (45).
** Independent t-test, $p<0.05$

Table 4-8 SNPs with significant association to food addiction in the subjects categorized solely based on symptom counts

Symptom counts	SNPs	Groups (N)	Minor allele	p	OR (95% CI)
3 and above	rs625413	HSC(131) LSC(617)	TT+CT	0.045	0.7(0.5-1.0)
4 and above	rs625413	HSC(69) LSC(679)	TT+CT	0.01	1.9(1.2-3.2)
	rs625413	HSC(69) LSC(679)	TT	0.03	1.5(1.0-2.2)

* SNPs- Single Nucleotide Polymorphisms, OR- Odds Ratio, CI- Confidence Interval, HSC-High Symptom Count, LSC-Low Symptom Count.

**The reference category was HSC

4.4 Discussion:

As the first exome study on food addiction, the unique study design with two equally obese groups but different in food addiction symptom counts, plus a healthy control group was likely the key for us to identify food addiction-related genes in a relatively small study. Several hundreds of genes have been linked to the development of the common form of human obesity (13) . It has been well documented that human obesity is a complex disease with multiple routes of etiology. One of the major challenges in finding food addiction genes in obese patients is to separate the genes causing obesity by food addiction from genes that cause obesity by other mechanisms. The two sets of equally obese patients were carefully selected. The key difference between the two groups was the food addiction clinical symptom counts. There are significant differences in different age groups and the prevalence is higher in women than men in food addiction (14, 97). Moreover, physical activity level is likely the most prominent factor in the development of human obesity (98, 99). Physical activity level affects energy expenditure, appetite, food intake (100, 101). It is one of the key confounding factors and should have strictly been controlled. Age, gender and physical activity level were matched among selected three subject groups in the present study. Furthermore, what makes the finding of our study reliable is the combination of the screening study using exome sequencing followed by a validation study using a genetic association approach. The exome sequencing study enables the unbiased discovery of coding variations for subsequent association testing for complex traits. One of the important advantages of exome sequencing is providing a well-defined and interpretable target for mutations in the locus. These mutations create variants at these loci that can be identified

as being associated with the trait (42). However, it is likely that many addiction-related genes were potentially missed because of the limitation of the technology and low power of the study owing to the small sample size.

Accumulating evidence from both animal studies as well as our own data on obesity in the general population strongly support the concept that food addiction is one of the distinctive etiologies of obesity (14). The most compelling evidence that an addiction to food exists is the similarities and overlaps in the neurobiological systems activated in both substance abuse and over consumption of hyper-palatable food (17-19). There are strong genetic components in the development of both obesity and addiction that have been well demonstrated by evidence from animal models and humans. Many studies have documented genes responsible for addiction in general and especially in substance abuse (31-37). However, the knowledge regarding the genetic background of food addition is still in the early stage and corresponding data are very scarce. Our present study is the first that employed two groups of equally obese patients with distinguished food-addiction symptom scores of clinical symptom counts of food addiction, to identify the candidate genes that are more likely responsible for food addiction.

The first gene identified in the present study as a candidate gene for food addiction is the DRD2 gene on chromosome 11. The DRD2 or dopamine receptor D2 gene encodes the D2 subtype of the dopamine receptor (102). It is well known that the dopaminergic pathways regulate neuronal systems associated with reward sensitivity in both food and substance consumption (103, 104). DRD2 is coupled to inhibitory G proteins and its activation results in a decrease in intracellular cyclic adenosine monophosphate (105). In

the current study, the major allele A of rs2511521 in the DRD2 gene in NFO subjects is associated with an increased risk of food addiction [OR=3.1(95% CI 1.1-8.2)]. Because both groups of FAO and NFO were obese and the only difference between them was the high symptom counts, therefore, the major allele A could represent a risk allele in the DRD2 gene for food addiction only in obese subjects. This is the first study that has directly demonstrated that the DRD2 is a candidate gene responsible for food addiction in obese subjects. Previously, a multi-loci genetic profile score [MLGP] as an index of elevated dopamine signaling based on six known dopamine-related polymorphisms (located in or close to ANKK, DRD2, SLC6A3 and COMT genes) was used to study of food addiction by investigating whether this score distinguished those with YFAS-diagnosed food addiction from controls (40). The investigators reported that the MLGP score was significantly higher in the food-addiction group than in the controls, suggesting the involvement of the dopamine-signaling pathway. In addition, in a GWAS of food addiction, using a modified Yale Food Addiction Scale (mYFAS) on 9314 women of European ancestry, it was reported that DRD2 was nominally significant for food addiction symptom counts ($p<0.05$; ref 106). However, it did not meet their pre-specified significant threshold for GWAS ($p <2.1\times10^{-4}$). In addition, one of the main weaknesses of this study was the older age of the population and therefore the lower prevalence of food addiction. The majority of the population was aged between 62-87 where the prevalence of food addiction was only 2.6% and as a result, this may not have been the ideal population in which to study food addiction.

DRD2 is also the gene that has been reported in several studies to be associated with substance dependence (90, 91). For instance, DRD2 (rs1076560) was associated with opioid dependence and was significantly associated with increased risk for drug dependence (105). Interestingly this reported SNP is on the same LD block as rs2511521 in the DRD2 gene identified in our food addiction study. The significant associations found in drug dependency and food addiction with the DRD2 gene suggest an overlap in the neurological pathway between food addiction and substance dependence.

The second gene identified in the present study is the TIRAP gene (toll-interleukin 1 receptor (TIR) domain containing adaptor protein) on chromosome 11. TIRAP is an adapter involved in Toll-like receptors (TLRs) signaling pathways in the innate immune response that results in activation of NF-kappa-B. The innate immune system recognizes pathogens through TLRs, which identify pathogen-associated molecular patterns. TLRs recognize different pathogen-associated molecular patterns and all TLRs have a Toll-interleukin 1 receptor (TIR) domain, which is responsible for signal transduction. The protein encoded by this gene is a TIRAP involved in the TLR4 signaling pathway of the immune system. It activates NF-kappa-B, MAPK1, MAPK3 and JNK, which then results in cytokine secretion and the inflammatory response. In addition, TIRAP positively regulates the production of TNF-alpha and interleukin-6 (107-109). Over-expression of the TIRAP gene in the brain was found after exposure to alcohol and nicotine in mice. Analysis of brain gene expression in mice that are genetically predisposed to alcohol consumption indicates a key role of TIRAP in regulating alcohol intake (109). In addition evidence in mice with dopaminergic neurodegeneration in the nigrostriatal pathway showed that nicotine and caffeine exposure

upregulates the gene expression of TIRAP (71). Our previous study on 58 food-addicted and non-food-addicted overweight/obese individuals (FAO, NFO) matched for age, sex, BMI and physical activity showed FAO group had lower levels of TNF-α as compared to NFO group. It indicates there is more likely a specific manifestation in obese food addicts contrary to the increased level of TNF-α in obese people (44). Since TIRAP positively regulates the production of TNF-alpha, therefore, this gene is also more likely to be responsible for increasing the likelihood of food addiction in obese subjects.

In addition to the two genes, we identified in stage I, the frequency of minor allele T for rs2739287 located in NTM in FAO remained significantly different compared to the other three groups (NFO, Ctrl, and NFO+Ctrl). However, these significant differences were not seen in the verification study. It has to be emphasized that although the total number of subjects in our second stage was 752, the statistical power is still relatively small for a genetic association study. It is likely many potential candidate genes may have been missed including the NTM because of the low statistical power of the present study. NTM or neurotrimin is closely linked to a related gene family member, opioid binding protein/cell adhesion molecule-like (OPCML), on chromosome 11 (81). In a GWAS for food addiction, NTM were significantly associated with clinical symptom counts. As well, the SNP rs75038630 located in NTM was significantly associated with a positive diagnosis of food addiction (106). Therefore, a verification study on a larger sample is warranted.

Despite the significant and novel findings, there were limitations in our study. The correction for multiple comparisons was not made in the stage I of screening study.

Furthermore, in the present study, the information on psychiatric comorbidities and family history of eating disorder were not available.

Another is related to the limitation of exome sequencing technology. In stage I of the current study, the sequencing platform had sufficient depth of coverage (≥50X) with high-quality sequencing data (Q20>90%, Q30>85%). However, exome sequencing has the disadvantage that this technology is unable to find non-protein coding genes. In addition, in stage I (the exome sequencing study) the medication use has been controlled for the selection of the subjects. However, in stage II because so many types of medications were used in 752 subjects, it was not possible to categorize the subjects based on medication use in a genetic association study.

The YFAS has been validated in different populations and is used as a tool for the calculation of symptom counts over the previous 12 months. The YFAS is the only tool available for the diagnosis of food addiction. Using this set of criteria can help to distinguish subjects who regularly indulge in hyper-palatable foods from those who have lost control over their eating behavior. However, since the questionnaire is self-reported, there may be a self-reporting bias (44).

4.5 Conclusion

In the current study we employed a strategy of combining the exome sequencing technology with a genetic association study to find food addiction related genes. Two genes related to addictive tendencies towards food were identified and validated: the DRD2 and

TIRAP genes in the Newfoundland population. This is the first time that TIRAP has been reported as to be associated with addictive tendencies towards food.

Author Contributions

Pardis Pedram is the first author: coordinating data collection, analyzing the data and interpreting the results, as well as the preparation of the manuscript. Guang Sun had the general scientific responsibility in the study design, data interpretation and manuscript revision.

Supplementary

Stage I

I.S1. SNPs with significant difference of allele frequencies in genes associated with psychological disorders among the three groups

I.S1.1. Comparison between FAO and NFO

Among the 100 SNPs with the most significant allele frequency differences between two groups of FAO and NFO, 19 SNPs located in 15 different genes related to psychological disorders has been discovered (Table S1). All the SNPs had significantly higher minor allele frequencies in FAO except for rs2991363 (A) located in SVEP1 in which the differences between allele frequencies were significantly lower in FAO. In the case of minor allele frequency equal to zero in any group, all the SNPs except rs1890100 were functional (based on the information obtained from F-SNP database (http://compbio.cs.queensu.ca/F-SNP/)).

Table S1. SNPs with significantly different frequencies associated with psychological disorders related genes between FAO and NFO*

CHR	SNP	Alleles	Minor allele	FreqFAO	FreqNFO	P	OR	Gene	Disease
8	rs11203929	T/C	T	0.5	0.1	0.01	15	PCM1	Schizophrenia (110, 111)
	rs28655174	G/A	G	0.4	0.1	0.01	11.7		
	rs208060	G/A	A	0.4	0.1	0.01	11.7		
	rs3780103	T/C	C	0.5	0.1	0.02	7		
6	rs1890100	G/A	A	0.3	0	0.01	NA	SYNE1	Bipolar Disorder and depression (112) (113)
2	rs10193313	C/A	C	0.3	0	0.02	NA	EPC2	Alzheimer Disease (114)
2	rs1980844	G/A	G	0.3	0	0.02	NA	ABCA12	Alzheimer's Disease (115)
13	rs4628819	T/C	T	0.3	0	0.02	NA	CARS2	Epilepsy (116)
19	rs1864113	G/C	G	0.3	0	0.02	NA	BABAM1	Schizophrenia (117)
23	rs5926304	T/C	T	0.5	0.1	0.02	7	PTCHD1	Autism (118, 119)
11	rs1837971	G/A	G	0.4	0.1	0.02	10.1	NAV2	Alzheimer (120), ADHD (121), schizophrenia (122)
2	rs2303606	C/A	C	0.5	0.1	0.02	7	DYSF	Alzheimer disease (123)
	rs2288355	A/T	A	0.2	0	0.03	NA		
3	rs4894810			0.2	0	0.03	NA	FNDC3B (intron)	Parkinson's Disease (124)
4	rs7694129	T/C	C	0.4	0.1	0.03	9	PPP2R2C (intron)	Bipolar disorder (125, 126)
6	rs195860	T/G	T	0.3	0	0.03	NA	POU3F2	Schizophrenia (127), bipolar disorder (128)
6	rs486881	T/G	T	0.4	0.1	0.03	9	CCNC	Alzheimer's disease (129)
9	rs2991363	A/T	A	0	0.3	0.03	0	SVEP1	Bipolar Disorder (130)
11	rs2445290	G/A	A	0.4	0.1	0.03	9	OR51L1	Anorexia nervosa (131)

* CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_FAO- minor allele frequencies in obese subject with high symptom counts, Freq_NFO- minor allele frequencies in obese subject with low/zero symptom counts, OR-odds ratio

** Independent t-test, $p<0.05$

I.S1.2. Comparison between FAO and Ctrl

Among the 100 SNPs with the most significant allele frequency differences between two groups of FAO and control, 14 SNPs located in 14 psychological disorders related genes has been found (Table S2). All the SNPs had significantly higher minor allele frequencies in FOA except for rs1042044 (A), rs2050831 (C), and rs2070179 (T) respectively located in GLP1R, VPS13A, and HCLS1. These SNPs had lower frequencies in FAO. Among the SNPs with minor allele frequency equal to zero in any group, based on the information obtained from F-SNP, no function has been reported for rs13135591, rs534286, rs2861575, and rs2272219.

Table S2. SNPs with significantly different frequencies associated with psychological disorders related genes between FAO and Ctrl*

CHR	SNP	Alleles	Minor Allele	Freq FAO	Freq Ctrl	P	OR	Gene	Disease
13	rs812808	T/C	T	0.4	0	0.007	NA	DOCK9	Bipolar disorder (132)
19	rs880090	C/G	C	0.4	0	0.01	NA	GMIP	Major depressive disorder (133)
22	rs2239766	T/G	T	0.5	0.1	0.01	13	SYN3	Schizophrenia (134)
5	rs306573	A/G	A	0.4	0.1	0.01	11.7	MCTP1	Bipolar Disorder (135)
6	rs1042044	A/C	A	0.1	0.4	0.01	0.09	GLP1R	Depression (136)
2	rs3770016	A/G	A	0.3	0	0.01	NA	PDE11A	Major depressive disorder (137)
4	rs13135591	C/T	C	0.3	0	0.01	NA	COL25A1	Alzheimer's disease (138)
9	rs534286	A/G	G	0.3	0	0.01	NA	TMEM245	Schizophrenia (139)
10	rs2861575	T/C	C	0.3	0	0.01	NA	OPALIN	Epilepsy (140)
9	rs2050831	T/C	C	0	0.3	0.02	0	VPS13A	Mood disorder and Schizophrenia (141)
15	rs2272219	T/C	C	0.3	0	0.02	NA	MTMR10	Seizure (142)
19	rs3746321	A/G	A	0.3	0	0.02	NA	ZNF224	Alzheimer disease (143)
21	rs13052645	T/C	T	0.3	0	0.02	NA	PRDM15	Parkinson disease (144)
3	rs2070179	T/C	T	0	0.3	0.02	0	HCLS1	Parkinson disease (145)

*CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_FAO- minor allele frequencies in obese subject with high symptom counts, Freq_Ctrl- minor allele frequencies in normal weight subject with low/zero symptom counts, OR-odds ratio
** Independent t-test, $p<0.05$

I.S1.3. Comparison between NFO and Ctrl

Among the 35 SNPs with the most significant allele frequency differences between two groups with low/zero symptom counts, 4 SNPs located in 4 psychological disorders related genes have been discovered (Table S3). All the SNPs had significantly higher

minor allele frequencies in Ctrl except for rs3800842 located in TMEM106B. Minor allele C in rs2303606 located in DYSF that was significantly lower in NFO compared to FAO (Table S2), remained significantly lower when it was compared to Ctrl.

Table S3. SNPs with significantly different frequencies associated with psychological disorders related genes between NFO and Ctrl*

CHR	SNP	Alleles	Minor allele	Freq NFO	Freq Ctrl	P	OR	Gene	Disease
7	rs3800842	A/G	A	0.5	0.1	0.04	6	TMEM106B	Alzheimer's disease (146)
1	rs3753527	T/C	T	0.1	0.4	0.05	0.2	SOAT1	Alzheimer's disease (147)
2	rs2303606	C/A	C	0.1	0.4	0.05	0.2	DYSF	Alzheimer's disease (123)
1	rs3753527	T/C	T	0.1	0.4	0.05	0.2	SOAT1	Alzheimer's disease (148)

*CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_NFO minor allele frequencies in obese subject with low/zero symptom counts, Freq_Ctrl- minor allele frequencies in normal weight subject with low/zero symptom counts, OR-odds ratio
** Independent t-test, $p<0.05$

I.S1.4. Comparison between FAO and NFO+Ctrl

When the subjects with low/zero symptom counts from NFO and Ctrl groups were combined (NFO+Ctrl), the allele frequencies were compared within FAO. Among the 100 SNPs discovered with the most significant differences in allele frequencies between two groups, 13 SNPs located in 13 psychological disorders related genes has been found (Table S4). All the aforementioned SNPs had significantly higher minor allele frequencies in FAO. In the case of minor allelic frequency equal to zero in any group, based on the information obtained from F-SNP, all the SNPs has been functional.

Among all the reported SNPs in Table S4, rs3746321 (A) and rs880090 (C) located in ZNF224 and GMIP, that the minor allele frequencies were significantly higher in FAO compared to healthy control (Table S2), the allele frequencies remained significantly higher in the subjects with high symptom counts when they were compared with NFO+Ctrl. In addition, rs28655174 (G) and rs7694129 (C) located in PCM1 and PPP2R2C that had significantly higher minor allele frequencies in FAO compared to NFO (Table S1) remained significantly higher when FAO was compared with all the subjects with low/zero symptom counts. Furthermore, the frequency of minor allele A in rs28477638 located in PRDM15 was significantly higher in FAO compared to all the subjects with low/zero symptom counts. More interestingly, different tagging SNPs (rs13052645 and rs28477638) located on PRDM15 was significantly higher in FAO when it was compared to healthy control or NFO+Ctrl (Table S2).

Table S4. SNPs with significantly different frequencies associated with psychological disorders related genes between FAO and NFO+Ctrl*

CHR	SNP	Alleles	Minor allele	Freq FAO	Freq NC	P	OR	Gene	Disease
7	rs858506	T/C	T	0.3	0.03	0.01	12.4	GATS	Depression (149)
19	rs3746321	A/G	A	0.3	0.03	0.01	12.4	ZNF224	Alzheimer Disease (143)
3	rs1480361	T/C	T	0.2	0	0.01	NA	DOCK3	ADHD-like phenotype (150, 151)
5	rs545358	C/A	C	0.2	0	0.01	NA	PLCXD3	Bipolar disorder (152)
14	rs4906357	C/A	A	0.2	0	0.01	NA	KLC1	Alzheimer Disease (153)
17	rs854625	A/G	A	0.2	0	0.01	NA	CCL15	Alzheimer Disease (154, 155)
19	rs880090	C/G	C	0.4	0.1	0.01	7.8	GMIP	Major depressive disorder (133)
17	rs4969350	A/G	G	0.5	0.2	0.01	5.4	BAIAP2	ADHD (156) and Autism (157)
8	rs28655174	A/G	G	0.4	0.1	0.01	5.4	PCM1	Schizophrenia (110, 111)
21	rs28477638	A/G	A	0.2	0	0.01	NA	PRDM15	Parkinson disease (144)
4	rs7694129	C/T	C	0.4	0.1	0.01	5.8	PPP2R2C (intron)	Bipolar disorder (125, 126)
1	rs2771122	A/G	A	0.2	0	0.2	NA	PGLYRP4	Parkinson's disease (158)
3	rs310764	A/G	A	0.2	0.03	0.2	10.33	SYN2	Epilepsy (159), Depression (160)

* CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_FAO- minor allele frequencies in obese subject with high symptom counts, Freq_NC- minor allele frequencies in obese and normal weight subject with low/zero symptom counts, OR-odds ratio

** Independent t-test, $p<0.05$

I.S2. SNPs with significant difference of allele frequencies in genes associated with energy metabolism and obesity or obesity-related disease among the three groups

I.S2.1. Comparison between FAO and NFO

Among the 100 SNPs with the most significant allele frequency differences between two groups of FAO and NFO, 22 SNPs located in 19 genes related to energy metabolism and obesity or obesity-related disease have been found (Table S5). All of the above mentioned SNPs had significantly higher minor allele frequencies in FAO except for 3 SNPs including rs2733743 (A), rs445664 (G) and rs1004976 (G) located respectively in ATP4A, REG3G, and GALNT10. In the case of minor allele frequency equal to zero in any groups, all the SNPs except rs2181687 (G), rs10443987 (A), and rs4832106 (G) were functional based on the information obtained from F-SNP (http://compbio.cs.queensu.ca/F-SNP/).

Table S5. SNPs with significantly different frequencies associated with the genes related energy metabolism and obesity or obesity-related disease between FAO and NFO *

CHR	SNP	Alleles	Minor allele	Freq FAO	Freq NFO	P	OR	Gene	Disease
6	rs3823310 rs6941075	A/C T/A	A T	0.4 0.5	0 0.1	0.003 0.01	NA 15	AKAP12	Abdominal Fat , Waist-Hip Ratio (161)
1	rs1409986	A/G	A	0.4	0	0.01	NA	PTGER3	Obesity (162)
6	rs2181687 rs6934692	A/G A/G	G G	0.4 0.4	0 0.1	0.01 0.03	NA 9	USP45	Insulin (163)
11	rs1466426	C/T	T	0.4	0.1	0.01	11.7	AMPD3	Energy metabolism (164)
2	rs4973588	A/G	A	0.3	0	0.02	NA	NGEF	Obesity (165)
10	rs10443987	T/A	A	0.3	0	0.02	NA	ANKRD26	Extreme obesity (166), insulin resistance (167)
16	rs9940089	G/C	G	0.3	0	0.02	NA	ABCC6	Cardiovascular disease (168) , Diabetes type 2 (169)
16	rs699444 rs740178	C/T T/A	T T	0.3 0.3	0 0	0.02 0.02	NA NA	ZFHX3	Cardiovascular Diseases (170, 171)
14	rs2273394	C/G	C	0.3	0	0.02	NA	SLC7A8	Blood cholesterol (172)
19	rs2733743	A/G	A	0	0.3	0.02	0	ATP4A	Type 1 diabetes (173)
2	rs7561798	A/G	A	0.4	0.1	0.03	9.8	SPHKAP	Body Weight (161)
1	rs3768436	G/T	G	0.4	0.1	0.03	9	CDC42BPA	Cholesterol, HDL (172)
2	rs445664	G/T	G	0.1	0.4	0.03	0.1	REG3G	Obesity (174)
2	rs4832106	A/G	G	0.3	0	0.03	NA	DNAH6	BMI (161)
2	rs2579387	C/T	C	0.3	0	0.03	NA	ANKRD44	Hip circumflex (161)
5	rs6453373	T/A	A	0.3	0	0.03	NA	AP3B1	Cardiovascular disease (175)
5	rs1004976	A/G	G	0.1	0.4	0.03	0.1	GALNT10	BMI (176)
9	rs4837213	C/T	T	0	0.3	0.03	0	FAM102A	Obesity-related disease (177)
9	rs7867211	A/G	G	0.3	0	0.03	NA	FNBP1	LDL (178)
11	rs2663168	A/G	A	0.4	0.1	0.03	9	ANO3	BMI (161)

CHR	SNP	Alleles	Minor allele	Freq FAO	Freq NFO	P	OR	Gene	Disease
11	rs293980	C/T	T	0.4	0.1	0.03	9	MUC15	Obesity (extreme) (179)
12	rs310791	A/G	A	0	0.3	0.03	0	E2F7	Subcutaneous Fat (161)
12	rs868158	A/G	A	0.3	0	0.03	NA	GPR133	Obesity (180)

* CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_FAO- minor allele frequencies in obese subject with high symptom counts, Freq_NFO- minor allele frequencies in obese subject with low/zero symptom counts, OR-odds ratio
** Independent t-test, $p<0.05$

I.S2.2. Comparison between FAO and Ctrl

Among the 100 SNPs with the most significant allele frequency differences between two groups of FAO and control, 26 SNPs located in 24 genes related to energy metabolism and obesity or obesity-related disease have been found (Table S6). All the aforementioned SNPs had significantly higher minor allele frequencies in the group with higher symptom counts except for rs1560511 (T), rs2886232 (T), rs1883790 (G), rs6009 (T), rs7802841 (C), rs605251 (G), and both rs6420479 (A) and rs6565491 (C) respectively located in PXDNL, ABCA10, JPH2, F5, KIAA1549, ME3, and RPTOR. Among the SNPs with minor allele frequency equal to zero in any groups, based on the information obtained from F-SNP, for rs734538 (G), rs1560511 (T), rs11243457 (A), rs2886232 (T), rs2235120 (G), rs605251 (G), rs6420479 (A), rs6565491 (C), and rs7163339 (T) no function has been reported.

Table S6. SNPs with significantly different frequencies associated with the genes related energy metabolism and obesity or obesity-related disease in between FAO and Ctrl*

CHR	SNP	Alleles	Minor allele	Freq FAO	Freq Ctrl	P	OR	Gene	Disease
4	rs2278576	C/G	C	0.4	0	0.01	NA	KIAA0922	Cardiovascular disease (181)
5	rs37567	C/G	C	0.4	0	0.01	NA	MAST4	Body Weight (182)
3	rs782427	A/G	A	0.4	0	0.01	NA	MCM2	Diabetes (183)
11	rs1044782	A/G	A	0.5	0.07	0.01	13	PGM2L1	Diabetes (184)
2	rs7426114	C/T	C	0.4	0.06	0.01	11.7	NEB	Waist Circumference (161)
11	rs10742699	A/G	A	0.4	0.06	0.01	11.7	ALX4	Diabetes (185)
17	rs12940161	A/G	G	0.4	0.06	0.01	11.7	MYH3	Cardiovascular disease (186)
2	rs3749022	C/T	C	0.3	0	0.01	NA	HIBCH	BMI (161)
5	rs1445804	C/T	T	0.3	0	0.01	NA	DNAH5	Blood Pressure (187)
5	rs2115500	C/T	C	0.3	0	0.01	NA	POLR3G	Cardiovascular disease (188)
6	rs734538	A/G	G	0.3	0	0.01	NA	SCUBE3	Cardiovascular disease (189)
8	rs1560511	T/G	T	0	0.3	0.01	0	PXDNL	Cardiovascular disease (190)
8	rs6991629	A/G	A	0.3	0	0.01	NA	FER1L6	Diabetes (169)
9	rs11243457	A/C	A	0.3	0	0.01	NA	RAPGEF1	Diabetes (191)
11	rs3802762	A/G	A	0.3	0	0.01	NA	CHST1	Glucose metabolism (192)
17	rs2886232	C/T	T	0	0.3	0.01	0	ABCA10	Cholesterol metabolism (193)
20	rs1883790	A/G	G	0	0.3	0.01	0	JPH2	Cardiovascular disease (194)
1	rs6009	C/T	T	0	0.3	0.02	0	F5	Cardiovascular disease (195)
7	rs7802841	C/A	C	0	0.3	0.02	0	KIAA1549	Cardiovascular disease (196)
8	rs2235120	C/G	G	0.3	0	0.02	NA	MYOM2	Cardiovascular disease (172)
11	rs11549076	A/G	A	0.3	0	0.02	NA	ME3 (intron)	Diabetes (197)
	rs605251	A/G	G	0	0.3	0.02	0		
17	rs6420479	A/G	A	0	0.3	0.02	0	RPTOR	Obesity (198, 199)
	rs6565491	C/T	C	0	0.3	0.02	0		
5	rs379707	C/A	C	0.3	0	0.02	NA	FYB	Diabetes(200)
15	rs7163339	C/T	T	0.3	0	0.02	NA	SPRED1	Obesity (201)

*CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_FAO- minor allele frequencies in obese subject with high symptom counts, Freq_Ctrl- minor allele frequencies in normal weight subject with low/zero symptom counts, OR-odds ratio

** Independent t-test, $p<0.05$

I.S2.3. Comparison between NFO and Ctrl)

Among the 35 SNPs with the most significant allele frequency differences between two groups with low/zero symptom counts, 5 SNPs located in 5 genes related to energy metabolism and obesity or obesity-related disease has been found (Table S7). Among these SNPs, rs1950501 (G), and rs10497553 (C), respectively located in RIPK3, and CWC22 had significantly higher minor allele frequencies in the group with higher BMI (NFO).

Table S7. SNP with significantly different frequencies associated with the genes related energy metabolism and obesity or obesity-related disease between NFO and healthy Ctrl*

CHR	SNP	Alleles	Mino allele	Freq NFO	Freq Ctrl	P	OR	Gene	Disease
12	rs2255301	T/C	T	0.1	0.5	0.03	0.1	CD4	Diabetes type 1(202)
14	rs1950501	G/C	G	0.5	0.1	0.03	7	RIPK3	Obesity (203)
2	rs10497553	T/C	C	0.5	0.1	0.04	6	CWC22	Obesity (204)
6	rs1874230	A/G	A	0.1	0.5	0.04	0.2	EEF1A1	Hypertension (205)
17	rs8531	T/G	T	0.1	0.4	0.05	0.2	C17orf59	Cholesterol regulation (206)

*CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_NFO minor allele frequencies in obese subject with low/zero symptom counts, Freq_Ctrl- minor allele frequencies in normal weight subject with low/zero symptom counts, OR-odds ratio
** Independent t-test, $p<0.05$

I.S2.4. Comparison between FAO vs. NFO+Ctrl

When the subjects with low/zero symptom counts from NFO and Ctrl groups were combined (NFO+Ctrl), the allele frequencies were compared within FAO. Among the 100 SNPs discovered with the most significant allele frequency differences between two groups, 25 SNPs located in 20 genes related to energy metabolism and obesity or obesity-related disease has been found (Table S8). All the SNPs had significantly higher minor

allele frequencies in the group with high symptom counts. In the case of minor allele frequency equal to zero in any groups, based on the information obtained from F-SNP, all the SNPs had been functional except for rs2854437 (T), rs228075 (A), rs4970433 (G), rs5909118 (T), rs556674 (G), and rs2482023 (A) respectively located in SORD, SLC37A1, GLTPD1, MAP3K15, COL12A1 and CAMK1D.

Among all the reported SNPs, the frequency of minor allele C in rs2278576 located in KIAA0922 that was significantly higher in FAO compared to the control group (Table S6) remained significantly higher when they were compared to NFO+Ctrl. In addition, the minor allele frequency of rs6941075 (T), rs1409986 (A), rs2273394 (C), rs2579387 (C), and rs2181687 (G) that was significantly higher in FAO compared to NFO (Table S5) remained significantly higher when FAO was compared with NFO+Ctrl (Table S8).

Table S8. SNPs with significantly different frequencies associated with the genes related energy metabolism and obesity or obesity-related disease between FAO and NFO+Ctrl

CHR	SNP	Alleles	Mino allele	Freq FAO	Freq NC	P	OR	Gene	Disease
4	rs2278576	C/G	C	0.4	0.03	0.001	18.6	KIAA0922	Cardiovascular disease (181)
8	rs10956163	C/G	C	0.3	0	0.004	NA	FER1L6	Diabetes (169)
15	rs2854437	T/G	T	0.3	0	0.004	NA	SORD	Diabetes (207, 208)
21	rs228075	A/G	A	0.3	0	0.004	NA	SLC37A1	Body Weight (161)
6	rs6941075	T/A	T	0.5	0.1	0.01	7	AKAP12 (intron)	Abdominal Fat , Waist-Hip Ratio (161)
1	rs1409986	A/G	A	0.4	0.1	0.01	9	PTGER3	Obesity (162)
2	rs4665119	T/G	T	0.4	0.1	0.01	9	RBM43	Diabetes (209)
9	rs2767012	T/G	G	0.2	0	0.01	NA	MUSK (intron)	Obesity (210)
	rs8011016	C/T	C	0.2	0	0.01	NA	SLC7A8	

CHR	SNP	Alleles	Mino allele	Freq FAO	Freq NC	P	OR	Gene	Disease
14	rs2273394	C/G	C	0.3	0.03	0.01	12.4		Blood cholesterol (172)
1	rs4970433	A/G	G	0.2	0	0.01	NA	GLTPD1	Obesity (211)
2	rs7565685	C/T	T	0.2	0	0.01	NA	ANKRD44	Hip circumflex (161)
	rs2579387	C/T	C	0.3	0.03	0.02	10.3		
23	rs5909118	T/A	T	0.2	0	0.01	NA	MAP3K15	Hypertension (212, 213)
1	rs2282366	A/G	A	0.2	0	0.01	NA	ACTN2	Cardiomyopathy (214)
4	rs10856978	C/T	C	0.2	0	0.01	NA	NPNT	Cardiovascular Diseases(215)
6	rs9348724	C/G	C	0.2	0	0.01	NA	SYCP2L	Cholesterol, LDL(172)
6	rs556674	A/G	G	0.2	0	0.01	NA	COL12A1	Cardiovascular Diseases(216)
10	rs2482023	A/C	A	0.2	0	0.01	NA	CAMK1D	Diabetes (217)
1	rs571026	C/T	T	0.4	0.1	0.02	5.8	RYR2	Cardiovascular Diseases(218)
	rs1759123	A/G	A	0.4	0.1	0.02	5.8		
2	rs2015983	A/C	A	0.4	0.1	0.02	5.8	ASB18	Cholesterol, LDL(219)
6	rs2181687	A/G	G	0.4	0.01	0.02	5.8	USP45	Diabetes (163)

* CHR- chromosome, SNPs- Single Nucleotide Polymorphisms, Freq_FAO- minor allele frequencies in obese subject with high symptom counts, Freq_NC- minor allele frequencies in obese and normal weight subject with low/zero symptom counts, OR-odds ratio

** Independent t-test, $p<0.05$

I.S3. SNPs associated with the genes related to cancer and the genes with unknown function or with other diseases out of the aforementioned disease

Among the 100 SNPs with the most significant differences in frequencies between different groups of FAO, NFO, Ctrl and NFO+Ctrl, the rest of the non-reported SNPs listed above were located in the genes associated with different types of cancers, congenital genetic diseases, immune system, reproductive system, some diseases like Crohn's disease, dermal disease, malaria, etc. However, some of the SNPs were located on genes whose the function has not been cleared yet.

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5

Chapter 5. Conclusions, Limitations, and Future Directions

5.1 Concluding remarks

Over the past 3 decades, the global prevalence of obesity has increased substantially and this disease has been recognized as the fifth principal cause of death in the world. There is a growing interest in the role of food addiction in increasing prevalence of human obesity. However, the exploration of food addiction in humans is at an early stage and many fundamental questions are yet to be answered. This thesis by a multi-tired approach has added distinguished findings in the field of food addiction. These findings open a novel avenue to assess the etiology of obesity and consequently may aid in finding new effective methods to treat and prevent obesity.

In my thesis, the approach for the understanding of the contributing factors in food addiction was in three phases and to the best of our knowledge, all the three studies were the first study of its kind.

In the first phase, we revealed that the prevalence of food addiction in the general Newfoundland population was 5.4% with a higher risk in women than men. In addition, on average 84% of food addicts were overweight/obese suggesting that obesity featured with food addiction may represent an important subgroup of the obese with a distinctive etiology. Interestingly, food addiction was significantly associated with the severity of obesity/amount of body fat from normal to obese individuals in the general population. These data provided a direct evidence that food addiction is strongly associated with obesity in the general population. In this phase of the study, we also found that food-addicted subjects' diet consisted of a higher percentage of calories from fat and protein,

possibly suggesting that these types of foods are more likely to be associated with compulsive overeating. Taken all these findings together, highlighted the necessity to understanding the probable etiology of food addiction. Therefore, in the second and third phase of our study, we set two equally obese groups matched by age, gender and physical activity levels but with the different phenotype of food addiction (food addicts and non-food addicts, or with high and low clinical symptom count). Thus, the key difference between the two groups was the food addiction phenotype. This study design provided us an excellent opportunity to discover the potential cause of obesity-related food addiction.

In phase II, we discovered significant differences in multiple aspects, including hormonal levels and nutritional intakes, between obese food addicts and obese non-food addicts. Among 34 neuropeptides, gut hormones, pituitary polypeptide hormones and adipokines that regulate appetite and metabolism, obese food addicts had lower levels of TSH, TNF-α, and amylin, but higher levels of prolactin, as compared to obese non-food addicts. Furthermore, in comparison between the two groups, obese food addicts consumed higher amount of total calorie intake (per kg body weight), the dietary fat intake (per g/kg body weight, per BMI and per percentage of trunk fat), the percent calorie intake from fat and carbohydrates (g/kg), sugar, minerals (including sodium, potassium, calcium and selenium), fat and its components (such as saturated, monounsaturated and trans fat), omega 3 and 6, vitamin D and gamma-tocopherol. Therefore, taken together, the data suggest that obese food addicts may consume more hyper-palatable foods that are known to have high amounts of fat, sugar, and salt (sodium).

The third phase of food addiction study was a two-stage study, a combination of exome sequencing method and a candidate gene association approach to find food addiction candidate genes. In this study, we used YFAS clinical symptom count as a phenotype of food addiction and we also added a healthy group as a control to the other two groups of obese with high and low/zero clinical symptom counts. In the first stage, which was the exome sequencing study, the 100 SNPs with the most significant allele frequency differences among 3 groups were categorized into 5 subgroups based on gene functions. The top 19 SNPs (TIRAP, MMADHC, ERAP1, NTM, MYPN, GRID1, ITPR2, GPSM1, ZCCHC14, TNN, PPARD, CACNA1C, SIM1, and DRD2) in the addiction subgroup were genotyped in the second stage on the entire food addiction population. In the stage II, we discovered that the major allele A of rs2511521 located in DRD2 (OR=3.1(95% CI 1.1-8.2)) and the minor allele T of rs625413 located in TIRAP (OR=2.5(95% CI 1.1-5.8)) in obese subjects with low/zero clinical symptoms significantly associated with increased risk of food addiction. What made the finding of our study reliable was the combination of the screening study using exome sequencing followed by a validation study using different methodology, i.e. a genetic association approach.

In conclusion, this thesis has provided an exclusive insight into the underlying causes of obesity and especially food addiction. The major strength of the current thesis was using different technologies that allowed us to explore the etiology of food addiction based on the fundamental factors responsible for obesity and addiction.

5.2 Limitations of the present work

Despite the significant and novel findings in each study of this thesis, there were limitations in our study. Aside from the limitations that were discussed in each paper, there are few additional concerns on the studies. The first limitation was attributed to the fact that our established food addiction study, as a part of CODING study, was a cross-sectional study. Therefore, unlike the longitudinal study, through a cross-sectional study, it was not possible to characterize inter-relationships and roles of disease risk factors and to supply evidence to cause-effect relationships, especially between some of the associations discovered.

Another limitation of our study is due to the greater number of females compared to males in the food addiction study. This could be justified by the notion that females are more likely to participate in population-based studies.

5.3 Future direction

The findings of the current thesis have opened up a number of avenues for the future work. As mentioned in above, a cross-sectional study has some limitations. Converting the existing cross-sectional CODING study to a new longitudinal design will greatly increase its value because a longitudinal study will provide valuable information to further characterize interrelationships and roles of appetite-regulating hormones and dietary intake in obesity and food addiction. Furthermore, this type of study will supply evidence to cause-effect relationships, especially between some of the associations discovered in the CODING study. At the current time, 10% of the subjects has participated from 2 to 8 times.

We have the plan to expand our CODING study to a longitudinal study in the near future and re-invite the subjects.

We recently started to add a further questionnaire to evaluate binge eating disorder in our CODING study. One of the limitations of our study was the lack of information on other eating disorders comorbidities. Evidence supports that there are overlaps between food addiction and other eating disorders like binge eating disorder. However, there is no study available to show, to what degree and in which aspect this overlap exists. Therefore, this information will provide valuable information on differences of the genes and appetite regulating hormones in the subjects with food addiction and binge eating disorder.

APPENDIX 1 - MANUSCRIPTS PUBLISHED DURING PHD

1. **P. Pedram**, G. Zhai, W. Zhang, Y Zhang, W Gulliver, G Sun; Metabolomics Characteristics in Obese Human Subjects with Food Addiction, in progress

2. **P. Pedram**, P. Gregory, A. Card, T. Bridger, H. Zhang, G. Sun; Serum GLP-1, PYY, ghrelin, amylin and TNFα are significantly associated with the severity of Childhood Obesity. PLOS ONE in progress

3. **P. Pedram**, W. Gulliver, G. Zhai, E. Aref-Eshghi, H. Zhang, G.Sun; Two novel candidate genes identified in adults from the Newfoundland population with addictive tendencies towards Food Appetite (invited for special issue), Appetite 2017 .

4. Xiang Gao, Yongbo Wang, Edward Randell, **Pardis Pedram**, Yanqing Yi, Wayne Gulliver, and Guang Sun. Higher dietary choline and betaine intakes are associated with better body composition in the adult population of Newfoundland, Canada. PLOS ONE. Accepted.

5. Yongbo Wang, Xiang Gao, **Pardis Pedram**, Mariam Shahidi, Jianling Du, Yanqing Yi, Wayne Gulliver, Hongwei Zhang, Guang Sun. Significant Beneficial Association of High Dietary Selenium Intake with Reduced Body Fat in the CODING Study. 2016 Jan 04; Nutrients 8 (1), 24

6. **Pedram P**, Sun G. Hormonal and Dietary Characteristics in Obese Human Subjects with and without Food Addiction. Nutrients (invited for special issue). 2014 Dec 31;7(1):223-38. doi: 10.3390/nu7010223.

7. Amini P, Cahill F, Wadden D, Ji Y, **Pedram P**, Vidyasankar S, Yi Y, Gulliver W, Paterno G, Zhang H, Rideout A, Sun G. Beneficial association of serum ghrelin and peptide YY with bone mineral density in the Newfoundland population. BMC Endocr Disord. 2013 Sep 23;13(1):35.

8. **Pedram P**, Wadden D, Amini P, Gulliver W, Randell E, Cahill F, Vasdev S, Goodridge A, Carter JC, Zhai G, Ji Y, Sun G. Food addiction: its prevalence and significant association with obesity in the general population. PLoS One. 2013 Sep 4;8(9):e74832. doi: 10.1371/journal.pone.0074832. eCollection 2013.

APPENDIX 2 – RESEARCH GRANTS DURING PHD

A2.1 The role of food addiction and gut hormones in obese Newfoundland children

Agency:

Janeway Foundation Research Award

Grant period

2013-2014

Budget:

$5,000

Summary

Background: Background: Childhood obesity is one of the most serious public health challenges. In Canada, Newfoundland has the highest rate of overweight and obese. The causes of childhood obesity are not yet completely understood. Our findings in our previous study on adult general population provide strong evidence that food addiction may represent a distinct etiology of human obesity. However, to the best of our knowledge there is no data are currently available regarding the role of food addiction in childhood obesity.

Objectives: To assess 1) the prevalence of food addiction in obese children, 2) the correlation of clinical symptom counts of food addiction with body composition measurements in obese children and 3) comparison of gut hormones level in food addicted and non-food addicted obese children to investigate if gut hormones are related to food addiction.

Method: With a previously awarded CIHR operating grant, a total of 125 families from the Canadian province of Newfoundland and Labrador with at least one obese child plus a parent or two parents recruited. And anthropometric measurements, body composition measurements, physical activity level and calorie intake was measured and the data entered in our data base. With the help of participant's parent or guardian the Yale Food Addiction Scale (the questionnaire for diagnosis of food addiction) will be filled. Gut hormones (Ghrelin, GLP-1, PYY, GIP, CCK, amylin, PP) will be measured using MAGPIX system and ELISA.

Expected results: For the first time, we will: 1) find prevalence of food addiction in obese children, which is extremely important for dissecting the etiology of childhood obesity, 2)

obtain solid evidence that food addiction contributes to childhood obesity, and 3) explore if gut hormones are related to food addiction.

A2.2 The Association of Dietary Selenium Intake and Level of Serum Selenium with Childhood Obesity

Agency:

Janeway Foundation Research Award

Grant period

2015-2016

Budget:

$6,000

Summary

Background: The rapidly rising prevalence of childhood obesity in Newfoundland and Canada has become a severe public health problem. However, the etiology of childhood obesity is not completely clear. Studies from our lab and others suggested that certain micronutrients might be associated with increased body fat deposition in adults, however little research has been done on obese children. In one of our previous studies on adults in the Newfoundland population we have discovered that dietary selenium has a significant beneficial effect on body composition. There are few studies available regarding the beneficial effect of serum selenium in obese children, however, the results from these studies were inconsistent. To the best of our knowledge there is no study available regarding the effect of dietary selenium on obesity indexes in children.

Objectives: In the current proposal, we will: 1) compare dietary selenium intake and serum selenium levels among obese/overweight, and normal weight children in Newfoundland, 2) analyze the correlation between dietary selenium intake and serum selenium levels with obesity indexes, 3) find out the beneficial dose of dietary selenium on obesity indexes

Method: The proposed study will be built on an existing CIHR previously funded childhood obesity cohort consisting of 125 obese children recruited from Newfoundland. In addition, 125 Normal weight children will be used from study funded by a 2013 Janeway Research Award. Serum selenium level will be measured in both groups using atomic absorption spectrometry. The Dietary selenium intake will be measured in both groups using Willet Food Frequency Questionnaire.

Expected results: We are expecting to obtain:1) Beneficial association of dietary selenium with obesity measurements, 2) Significant difference of dietary selenium intake and serum

selenium level among obese/overweight and normal children. The findings will provide solid evidence for policy makers to create policy or program to reduce the prevalence of child obesity in Newfoundland by implanting new program to supplement selenium in diet (school lunch for example).

A2.3 The Association of Dietary Calcium Intake and Level of Serum Calcium with Obesity and Insulin Resistance in NL Children

Agency:

Janeway Foundation Research Award

Grant period

2016-2017

Budget:

$5,000

Summary

Background: Childhood obesity is one of the most serious public health challenges in Newfoundland and Labrador and Canada. Dietary Calcium (Ca) as an important micronutrient is well recognized as a critical nutrient, beyond its key role in the maintenance of skeletal integrity, in modulating chronic disease risk in both children and adults. There are numerous studies available in supporting the beneficial association of dietary calcium on body composition in adults and also children. However, it is not clear how and in which level the extra cellular calcium can affect the body composition and sensitivity to insulin in children. There have been few isolated reports with inconsistent results on the relationship between serum calcium and obesity in adults. However, to the best of our knowledge there is no study available on the effect of serum calcium on obesity indexes in children.

Objectives: in the current proposal, we will 1) compare the dietary calcium intake and serum calcium levels among obese/overweight, and normal weight children in Newfoundland, 2) analyze the correlation between serum calcium levels and dietary calcium intake with obesity indexes (BMI, waist and hip circumference, percentage of body fat, trunk fat, gynoid and android fat and visceral fat), 3) analyze the correlation between serum calcium levels and dietary calcium intake with insulin resistance

Method: The proposed study will be built on an existing CIHR previously funded childhood obesity cohort consisting of 125 obese children recruited from Newfoundland. In addition, 125 Normal weight children will be used from study funded by a 2013 Janeway Research Award. Fasting serum calcium, insulin level, PTH and 25-OH vitamin D will be

measured in both groups. HOMA-β and HOMA-IR will be calculated. The Dietary calcium intake will be measured in both groups using Willet Food Frequency Questionnaire.

Expected results: We are expecting to obtain the following research findings: 1) Beneficial association of dietary calcium with obesity indexes, 2) Beneficial association of serum calcium with obesity indexes and insulin resistance.

APPENDIX 3 – PUBLISHED ABSTRACTS & PRESENTATIONS DURING PHD

ORAL PERESENTAION

1. Two new candidate genes of food addiction identified, **34th Annual Scientific Meeting of The Obesity Society;** New Orleans, LA , November 2016.

2. Food Addiction: Its Prevalence and Significant Association with Obesity in the General Population", **31st Annual Scientific Meeting of The Obesity Society**, Atlanta, Georgia, November, 2013.

3. Prevalence of food addiction and its association with obesity in the Newfoundland population, **3rd Canadian obesity summit**, May.2013, Vancouver, Canada.

4. The comparison between efficacy of Tamsulosin and Terazosin in alpha-adrenergic blockers in spontaneous passage of ureter stones after ESWL: **10th International Students' Conference on Biomedical and Interdisciplinary Researches (SICOBAIR)** May,2009, Iran University of Health and Medical Sciences, Tehran, Iran.

POSTER PERESNETATION

1. **P Pedram**, Tracy Bridger, Yun Huang, Haicheng Zhou, Wayne Gulliver, Guangju Zhai, Sudesh Vasdev, Mike Whal, Guang Sun; The Associations of Dietary Selenium Intake and Level of Serum Selenium with Childhood Obesity; **34th Annual Scientific Meeting of The Obesity Society**; New Orleans, LA , November 2016

2. **P Pedram**, Tracy Bridger, Yun Huang, Haicheng Zhou, Wayne Gulliver, Sudesh Vasdev, Guangju Zhai, Mike Whal, Guang Sun; Negative associations of dietary and serum selenium with insulin resistance in children; **34th Annual Scientific Meeting of The Obesity Society**; New Orleans, LA , November 2016

3. Matthew Nelder, **Pardis Pedram**, Farrell Cahill, Hongwei Zhang, Guangjo Zhai, Wayne Gulliver, Guang Sun; The Association between Food Addiction and Body Fat Distribution in Men and Women of the General Newfoundland Population; **34th Annual Scientific Meeting of The Obesity Society**; New Orleans, LA , November 2016

4. Haicheng Zhou, **Pardis Pedram,** Yun Huang, Hongwei Zhang, Guang Sun; Serum selenium level in response to short term overfeeding in young men; **34th Annual Scientific Meeting of The Obesity Society**; New Orleans, LA , November 2016

5. **P. Pedram**, P. Gregory, A. Card, T. Bridger, H. Zhang, G. Sun; Serum GLP-1, PYY, ghrelin, amylin and TNFα are significantly associated with the severity of Childhood Obesity; **33rd Annual Scientific Meeting of The Obesity Society**; Los Angeles (CA), November 2015 (**it has been selected as a top 10 abstract presentation in The Paediatric Obesity Section**).

6. **P. Pedram**, W. Gulliver, G. Zhai, E. Aref-Eshghi, H. Zhang, G.Sun; Identifying candidate genes of food addiction by exome sequencing; **33rd Annual Scientific Meeting of The Obesity Society**; Los Angeles (CA), November 2015.

7. Yongbo Wang, Xiang Gao, **Pardis Pedram**, Mariam shahidi, Jianling Du, Yanqing Yi, Wayne Gulliver, Guang Sun. Significantly beneficial association of dietary selenium intake with reduced body fat. **33rd Annual Scientific Meeting of The Obesity Society**, 2015. Los Angeles, November 2-7, 2015

8. **P. Pedram**, W. Gulliver, D. Wadden, F. Cahill, E. Randell and G. Sun; Hormonal and Dietary Characteristics in Obese Human Subjects with and without Food Addiction; **32nd Annual Scientific Meeting of The Obesity Society**; Boston (MA), November 2014.

9. **P. Pedram**, G. zhai, W. Zhang, Y Zhang, W Gulliver, F Cahill, G Sun; Metabolomics Characteristics in Obese Human Subjects with Food Addiction; **32nd Annual Scientific Meeting of The Obesity Society**; Boston (MA), November 2014.

10. **P. Pedram**, P. Amini, D. Wadden, F. Cahill, W. Gulliver, G. Zhai, G. Sun, Hormonal, metabolic and dietary characteristics in food addicted obese. **31st Annual Scientific Meeting of The Obesity Society**, Atlanta, Georgia, November 2013.

11. P. Amini, D. Wadden, F. Cahill, **P. Pedram**, S. Vidyasankar, W. Gulliver, E.W. Randell, H. Zhang, G. Sun. Serum Acylated Ghrelin Is Negatively Correlated with High Sensitivity C - Reactive Protein in the Newfoundland Population. **31st Annual Scientific Meeting of The Obesity Society**, Atlanta, Georgia, November 2013.

12. P. Amini, F. Cahill, D. Wadden, **P. Pedram**, S. Vidyasankar, W. Gulliver, E.W. Randell, G. Sun. High Dietary Selenium Intake Is Associated with a Low Percentage of Body Fat in the Newfoundland Population. **31st Annual Scientific Meeting of The Obesity Society**, Atlanta, Georgia, November 2013.

13. P. Amini, F. Cahill, D. Wadden, **P. Pedram**, W. Gulliver, E.W. Randell, T. Bridger, H. Zhang, G. Sun. Correlation of Gut Hormones with Body Composition Characteristics in Obese Children. **31st Annual Scientific Meeting of The Obesity Society**, Atlanta, Georgia, November 2013.

14. D. Wadden, P. Amini, F. Cahill, **P. Pedram**, T. Bridger, W. Gulliver, E.W. Randell, G. Sun. Gut Hormones and Childhood Obesity in the Newfoundland Population. **31st Annual Scientific Meeting of The Obesity Society**, Atlanta, Georgia, November 2013.

APPENDIX 4 – POSTERS PRESENTED AT INTERNATIONAL CONFERENCES

A4.1 Hormonal and Dietary Characteristics in Obese Human Subjects with and without Food Addiction

Presented in 32nd Annual Scientific Meeting of The Obesity Society; Boston (MA), November 2014.

Background: The concept of food addiction (FA) is a potentially important contributing factor to the development of obesity in the general population; however, little is known about the hormonal and dietary differences between obesity with and without FA. **Objectives:** The aim of our study was to explore potential biomarkers, including various hormones and neuropeptides, which regulate appetite and metabolism, and dietary components that could potentially differentiate obesity with and without FA.

Design: Of the 737 adults recruited from the general Newfoundland population, 58 food-addicted and non-food-addicted overweight/obese individuals (FAO, NFO) matched for age, sex, BMI and physical activity were selected. A total of 34 neuropeptides, gut hormones, pituitary polypeptide hormones and adipokines were measured in fasting serum.

Results: We found that the FAO group had lower levels of TSH, TNF-α and amylin, but higher levels of prolactin, as compared to NFO group. The total calorie intake (per kg body weight), the dietary intake of fat (per g/kg body weight, per BMI and per percentage of trunk fat) and the percent calorie intake from fat and carbohydrates (g/kg) was higher in the FAO group compared to the NFO group. The FAO subjects consumed more sugar, minerals (including sodium, potassium, calcium and selenium), fat and its components

(such as saturated, monounsaturated and trans fat), omega 3 and 6, vitamin D and gamma-tocopherol compared to the NFO group.

Table 1: Hormonal and neuropeptide characteristics in food addicted (FAO) and non – food addicted (NFO) overweight/obese

	Hormones (pg/ml)	FAO mean± SD (14-29)	NFO mean± SD (14-29)	P
Neuropeptides	NPY	8.81±3.74	5.71±3.82	ns
	α-MSH	148.06±84.16	147.2±89.12	ns
	β-Endrophin	377.86±90.82	396.54±108.3	ns
	Cortisol	230056±100323	232807.9±138900	ns
	Melatonin	3320.9±1377.7	3652.75±1652.43	ns
	MCP1	294.43±88.2	282.56±90.11	ns
	Neurotensin	379.6±103.05	379.32±100.7	ns
	Oxytocin	119.5±49.13	120.22±57.86	ns
	Orexin A	969.6±438.2	974.5±347.5	ns
	AGRP	16.11±6.94	16.18±8.26	ns
	Substance P	39.16±12.51	39.7±15.06	ns
Gut hormones	**Amylin**	24.9±11.3	32.05±18.75	**0.04**
	GLP-1	19.91±22.54	21.4±22.1	ns
	Gherelin	25.4±15.8	25.91±17	ns
	Leptin	20795.4±12173.3	18206.72±10765.9	ns
	GIP	17±16.31	17.05±12	ns
	Glucagon	22.61±10.5	45.1±52.02	ns
	PP	49.3±79.4	46.85±53.4	ns
	PYY	68.33±122.3	93±109.3	ns
	C-peptide	1373.7±740.15	1269±506.74	ns
Pituitary polypeptide hormones	**Prolactin**	2335.1±1197.8	1938.3±745.5	**0.02**
	ACTH	3.05±2.56	5.55±6.93	ns
	BDNF	2219.3±658.73	2138.38±931.52	ns
	LH	6.2±7.3	6.21±8.6	ns
	FSH	13.27±18.75	9.58±14.12	ns
	GH	505.76±635.84	810.83±1019.56	ns
	TSH	0.23±0.32	1.1±2.14	**0.01**
	CNTF	148.1±324.22	1647.4±6280.6	ns
Adipokines	**TNF-α**	4.21±1.23	4.5±2.2	**0.02**
	Adiponecin	65700.8±68327.1	71437.3±56215.3	ns
	Lipocalin	357±151.7	462.2±153	ns
	Adipsin	7167.66±2888.25	8009.9±2733	ns
	PAL1	261.3±88.8	261.31±88.84	ns
	Resistin	82±43.4	109±55.8	ns

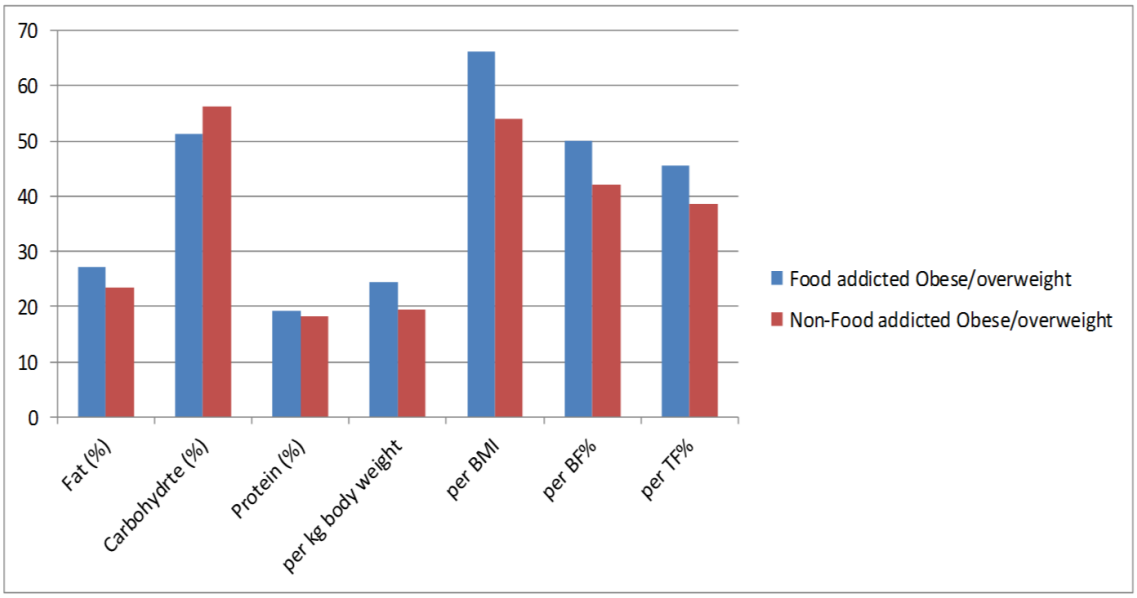
Mean ± standard deviation (SD), FAO– food addiction overweight/obese NFO – non-food addiction overweight/obese, BMI – body mass index. Independent t-test was set to $p < 0.05$.

Table 2:The significant differences of micronutrient intakes per kg body weight between food addicts (FAO) and non-food addicts (NFO) overweight/obese.

BMI			
Dietary intakes	FAO (29) (mean ± SD)	NFA (29) (mean ± SD)	p*
Sugar (g/kg)	1.4 ± 0.8	0.17 ± 0.54	**0.03**
Saturated fat (g/kg)	0.25 ± 0.14	0.16 ± 0.06	**0.01**
Trans fat (g/kg)	0.001 ± 0.00	0.0007 ± 0.0	**0.01**
Monounsaturated fat (g/kg)	0.30 ± 0.13	0.20 ± 0.06	**0.01**
Poly saturated fat (g/kg)	0.14 ± 0.07	0.10 ± 0.03	**0.00**
Omega 3 (g/kg)	0.007 ± 0.0	0.005 ± 0.0	**0.01**
Omega6 (g/kg)	0.05 ± 0.03	0.03 ± 0.02	**0.00**
Vitamin B1 (mg/kg)	0.02 ± 0.01	0.017 ± 0.00	0.04
Vitamin D (IU/kg)	2.5 ± 2.1	1.9 ± 1.0	0.04
Dihydrophylloquinone (mcg/kg)	0.3 ± 0.03	0.2 ± 0.02	**0.03**
Gamma tocopherol (mg/kg)	0.3 ± 0.02	0.02 ± 0.01	**0.04**
Sodium (mg/kg)	26.1 ± 12.0	19.4 ± 6.3	0.01
Calcium (mg/kg)	13.0 ± 7.1	10.0 ± 4.0	0.02
Potassium (mg/kg)	50.8 ± 21.3	41.2 ± 16.8	0.04
Selenium (mg/kg)	1.4 ± 0.6	1.1 ± 0.3	0.02

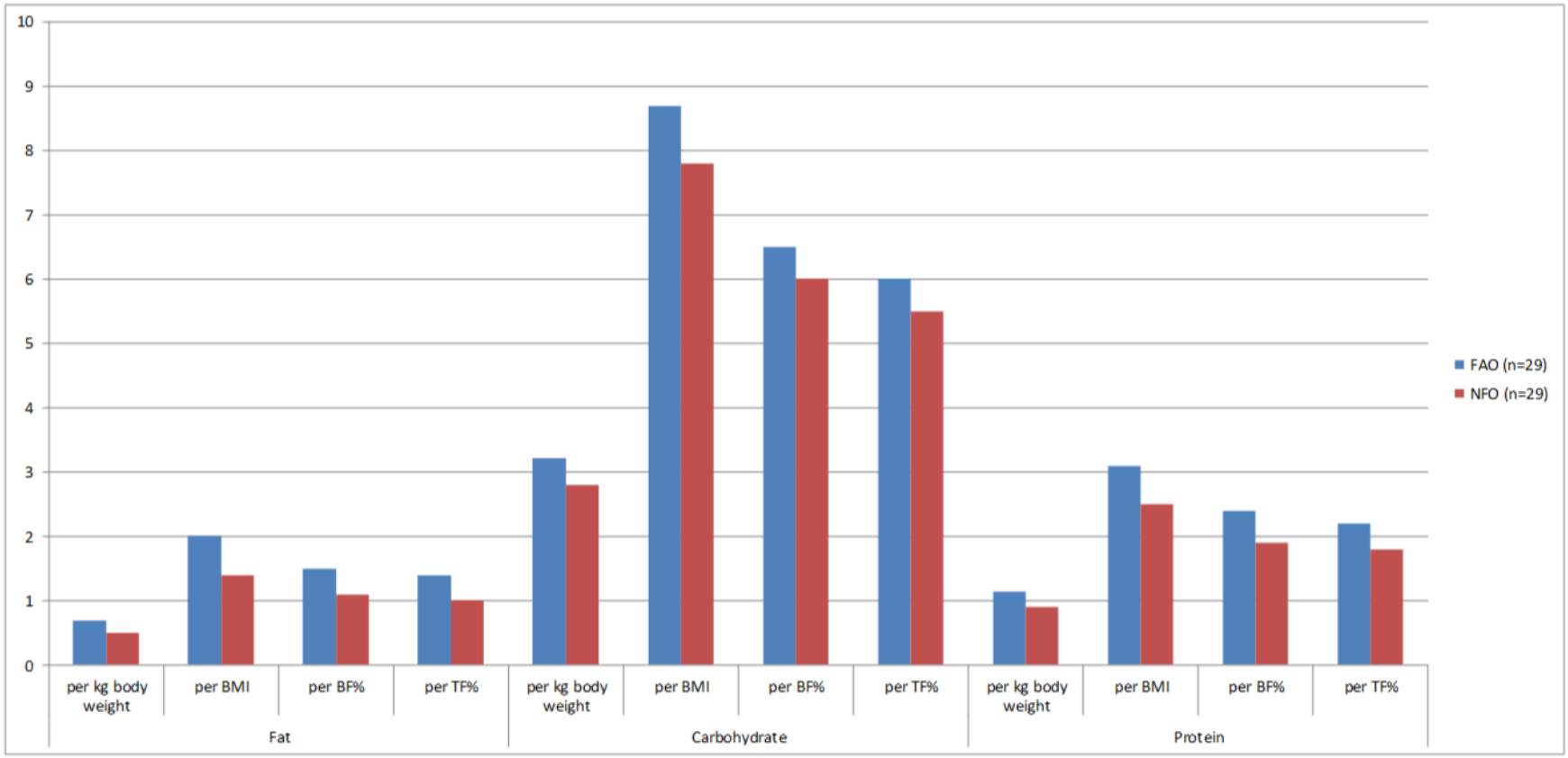
* Independent t-test was set to $p < 0.05$. Bolded p indicates $p < 0.05$ between FAO and NFO based on percentage of the body fat as well.

Fig.1: Calorie intake characteristics in food addiction and non- food addiction overweight/obese groups



Independent t-test test significance level was set to $p < 0.05$

Fig. 2: Macronutrients intake characteristics in food addiction (FAO) and non- food addiction (NFO) overweight/obese group



* Independent t-test test significance level was set to $p < 0.05$

A4.2 Metabolomics Characteristics in Obese Human Subjects with Food Addiction

Presented in 32nd Annual Scientific Meeting of The Obesity Society; Boston (MA), November 2014.

Background: Food addiction (FA) is a clinical trait in 5% of the adult population. However, the metabolomics (chemical fingerprint of metabolism processes) methodology has not been applied to obese with FA.

Objectives: This study explored the potential differentiation of metabolites among FA and non-FA obese.

Methods: From a total of 737 adults recruited from the general Newfoundland (NL) population 25 FA overweight/obese (FAO) and 25 non-FA overweight/obese (NFO) matched for sex, age and physical activity were selected. Obesity was evaluated by BMI and FA was assessed using the Yale Food Addiction Scale. Serum metabolomics profile including 183 metabolites (Acylcarnitines (AC), sphingolipids (SP), amino acids (AA), biogenic amines (BA) and glycerophospholipids (GP)) were measured by high performance liquid chromatography (HPLC).

Results: FAO had lower citrulline,SM(OH)C20:2,Ac-Orn and nitrotyrosine vs. NFO. Moreover, citrulline and histidine are positively and isoleucine negatively correlated to FA. AC (C16:1 and C16:2), SP [SM(OH)C20:2] and BA (Ac-Orn,dopamine,nitrotyrosine) are negatively associated with FA in obese. In FAO, C10 (r:0.43) was positively and C5-M-DC (r:-0.44) negatively among AC,GP (lysoPC a C16:0(r:-0.43) and lysoPC a C20:4(r:-

0.408) and AA (asparagine (r:-0.40) were negatively correlated to FA symptoms. However no metabolites was related to FA symptoms in NFO.

Conclusions: We have discovered, for the first time, that a number of metabolites may be associated with overweight/obesity with food addiction. These metabolites are valuable candidates in the study of food addiction.

Fig. 1: Significant differences of metabolites level between food addicts and non-food addicts overweight/obese

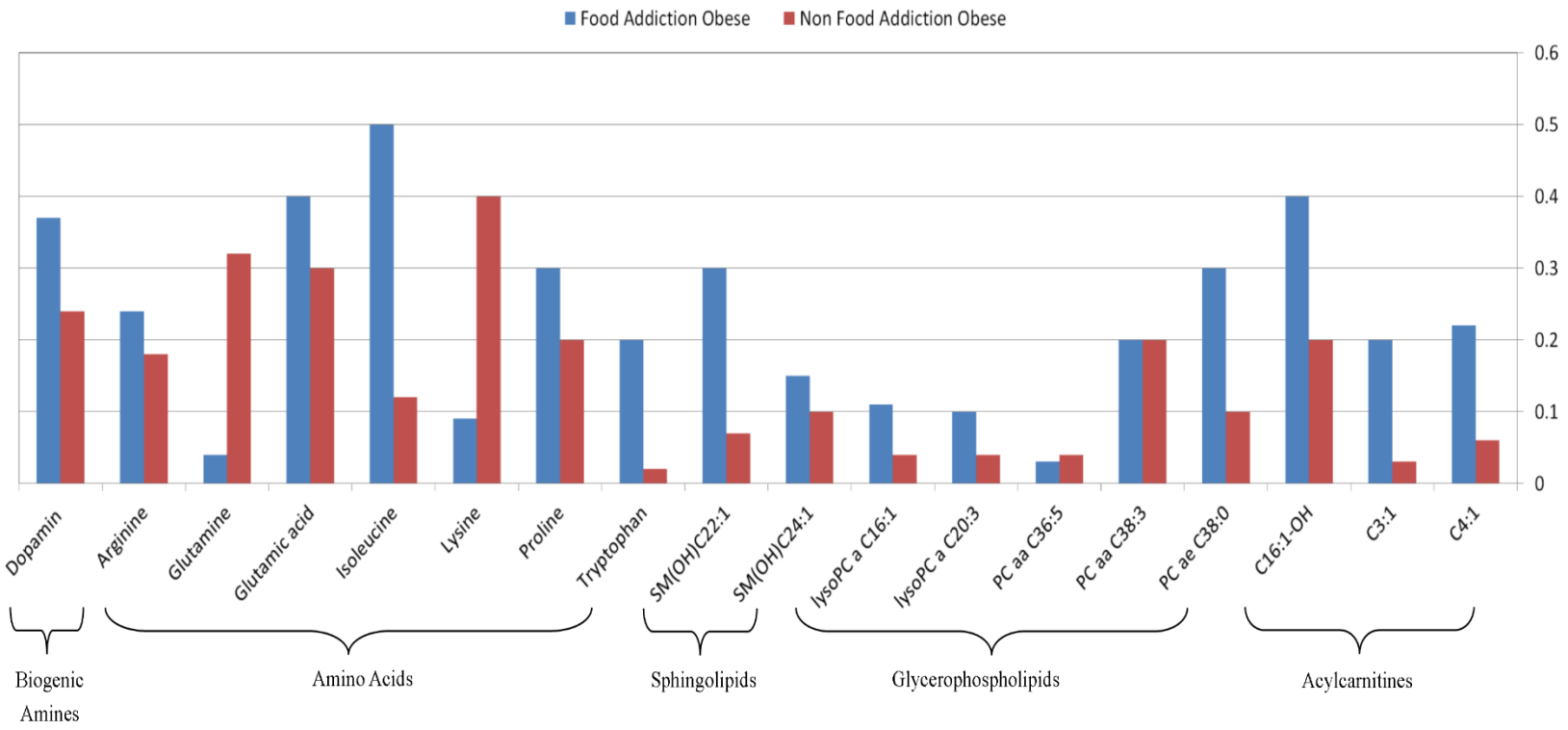


Table 1. The significant association between food addiction and serum metabolites

Metabolites		r	p
Acylcarnitines	C16:1	-1.33	0.02
	C16:2	-1.14	0.03
Sphingolipids	SM(OH)C20:2	-1.22	0.01
Biogenic amines	Ac-Orn	-1.3	0.02
	Dopamine	-1.23	0.02
	Nitrotyrosine	-0.8	0.01
Amino Acids	Citrulline	-0.93	0.01
	Histidine	-0.9	0.03
	Isoleucine	2.22	0.01

Table 2. the significant association between severity of food addiction and serum metabolites

Metabolites		r	p
Acylcarnitines	C10	0.43	0.03
	C5-M-DC	-0.44	0.03
Glycerophospholipids	lysoPC a C16:0	-0.43	0.03
	lysoPC a C20:4	-0.41	0.04
Acylcarnitines	Asparagine	-0.4	0.05

A4.3 Serum GLP-1, PYY, ghrelin, amylin and TNFα are significantly associated with the severity of Childhood Obesity

Presented in 33rd Annual Scientific Meeting of The Obesity Society; Los Angeles (CA), November 2015 (it has been selected as a top 10 abstract presentation in The Paediatric Obesity Section).

Background: The gastrointestinal tract and adipose tissue secrete many hormones that are actively involved the regulation of appetite and energy metabolism. The differences between obese and normal weight children are rarely reported.

Objectives: In the current study, we investigated the levels of an adipokine (TNFα) and 9 gut hormones: amylin, ghrelin, leptin, GLP-1, GIP, PP, PYY, C-peptide and insulin and the correlation to obesity measurements in obese and normal weight children.

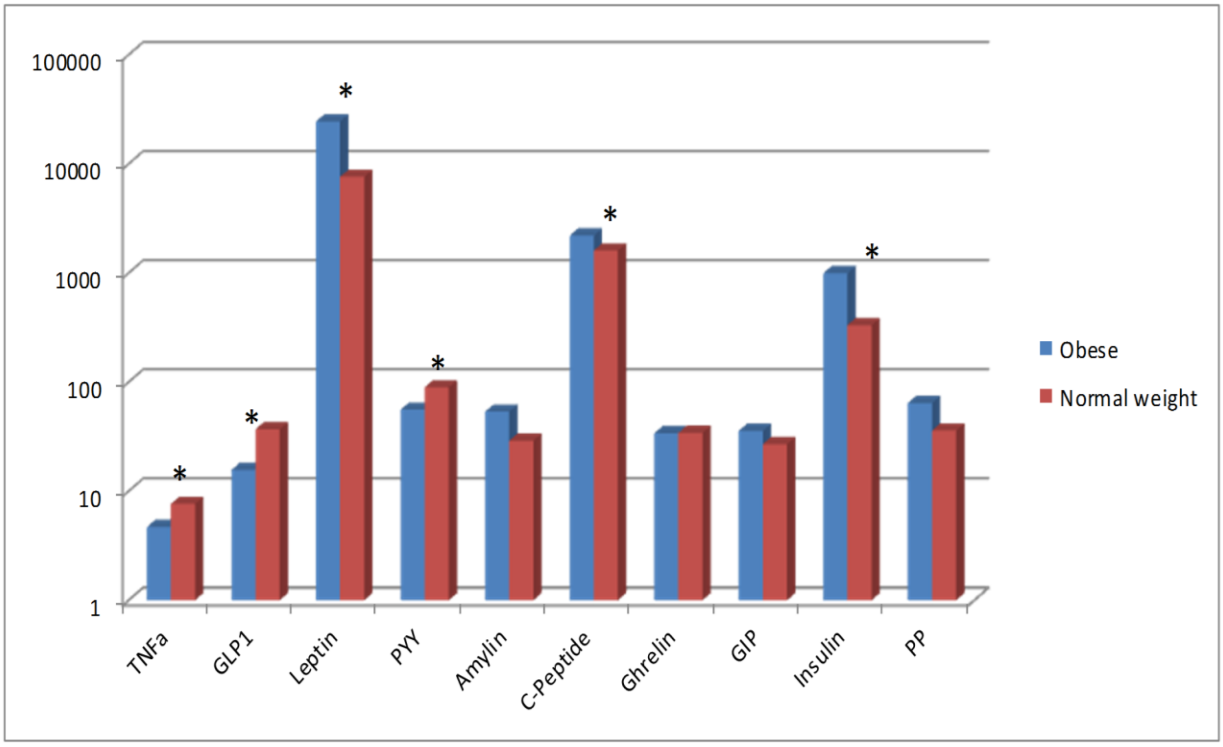
Method: 120 obese children from the on-going Childhood Obesity Study were compared with 38 normal weight children in the province of Newfoundland (matched with the same F:M). Obesity was evaluated using BMI based on the criteria recommended by The Center for Disease Control. Gut hormones and TNFα were measured using MAGPIX system on fasting serum samples. Body fat percentage (total, trunk, gynoid and android) were determined using Dual-energy X-ray absorptiometry.

Results: ANCOVA analysis, controlling for age showed that obese children have significantly lower levels of GLP-1, PYY and TNFα than normal weight group, contrary to the report of PYY and TNFα in obese adults. In obese children TNFα and ghrelin were negatively while amylin was positively correlated with body composition measurements,

further suggesting the uniqueness of relationship between these hormones and obesity in children.

Conclusion: Our results for the first time demonstrated that the severity of childhood obesity might be associated with the serum concentration of TNFα, PYY, ghrelin and amylin in a different way than adults.

Fig. 1: Comparison between adipokine and gut hormones controlling for age in obese and normal weight children



GLP-1, glucagon-like peptide 1, PYY, peptide tyrosine tyrosine, PP, pancreatic peptide, C-Peptide, connecting peptide, GIP, glucose-dependent insulinotropic peptide, TNF- α, tumor necrotic factor alpha, MCP-1, monocyte chemoattractant protein 1

*Independent t-test test significance level was set to $p < 0.05$

Table 1. Correlation between obesity indexes and adipokine and gut hormones in obese children

		TNF-α	GLP1	leptin	PYY	Amylin	C-peptide	Ghrelin	GIP	Insulin	PP
%Body fat	R	**-0.2**	0.1	**0.7**	-0.1	0.2	-0.1	**-0.3**	-0.4	0.1	-0.1
	P	**0.02**	n.s	**0.0**	n.s	n.s	n.s	**0.02**	n.s	n.s	n.s
%Android fat	R	-0.2	0.2	**0.6**	-0.03	-0.0	-0.1	**-0.3**	-0.1	0.02	-0.03
	P	n.s	n.s	**0.0**	n.s	n.s	n.s	**0.01**	n.s	n.s	n.s
% Trunk fat	R	**-0.2**	0.1	**0.7**	-0.1	0.2	0.02	**-0.2**	-0.0	0.2	-0.2
	P	**0.02**	n.s	**0.0**	n.s	n.s	n.s	**0.04**	n.s	n.s	n.s
% Gynoid fat	R	**-0.2**	0.01	**0.6**	-0.1	0.04	-0.2	**-0.2**	-0.1	-0.1	-0.1
	P	**0.03**	n.s	**0.0**	n.s	n.s	n.s	**0.04**	n.s	n.s	n.s
Weight	R	-0.1	0.1	**0.4**	-0.1	**0.3**	**0.0**	-0.2	0.2	**0.5**	-0.1
	P	n.s	n.s	**0.0**	n.s	**0.003**	**0.03**	n.s	n.s	**0.0**	n.s
BMI	R	**-0.2**	0.1	**0.5**	-0.2	**0.2**	0.1	**-0.2**	0.1	**0.4**	-0.1
	P	**0.04**	n.s	**0.0**	n.s	**0.02**	n.s	**0.02**	n.s	**0.0**	n.s

GLP-1, glucagon-like peptide 1, PYY, peptide tyrosine tyrosine, PP, pancreatic peptide, C-Peptide, connecting peptide, GIP, glucose-dependent insulinotropic peptide, TNF- α, tumor necrotic factor alpha, MCP-1, monocyte chemoattractant protein 1

*Independent t-test test significance level was set to $p < 0.05$

A4.4 The Associations of Dietary Selenium Intake and Level of Serum Selenium with Childhood Obesity

Presented in 34th Annual Scientific Meeting of The Obesity Society; New Orleans, LA , November 2016

Background: Selenium (Se) is a trace element that plays an important role in adipocyte hypertrophy and adipogenesis. Our previous study found that high dietary Se intake (SeD) is associated with a beneficial body composition profile in adult Newfoundland (NL) population. However, to the best of our knowledge there is no study available in children.

Objectives: To investigate the association of dietary Se intake and Selenium serum level with major obesity indexes

Method: 120 obese children (Ob) were compared with 38 normal weight children (NW) all recruited from NL (matched with gender ratio). Obesity was evaluated using BMI based on the CDC criteria for children. Body fat percentages (total and trunk) and visceral fat were determined using DXA. SeS (µg/L) was measured by atomic absorption spectrometry. SeD (µg/kg/day) was evaluated using the Willett FFQ.

Results: t-test controlling for age and calorie intake showed that Ob consumed lower amount of Se and have lower SeS ($p<0.05$). In the entire population and Ob, negative correlations were found between SeD and all obesity indexes (r:-0.5_-0.2, $p<0.03$). However, SeS in both groups had a significant correlation to only hip and waist circumference (r:-0.02 $p<0.04$). When subjects were grouped into tertiles (low, medium, or high) according to SeD (µg /day) and SeS controlled for covariates, those in the highest SeD consuming group (top 33.3% or above 128µg /day) had significantly lower level of

all obesity indexes. This level is higher than recommended dietary allowances in children and even in adults. However only weight, body fat percentages and visceral fat was significantly different between different levels of SeS.

Conclusion: Our results for the first time showed that in obese children lack of Se intake and low level of Se are associated with obesity, especially central obesity marked by higher trunk and visceral fat.

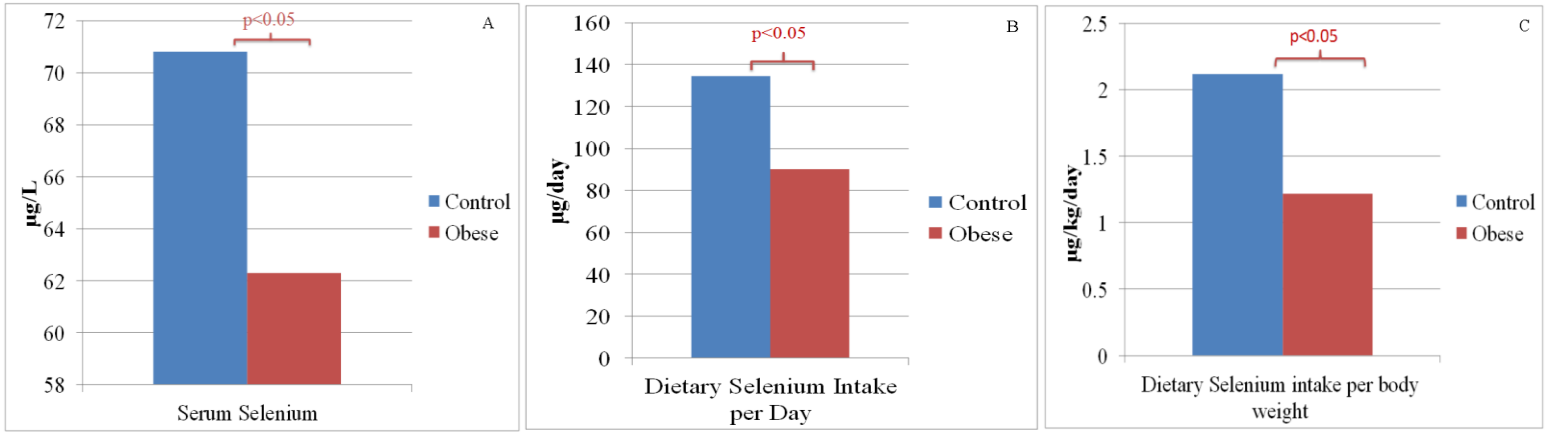


Figure 1.Comparison of A. serum selenium, B. dietary selenium intake per day and C. dietary selenium intake per body weight between obese and normal weight children

*Independent t-test test significance level was set to $p < 0.05$

Table 1.Correlation of serum selenium, dietary selenium intake per day and dietary selenium intake per body weight with major obesity indexes

Variables			Selenium intake(µg/d)	selenium intake (µg/kg/day)	Serum selenium(µg/L)
Age & Calorie	**Weight (kg)**	**r**	-0.02	**-0.5**	-0.1
		p	NS	**0.001**	NS
	BMI (kg/m2)	**r**	-0.1	-0.3	-0.2
		p	NS	NS	NS
	Waist (cm)	**r**	-0.1	-0.3	-0.2
		p	NS	NS	NS
	Hip (cm)	**r**	-0.09	**-0.4**	-0.2
		p	NS	**0.03**	NS
	Waist/Hip	**r**	-0.04	0.01	-0.04
		p	NS	NS	NS
	BF%	**r**	-0.2	0.02	0.03
		p	NS	NS	NS
	TF%	**r**	-0.2	0.02	0.04
		p	NS	NS	NS
	Visceral Fat Volume (cm3)	**r**	-0.2	-0.1	**-0.3**
		p	NS	NS	**0.05**
	Visceral Adipose Fat Mass (g)	**r**	-0.2	-0.1	**-0.3**
		p	NS	NS	**0.05**

BF% – percent, body fat and TF% – percent trunk fat.
*Independent t-test test significance level was set to $p < 0.05$

A4.5 Negative associations of dietary and serum selenium with insulin resistance in children

Presented in 34th Annual Scientific Meeting of The Obesity Society; New Orleans, LA , November 2016

Background: Selenium (Se) can mimic insulin function. Se has been shown to be protective against the development of diabetes. Recent studies in adults have shown that high Se exposure may increase the risk of type 2 diabetes and insulin resistant (IR). However, to the best of our knowledge, there is no study in children.

Objectives: In the current study, we investigated the link between dietary Se (SeD) and serum Se (SeS) with IR.

Method: 120 obese children (Ob) were compared with 38 normal weight children (NW) in Newfoundland (matched with gender ratio). Obesity was evaluated using BMI based on the CDC criteria for children. SeS (µg/L) was measured by atomic absorption spectrometry. SeD (µg/kg/day) was evaluated using the Willett FFQ. Fasting serum glucose (FSG) was measured by Immulite 2500 autoanalyzer. Fasting insulin (FIN) was measured by MAGPIX. Homeostatic Model Assessment of β cell function (HOMA-β) and Insulin Resistance (HOMA-IR) were used for measurement of insulin resistance.

Results: Partial correlation controlling calorie intake and age in the entire study showed a significant negative correlation between SeD and FIN, HOMA-β and HOMA-IR (r:-0.3, $p<0.0001$) but no correlation with SeS was found. In obese children no association was found between SeD and SeS and IR. However in the NW there was a negative correlation between SeS and FIN, HOMA-β and HOMA-IR (r:-0.5, $p<0.01$) but no association was

found between SeD and IR. When subjects were grouped into tertiles (low, medium, or high) according to SeD (µg /day) and SeS controlled for covariates, those in the lowest SeD consuming group (lowest 33.3% or below 87µg /day) had significantly lower FIN, HOMA-β and HOMA-IR. However SeS between 59-74 µg/L (medium level) had significantly lower level of HOMA-β and HOMA-IR.

Conclusion: Our results showed the beneficial association of dietary Se on IR is consistent in both obese and normal weight groups. The beneficial association of serum Se was seen in NW children in low to medium level of serum Se.

Table 1. Correlation of Serum Selenium (µg/L), dietary Selenium intake (µg/day), dietary Selenium intake per body weight (µg/kg/day) in entire population

Variables			Serum Selenium (µg/L)	Selenium Intake (µg/d)	Selenium per weight (µg/kg/day)
Age & Calorie	**Fasting Blood Glucose (mmol/L)**	r	0.02	-0.02	0.03
		p	NS	NS	NS
	Insulin (pmol/L)	r	-0.08	**-0.4**	**-0.4**
		p	NS	**0.00**	**0.00**
	HOMA-IR	r	-0.06	**-0.3**	**-0.3**
		p	NS	**0.001**	**0.00**
	HOMA-β	r	-0.1	**-0.3**	**-0.4**
		p	NS	**0.00**	**0.00**

HOMA-β-Homeostatic Model Assessment of β cell function, HOMA-IR, Homeostatic Model Assessment of Insulin Resistance, NS-Not Significant

Table 2. Correlation of Serum Selenium (µg/L), dietary Selenium intake (µg/day), dietary Selenium intake per body weight (µg/kg/day) in normal weight children

Variables			Serum selenium(µg/L)	Selenium intake(µg/d)	selenium per weight(µg/kg/day)
Age & Calorie	**Fasting Blood Glucose (mmol/L)**	r	0.07	-0.3	-0.1
		p	NS	NS	NS
	Insulin (pmol/L)	r	**-0.4**	-0.2	-0.2
		p	**0.006**	NS	NS
	HOMA-IR	r	**-0.5**	-0.2	-0.3
		p	**0.002**	NS	NS
	HOMA-β	r	**-0.4**	-0.1	-0.3
		p	**0.02**	NS	NS

HOMA-β-Homeostatic Model Assessment of β cell function, HOMA-IR, Homeostatic Model Assessment of Insulin Resistance, NS-Not Significant

Table 3. Correlation of Serum Selenium (µg/L), dietary Selenium intake (µg/day), dietary Selenium intake per body weight (µg/kg/day) in obese children

Variables			Serum selenium(µg/L)	Selenium intake(µg/d)	selenium p weight(µg/
Age & Calorie	Fasting Blood Glucose (mmol/L)	r	0.02	-0.01	0.08
		p	NS	NS	NS
	Insulin (pmol/L)	r	-0.1	-0.1	-0.09
		p	NS	NS	NS
	HOMA-IR	r	-0.09	-0.2	-0.1
		p	NS	NS	NS
	HOMA-β	r	-0.2	-0.2	-0.2
		p	NS	NS	NS

HOMA-β-Homeostatic Model Assessment of β cell function, HOMA-IR, Homeostatic Model Assessment of Insulin Resistance, NS-Not Significant