THE RESPONSE OF FUNCTIONALLY RELATED GUT HORMONES, GHRELIN AND GLP-1 TO OVERFEEDING

by

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Abstract

Introduction: Ghrelin and glucagon-like peptide-1 (GLP-1) are peripherally secreted hormones from the gut. GLP-1 is secreted from the distal gastrointestinal tract in response to a meal and is involved in the insulin response and energy homeostasis. Ghrelin is secreted mainly from the fundus of the stomach and has been shown to increase appetite. Although both hormones have been linked to the development of obesity and diabetes, data is lacking as to how GLP-1 and ghrelin respond to a positive energy challenge (PEC) and whether the response differs according to obesity status. Thus the present study was designed to investigate the response of these functionally-related gut hormones to a period of energy surplus (overfeeding).

Methods: A total range of 68-72 young men (68 in the ghrelin study, 72 for GLP-1) were overfed 70% more calories than baseline requirements for 7 days. Fasting blood samples, anthropometric measures and body composition utilizing dual-energy X-ray absorptiometry (DXA) were taken pre- and post- overfeeding. Biochemical markers measured included glucose, insulin, cholesterol, HDL-C, LDL-C, and triglycerols. Serum total GLP-1 and acylated ghrelin were measured using enzyme-linked immunosorbent assays (ELISA) and enzyme immune assays (EIA), respectively.

Results: As expected, serum GLP-1 increased in response to the energy surplus, however unexpectingly, circulating acylated ghrelin also increased. The increase in both GLP-1 and ghrelin were independent of adiposity status. At baseline, there was no difference in fasting GLP-1 and ghrelin between the normal weight, overweight, and obese groups. In the overweight/obese cohort, baseline GLP-1 concentration was negatively associated

with HDL-cholesterol and positively associated with triacylglycerols and markers of insulin resistance. Also in the overweight/obese subjects, a negative relationship was present between baseline GLP-1 concentration and change in percent gynoid fat. Baseline acylated ghrelin was inversely correlated with weight and BMI in the normal weight group and inversely correlated with BMI in the overweight group. Additionally, baseline acylated ghrelin was negatively associated with change in weight and BMI in the overweight group and positively associated with the same variables in the obese group. Percentage change in GLP-1 was positively associated with percentage change in triacylglycerols in both the normal weight and overweight/obese groups. Percent change in GLP-1 was negatively correlated with percent change in gynoid fat in the normal weight group and positively correlated with percent change in cholesterol in the overweight/obese group.

Conclusion: Serum GLP-1 and ghrelin increased in response to a 7-day overfeeding period in young Newfoundland men, regardless of obesity status. Our results suggest a protective role for GLP-1, increasing to counteract the energy surplus. We also suggest that the increase in ghrelin is attempting to offset the rise in insulin resistance.

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List of Symbols, Nomenclature or Abbreviations

Abbreviation	Full name
AACE	American Association of Clinical Endocrinologists
ADP	air displacement plethysmography
AF	android fat
ANOVA	analysis of variance
BF	body fat
BIA	bioelectrical impedance analysis
CCK	cholecystokinin
CDC	Center for Disease Control
CNS	central nervous system
CT	computed tomography
DPP-IV	dipeptidyl peptidase-IV
DXA	dual-energy X-ray absorptiometry
ELISA	enzyme-linked immunosorbent assay
FTO	fat mass and obesity-associated gene
GCG	glucagon gene
GF	gynoid fat
GHRL	ghrelin/obestatin prepropeptide gene
GHS-R	growth hormone secretagogue receptor
GI	gastrointestinal
GLP-1	glucagon-like peptide-1
GLP1R	glucagon-like peptide-1 receptor
GWAS	genome-wide association study
HDL-c	high-density lipoprotein cholesterol
HOMA	homeostatic model assessment
HOMA-β	homeostatic model assessment-β-cell function
HOMA-IR	homeostatic model assessment-insulin resistance
ICV	intracerebroventricular
LDL-c	low-density lipoprotein cholesterol
MC4R	melanocortin receptor 4
mRNA	messenger RNA
MRI	magnetic resonance imaging
NL	Newfoundland and Labrador
NPY	neuropeptide Y
PEC	positive energy challenge
POMC	pro-opiomelanocortin
PP	pancreatic polypeptide

PYY	peptide tyrosine tyosine (peptide YY)
QTL	quantitative trait loci
TF	trunk fat
TG	triacylglycerol
TRKB	tropomyosin receptor kinase B
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

INTRODUCTION TO OBESITY

Prevalence and health consequences

The World Health Organization (WHO) estimates that more than 200 million men and 300 million women are obese, globally [1]. Additionally, the organization has predicted that by 2015, approximately 2.3 billion and 700 million adults will be classified as overweight and obese, respectively [1]. Not only is the current obesity epidemic present in adult populations, but childhood rates of obesity have drastically increased [2]. Once thought to be a problem of only developed nations, obesity has now become prevalent in developing nations [3]. This is evident as about 65% of the global population reside in countries in which carrying excess body fat results in greater mortality than being underweight [1]. Obesity, or the excessive accumulation of adipose (fat) tissue, is a major health concern as it is associated with a number of health complications including type-II diabetes, hypertension, cardiovascular diseases, and certain forms of cancer [4]. This is of great interest to Newfoundland and Labrador as the province has one of the highest rates of obesity in Canada [5]. Moreover, diabetes places immense stress on the health care system. The Canadian Diabetes Association has projected that by 2020, more than 15% of Newfoundlanders and Labradoreans will be classified as diabetic with an estimated health care cost exceeding $360 million dollars, annually [6].

Currently, there are many methods to define obesity status; in other words, classifying an individual as underweight, normal weight, overweight or obese. The most widely used method to determine obesity status has been utilizing body mass index (BMI): dividing a person's weight in kilograms by the square of his/her height in metres

(kg/m^2). According to the WHO, a BMI of 25 kg/m^2 or greater is classified as overweight, while a BMI of 30 kg/m^2 or greater is classified as obese. BMI has been criticized for inaccurately representing body composition but many large population based studies use the method for its simplicity [7]. Additionally waist circumference measurements and the ratio of waist to hip circumferences have been utilized to estimate central adiposity. Skin fold measures using caliper [8], bioelectrical impedance analysis (BIA) [9] and air displacement plethysmography (ADP) [10] have also been used to estimate body fat. The gold standard for quantifying body fat was originally hydrodensitometry (underwater weighing) [11] but now greater precision and simplicity is found using dual-energy X-ray absorptiometry (DXA) [12]. DXA works on the principle that adipose tissue is less dense than muscle (and bone) and can differentiate between the three tissues based on the different densities. Still more accurate measures are evident when using MRIs [13] and CT scans [14], but due to the expensive operative costs, these are not usually employed in large-scale studies.

Aetiology

Recently, the American Association of Clinical Endocrinologists (AACE) has declared obesity as a disease [15]. In general, obesity results from 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) [16]. The easy accessibility/overconsumption of calorically dense foods and the lower physical activity requirements contribute to the chronic energy imbalance. However, obesity is a multifaceted condition and thus, factors including genetic predisposition and the environment do influence one's susceptibility to gain weight (or lose weight). Various

environmental cues including the weather, culture, industry marketing, portion size, price of food and potential food addictions have been thought to have a role in the increasing prevalence of obesity [17]. Currently, walkability scores of communities have been calculated, and used to assess relationships with obesity [18, 19]. However, strong evidence that environmental factors have an influence on body weight and adiposity are found in populations which have a common genetic background but due to a changing environment, weight gain has ensued. For example, Pima Indians who reside in the United States carry 25 kg more body weight as compared to Pima Indians residing in Mexico [20].

Moreover, genetics factors are theorized to play a pertinent role in obesity as variants in many candidate genes have been associated with human obesity, and rare monogenic forms of obesity have also been observed [21, 22]. Twin studies have estimated the heritability (how much is genetics playing a role in differences of a given trait) of obesity ranges from 64-84% (based on BMI) [23-25]. Interestingly, studies have found strong relationships between individuals who were adopted and their biological parents [23]. Various linkage studies have found evidence for quantitative trait loci (QTL) affecting obesity-related traits [26, 27]. Additionally, several polymorphisms have been found in association studies to be linked with obesity-phenotypes [28, 29]. For example, FTO (fat mass and obesity-associated gene) was found to be associated with obesity in both a genome-wide association study (GWAS) and a genetic association study [30, 31]. Interestingly, a polymorphism in the MC4R receptor gene (V103I) has been associated with protection against weight gain and obesity [32]. Many monogenic obesity syndromes exist with mental retardation and/or the developmental anomalies including Prader-Willi

Syndrome [33, 34], Bardet-Biedl Syndrome [35, 36], and TRKB deficiency [37]. As well, monogenic forms of obesity have been observed without the presence of the stated developmental issues. Congenital leptin deficiency (ob/ob mice) [38], leptin receptor deficiency (db/db mice) [39, 40], POMC deficiency [41] and melanocortin 4 receptor deficiency are all examples of rare monogenic forms of obesity [42, 43].

It must be noted however, that physiological mediators of the endocrine system (hormones) influence energy equilibria and thus, obesity status [44]. Various hormones play an important role in energy homeostasis, appetite regulation, adipose distribution and therefore, human obesity and related health complications. Hormones secreted into the circulation from peripheral tissues interact with complex metabolic pathways including regulation in the central (CNS) [45]. For example, the adipokine leptin, is secreted in proportion to fat mass and inhibits food intake [46]. The ob/ob mouse model, first discovered in the 1950s, lacks the ability to code for the functional leptin protein, and thus ob/ob mice become severely obese [38, 47]. Furthermore, hormones secreted from endocrine cells of the gastrointestinal (GI) tract also play a role in energy homeostasis [48, 49]. Peripheral secreted signals from the GI tract are referred to as gut hormones, and have a wide range of functions including: decreasing (or increasing) appetite, modulation of glucose and lipid metabolism or insulin sensitivity, and GI motility. The hormones are secreted into circulation from specific locations through the GI tract (i.e. stomach, duodenum) and bind to receptors in various tissues and as mentioned, the CNS [48, 49]. Ghrelin, glucagon-like peptide-1 (GLP-1), peptide YY (PYY), pancreatic polypeptide (PP), cholecystokinin (CCK), and amylin are all examples of hormones released into the blood from the gut. In all there are over 20 gut hormones present in humans. However,

the remainder of this thesis manuscript will focus on the gut hormones ghrelin and GLP-1.

INTRODUCTION TO GHRELIN

Ghrelin is 28-amino acid peptide secreted mainly from the endocrine cells (X/A-like cells) of the fundus (stomach) but is also found in the central nervous system (CNS) [50-52]. It was discovered in 1999, and is the only endogenous ligand of the growth hormone secretagogue receptor (GHS-R) [53]. The ghrelin gene (GHRL) is found on human chromosome six which encodes a preproghrelin molecule, that when cleaved, forms ghrelin [54]. The active ghrelin molecule contains a posttranslational acylation modification: its 3rd amino acid (serine) is n-octanoylated [52, 55]. Ghrelin rises in response to lack of nutrients in the gut; its circulating concentration rises during fasting, and peaks just before consumption of a meal [56, 57]. The concentration then decreases in proportion to the food intake and drops to a minimum value roughly one hour post meal. Because of this, ghrelin is thought to be a hunger signal, increasing appetite and thus inducing food intake [58, 59]. Circulating active ghrelin binds to its receptor in various tissues including the central nervous system and has a half-life of about 30 minutes [60]. Ghrelin can act directly in the CNS, specifically at the arcuate nucleus of the hypothalamus, to increase appetite [61]. Injections of ghrelin (ICV) increase the level of the orexigenic hormone neuropeptide Y's (NPY) mRNA in the arcuate nucleus [62, 63]. It is interesting to note that most cross-sectional studies have found a negative association between adiposity and ghrelin: lean individuals have higher levels of circulating ghrelin as compared to their obese counterparts [64-69]. However other studies have found

dissimilar findings: both positive and lack of association between ghrelin and increased adiposity [70, 71]. Moreover, high ghrelin concentrations are found in the inherited disease of Prader-Willi [72-74], which is characterized by uncontrollable appetite, and also in cases of anorexia nervosa, where there is little appetite [75, 76]. Administration of ghrelin in animal models and humans increases appetite and food intake [77-80]. Interestingly, it has been shown that, when ghrelin receptor knockout mice (GHSR-null) are fed high fat diets, they consume less food and have less body fat as compared to controls [81]. When ghrelin receptor knock-out mice are administered ghrelin, the signature enhancement of appetite is absent (as well as the increase in growth hormone) [82]. Additionally nutritional factors could influence ghrelin concentration as high-fat meals decrease circulating levels of the hormone [83, 84]. Therefore ghrelin is thought to be involved in energy homeostasis regulation and related conditions (i.e. human obesity) and has even been ascribed in the newly investigated concept of 'food addiction' [85].

INTRODUCTION TO GLP-1

Glucagon-like peptide-1 (GLP-1) is a 30 amino acid peptide secreted into circulation by the L-cells of the distal GI tract in response to nutrients in the gut [86]. GLP-1 is encoded from same gene as glucagon (GCG) which is located on human chromosome two. Due to tissue-specific posttranslational modification, the gene product proglucagon, is cleaved by prohormone convertase forming active GLP-1 [87]. The half-life of GLP-1 is extremely short; it is degraded in circulation within ~2 minutes by dipeptidyl peptidase-IV (DPP-IV) [87, 88]. There is one known receptor for GLP-1 (GLP1R) which has been found both centrally and peripherally [89-91]. Once bound to its

receptor, GLP-1 elicits a number of responses which in general, helps to establish a more favourable insulin response. GLP-1 is considered an incretin hormone, as it facilitates the glucose-dependent insulin secretion [92, 93]. In fact, it has been suggested that GLP-1 and related incretin hormones are responsible for about 2/3 of the insulin response once food is ingested [94]. Additionally, GLP-1 has been shown to decrease the secretion of glucagon from the alpha cells of the pancreas [95-97]. A study on type-I diabetics, who have little or no β-cell activity, observed that GLP-1 could lower fasting blood glucose, independent of influencing insulin secretion [95]. GLP-1 also acts to inhibit gastric motility and GI secretions [98, 99]. Satiation has also been shown to be influenced by GLP-1 as the hormone has been observed to decrease appetite [100-102]. It is of interest to note GLP-1 receptor knockout (GLP1R -/-) mice are deemed glucose intolerant though they do not become obese [103]. Holst *et al.* credits this phenomena to the redundancies present in appetite regulation pathways [86]. In regards to adiposity status, GLP-1 secretion is greater in lean individuals as compared to their obese counterparts [104]. Also type-II diabetics who are insulin resistant, have a blunted GLP-1 response [105] and therefore, treatment with GLP-1 or GLP-1 receptor agonists have resulted in an improved insulin response [106, 107]. In addition, DPP-IV inhibitors have been utilized in the condition as they act to increase the half-life of GLP-1 [108]. Recently, both GLP-1 agonists and DPP-IV inhibitors have been studied in regards to human obesity [109, 110].

OVERFEEDING AS A METHOD TO STUDY OBESITY: ENDOCRINE INVOVLEMENT

Aforementioned, obesity is caused by a chronic energy surplus (positive energy balance) in which energy intake exceeds energy expenditure [4]. Individuals overfed more calories than they expend, will store this excess fuel in the form of triglycerides in adipose tissue, consequently causing increased fat mass. In the study of obesity, the positive energy challenge is of great interest to researchers as metabolic and physiological changes induced during a period of overfeeding aid in further understanding human obesity. Many animal models have been used in overfeeding intervention studies. Specifically in rat and mice models, high fat diets have been utilized to induce overfeeding as fat is the most calorically dense macronutrient [111]. Studies in the past have provided rats/mice with diets containing 30-78% of calories from fat [112], through feeding of a cafeteria style diet or by simply adding a portion of lard to food [113-116]. Moreover, researchers have implemented overfeeding studies on humans, as due to underlying physiological differences, animal model intervention-based research does not always translate into similar results in humans. In fact, a number of overfeeding studies in human have been completed, however they differ in various aspects including: length of overfeeding period, macronutrient composition of overfeeding, degree of overfeeding, participant demographics and number of participants. One of the earlier human overfeeding studies was completed between 1964 and 1970 by Ethan Sims, a researcher at the University of Vermont [117]. This study named “The Vermont Prison Experiments”, took place through a 10-week period in which inmates were overfed nearly three times the amount of calories they would normally intake [117-119]. On average, the subjects’ caloric intakes ranged from about 8,000-10,000 kilocalories per day. After the 10-weeks of overfeeding, the men gained 15-25% of their originally weight (average

weight gain of 36 pounds) and most of the gain due to an increase in total body fat. The study was used for many investigations including: 1) observing adipose tissue changes through the duration of overfeeding (adipose tissue biopsies) and 2) observing changes in various hormones (i.e. thyroid hormone) through the duration of obesity [119-121]. Another human overfeeding study was completed by Claude Bouchard's research group and published in the New England Journal of Medicine in 1990 [122]. In this study twelve pairs of male twins (monozygotic) were fed an energy surplus of 1000 kcal per day, for six days out of a seven-day week. The duration of the study was 100-days so each subject was overfed for 84 days totalling an excess of 84,000 kcalories. The weight gain ranged from 4.3 – 13.3 kilograms with a mean weight gain of 8.1 kg [122]. The group theorized that similarity in weight gain found between twins was due to genetic influences. Therefore as indicated through the previously mentioned studies, human overfeeding studies have been utilized to study obesity in the past.

From overfeeding studies in both our lab and others, it has been observed that an energy surplus can change anthropometrics, body composition, genetic expression and circulating hormones [123-127]. However human positive energy challenge studies investigating the response of various gastrointestinal hormones, specifically peptides (ghrelin, GLP-1, etc) released from the gut involved in energy homeostasis are few in number [128]. Therefore the current research goal of this thesis was to investigate the potential role of circulating ghrelin and GLP-1 on the development of human obesity by examining: 1) the response of fasting circulating acylated ghrelin and GLP-1 concentrations in normal weight, overweight, and obese males to short-term overfeeding; 2) fasting circulating acylated ghrelin and GLP-1 concentrations in each adiposity group

before/after overfeeding; and 3) the relationships of fasting circulating acylated ghrelin and GLP-1 concentrations with anthropometrics, body composition, fasting glucose, insulin, and blood lipids concentrations, and insulin resistance state, before and after overfeeding.

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CHAPTER 2: Serum acylated ghrelin concentrations in response to short-term overfeeding in normal weight, overweight, and obese men

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ABSTRACT

Background: Ghrelin, an orexigenic gut hormone secreted primarily from the stomach, is involved in energy homeostasis. However, little data is available regarding its response to energy surplus and the development of human obesity.

Objective: The present study investigated the response of circulating acylated ghrelin to a 7-day positive energy challenge.

Design: A total of 68 healthy young men were overfed 70% more calories than required, for 1-week. Subjects were classified based on percent body fat (measured by dual-energy X-ray absorptiometry) as normal weight, overweight, and obese. Serum acylated ghrelin concentration was measured before and after the positive energy challenge. Additionally, the relationship between acylated ghrelin and obesity-related phenotypes including weight, body mass index, percent body fat, cholesterol, HDL-c, LDL-c, glucose, insulin and homeostasis model assessment of insulin resistance and β-cell function at baseline and change due to overfeeding, were assessed.

Results: Contrary to our expectations, serum acylated ghrelin was significantly increased in response to overfeeding and the increase was independent of obesity status. There was no significant difference in fasting acylated ghrelin between normal weight, overweight, and obese men at baseline. Acylated ghrelin was negatively correlated with weight and BMI for normal weight and with BMI in overweight men. Also ghrelin was correlated with change in weight and BMI in overweight (negative relationship) and obese (positive relationship) groups.

Conclusion: Our results showed that circulating acylated ghrelin was increased after a 7-day positive energy challenge regardless of adiposity status. However, acylated ghrelin was

correlated with change in weight and BMI in opposing directions, in overweight and obese subjects respectively, thus dependent on obesity status.

INTRODUCTION

Although it has been well-established that appetite is controlled through complex mechanisms mainly in the central nervous system (CNS), appetite-regulating hormones secreted from the periphery, including the gastrointestinal tract (gut), communicate with the CNS to play an important role in energy homeostasis [1-3]. Ghrelin, an endogenous ligand of the growth hormone secretagogue receptor (GHS-R), is the only known orexigenic gut hormone, which increases appetite and food intake [4-6]. Ghrelin is found in both the gastrointestinal tract and hypothalamus, though it is primarily synthesized and released from X/A like cells of the gastric mucosa of the stomach [4, 7-9]. Both acylated and non-acylated forms of ghrelin exist, however the active form is n-octanoylated on the third amino acid (serine) residue [4, 10, 11]. Ghrelin is thought to be a meal initiator: concentration rises prior to feeding, and continually decreases to a minimum one-hour after the meal [12, 13]. The postprandial suppression of circulating ghrelin is proportional to the caloric content of the consumed meal [14].

Many studies have investigated ghrelin's role in the regulation of appetite and energy homeostasis. Administration of ghrelin in both rats and humans has been shown to increase appetite and food intake [6, 15-18]. However in humans, most studies have found a negative relationship between ghrelin and adiposity: higher circulating ghrelin concentrations in lean individuals as compared to obese [19-24]. In contrast, other studies have found positive relationships between acylated ghrelin concentrations and markers of adiposity [25] or a negative relationship with BMI and no relationship to body fat [26]. Additionally, a growing body of evidence shows that ghrelin might have a role in insulin resistance and development of type-II diabetes. Animal studies have shown that ghrelin can inhibit insulin secretion, but increases glucagon secretion from pancreatic islet cells [27]. Moreover human studies reveal that patients

with insulin resistance have lower ghrelin concentrations compared with insulin-sensitive patients [28].

Circulating ghrelin concentration seems to be influenced through dietary regulation and specific nutrient intakes. For example, a high-fat meal has been shown to decrease circulating ghrelin concentration in humans [29, 30]. Moreover, our laboratory and others have revealed changes in nutritional status, such as overfeeding influence adipokine and gut hormone concentrations [31, 32], adipose tissue metabolism [33, 34] and genomic expression [35]. Intervention based studies, observing nutritional regulation via a positive energy challenge, are also important in understanding the role of ghrelin in the development of human obesity where energy surplus is the major driving factor [36-40]. Currently, data is missing in this aspect.

Our current research goal was to investigate the potential role of acylated ghrelin on the development of human obesity by examining: 1) the response of fasting serum acylated ghrelin concentrations in normal weight, overweight, and obese males to short-term overfeeding; 2) fasting serum acylated ghrelin concentrations in each adiposity group before and after overfeeding; and 3) the relationships of fasting serum acylated ghrelin concentrations with fasting glucose and insulin concentrations, blood lipids, and insulin resistance state, before and after overfeeding.

MATERIALS AND METHODS

Ethics Statement

All participants provided informed and written consent. The current study received ethical approval from the Human Investigations Committee for the Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada.

Subjects

A total of 68 young males from the Canadian province of Newfoundland and Labrador were recruited to study the effects of an acute positive energy balance on metabolism and various endocrine factors (original study contained 72, underweight participants were eliminated from analyses). Participation criteria included: 1) age 19-29 years; 2) at least of 3rd-generation Newfoundland descent; 3) no serious cardiovascular, metabolic, or endocrine diseases; 4) no medications intended for lipid metabolism change; and 5) a stable 6-month reported body weight value (±2.5 kg). Recruited subjects were asked to refrain from 1) consuming alcoholic or additional calorie-containing beverages and from 2) taking any drugs or medication, throughout the duration of the research study.

Serum Measurements

Blood samples were collected from all subjects both before and after completion of the positive energy challenge (explained below). Serum was prepared from clotted samples collected after a 12 hour fast which were stored at -80 ^{o}C until time of further analysis. Serum acylated ghrelin concentrations were measured in duplicate with enzymeimmunosorbent assay (EIA) kits (Bertin Pharma; Montigny le Bretonneux, France). Acylated ghrelin analyses were performed on ice. The concentrations of serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triacylglycerols (TG), and glucose were analyzed using Synchron reagents by an Lx20 clinical chemistry analyzer (Beckman Coulter Inc., CA, USA). Low-density lipoprotein (LDL) cholesterol was calculated by the Friedwald equation: total cholesterol – HDL – TG/2.2 (which is a reliable estimate in the absence of severe hypertriglyceridemia >4.6 mmol/L). Serum insulin

was evaluated using an Immulite 2500 immunoassay analyzer (Siemens, Los Angeles, CA). Insulin resistance and pancreatic β-cell function were assessed using the homeostasis model assessment (HOMA). Insulin resistance was quantified using HOMA-IR [insulin (mU/L) x glucose (mmol/L)/22.5)] while β-cell function was measured using HOMA-β [20 x insulin (mU/L/(glucose (mmol/L) - 3.5)].

Body composition assessment

Dual-energy X-ray absorptiometry (DXA Lunar Prodigy; GE Medical Systems, Madison, WI) was used to measure body composition (lean tissue, body fat, and bone mineral composition). Following a method previously described by us [41] the participant was scanned in a supine position after a 12-hour fast and the following parameters were determined from the DXA scan: total percent body fat (%BF), percent trunk fat (%TF), percent android fat (%AF), and percent gynoid fat (%GF). All measurements were performed before overfeeding and on the day after overfeeding.

Anthropometric measurements

Measurements of body weight, height, waist circumference, and hip circumference were performed after a 12-hour fasting period. Body weight was measured to the nearest 0.1 kilogram on a platform manual scale balance (Health O Meter, Bridgeview, IL) with subjects wearing a standardized hospital gown. A fixed stadiometer was used to measure height to the nearest 0.1 centimeters (shoes removed). A flexible measuring tape was employed to measure waist circumference to the nearest 0.1 centimeters at the level of the umbilicus. The same procedure was used to measure hip circumference at the level of largest circumference between the waist

and thighs. Body mass index (BMI) was calculated by dividing participants' weight in kilograms by the square of height in centimeters [(weight-kg)/(height-m)^2].

Overfeeding protocol

Participants enrolled into a 7-day positive energy challenge and were required to consume 70% more calories than their normal food intake. Throughout the challenge period, subjects followed a diet consisting of 15% protein, 35% fat, and 50% carbohydrates, thus mimicking typical North American dietary patterns. A 7-day positive energy challenge was selected to ensure that metabolic changes were induced throughout its duration. Individual energy requirements were recorded and estimated before commencing the overfeeding protocol by the use of three 24-hour recalls and a 30-day dietary inventory. For one week at time 0900, 1200, and 1700, participants were offered meals of which caloric and macronutrient content was assessed using FOOD PROCESSOR SQL software (version 9.5.0.0; ESHA Research, Salem, OR). The average baseline energy intake before and during overfeeding were 2969 kcal and 5471 kcal, respectively. A detailed overfeeding protocol has been described in our previously published papers [33, 34].

Statistical analysis

Data are presented as means ± SE unless otherwise stated. Prior to analysis, data that showed a skewed distribution was logarithmically transformed to approximate a normal distribution. Concentration data for serum acylated ghrelin, triacylglycerols, insulin, and HOMA-IR and HOMA-β, all at baseline, after, and change during overfeeding were log transformed prior to analysis. Using criteria suggested by Bray [42] subjects were classified as either normal

weight, overweight or obese based upon %BF (8-20.9, 21-25.9 and ≥26%, respectively). Subjects were also classified using the World Health Organization BMI-based classification (BMIs of ≤24.9, 25.0-29.9, and ≥30 kg/m^2 for normal weight, overweight, and obese respectively). Because of its more accurate classification of obesity status, Bray Criteria was used for subsequent analyses.

Using a two-factor analysis of variance (ANOVA) with repeated measures before and after overfeeding, differences in physical and biochemical variables in response to overfeeding were assessed between the three groups. One-factor analysis of variance was used to compare baseline values between the three adiposity groups. Variables which showed a significant overfeeding-adiposity interaction underwent within-group analysis of the response to overfeeding using a paired t-test. In both one factor and two-factor ANOVA analyses, Bonferroni post hoc tests were employed.

Initial Pearson's correlation analyses were performed to assess relationships between fasting acylated ghrelin concentration and physical/biochemical variables of interest. Next we performed two correlation analyses: 1) baseline acylated ghrelin concentration was compared with all variables at baseline and 2) baseline acylated ghrelin concentration was compared with changes in all variables in response to overfeeding thus examining if baseline acylated ghrelin could predict the changes in related physical and biochemical markers. All statistical analyses were completed using SPSS, version 19.0 (SPSS Inc, Chicago) in which: 1) all tests were two-sided; and 2) a P value < 0.05 was deemed statistically significant.

RESULTS

Descriptive Statistics

Basal physical and biochemical characteristics of subjects are shown in **Table 1**. Differences in body composition and glucose, and lipid metabolism between normal-weight, overweight, and obese (based on %BF) young healthy men were previously described by us [31, 33-35].

Baseline fasting acylated ghrelin concentrations (mean ± S.E.) for normal weight, overweight, and obese individuals were 222.19 ± 41.60, 274.91 ± 92.4, and 352.03 ± 112.4 ng/L, respectively. There was no statistically significant difference between adiposity groups for acylated ghrelin concentration at baseline. Similar results were obtained when participants were divided into normal weight, overweight and obese groups based on the World Health Organization's BMI classification (data not shown).

Changes in body composition and phenotypes of glycemic control and lipid metabolism in response to the 7-day overfeeding challenge are also described in Table 1. Within all adiposity groups, the positive energy challenge increased body composition, serum lipids, insulin resistance, and pancreatic beta cell function, which was previously described by us [31, 33-35]. In response to the 7-day overfeeding challenge, circulating acylated ghrelin concentration significantly increased by 62.14 ng/L in the entire cohort (P=0.042). However, no adiposity-overfeeding interaction was present; the response of acylated ghrelin to overfeeding was not significantly different between normal weight, overweight, and obese groups (P=0.523).

Correlations of acylated ghrelin with adiposity and phenotypes of glucose and lipid metabolism

Partial correlation analyses, controlling for age, were used to assess the relationships between baseline fasting acylated ghrelin concentration and baseline phenotypes of adiposity,

and serum glucose and lipid concentrations (**Table 2**). For normal weight subjects, acylated ghrelin was negatively correlated with weight and BMI ($P < 0.05$). Furthermore, fasting glucose concentration was positively correlated with acylated ghrelin concentrations. The significant negative correlation between acylated ghrelin and BMI was also observed in overweight subjects, but not in obese subjects. LDL cholesterol was negatively correlated with acylated ghrelin concentration only in obese subjects.

Additionally, partial correlation analyses examining the relationship between baseline acylated ghrelin concentration and changes in variables after the overfeeding period were used to examine if acylated ghrelin concentration could predict variable change under a positive energy challenge (**Table 3**). Significant negative relationships were found for the overweight group, between baseline acylated ghrelin concentration and change in body weight and BMI. However, the relationship between baseline acylated ghrelin and change in body weight and BMI, were positive for obese subjects.

Acylated ghrelin Tertiles: Correlations of acylated ghrelin with phenotypes of adiposity and concentrations of glucose and lipids

We repeated all of the above analyses after participants were divided into acylated ghrelin tertile subgroups (low, medium, and high ghrelin; data not shown). Only one baseline correlation existed within the analysis. For the high ghrelin subgroup, baseline acylated ghrelin was positively correlated with baseline fasting triacylglycerols. No significant relationships were found when partial correlation analyses were performed examining baseline acylated ghrelin concentrations and changes in the aforementioned variables.

DISCUSSION

The most important finding from the present study was the discovery of the significant increase in serum acylated ghrelin concentration after the 7-day positive energy challenge. Conventional knowledge would lead us to expect that to counteract a positive energy balance (thus limiting caloric intake) during overfeeding, secretion of ghrelin would be diminished to decrease appetite. However, the results from our study was surprisingly the opposite; acylated ghrelin concentration significantly increased after the 7-day overfeeding challenge regardless of adiposity status. Pathologically high circulating concentrations of ghrelin are known to occur in the inherited disease of Prader-Willi Syndrome, which is characterized by uncontrollable appetite [43-45], but also in cases of anorexia nervosa, where there is little appetite [46, 47].

At the present time, results are not clear based on limited studies examining the responsiveness of ghrelin to a positive energy challenge. Moreover, the few available studies with positive energy challenge interventions differ in many aspects including length of overfeeding, macronutrient composition, amount of food consumed above daily caloric requirements, and also the physical and demographic characteristics of the study participants. Hagobian et al. overfed 9 healthy subjects (6 men and 3 women) 25% more calories than required for weight maintenance for 3 days, retaining a macronutrient composition of 56% carbohydrate, 29% fat, and 15% protein, and found no significant change in circulating ghrelin concentration [38]. Votruba et al. overfed 69 non-diabetic, mainly obese individuals (40 men and 29 women) 60% more calories than required at baseline from a “vending machine diet” over a 3-day period [37]. Circulating ghrelin concentration did not change through the 3-day duration. A 5-day high fat (60% fat, 32.5% carbohydrates and 7.5% protein) overfeeding study (50% more calories than required) found a non-significant increase in circulating ghrelin in a cohort of 26

healthy young men [36]. Robertson et al. overfed 6 healthy lean male subjects by ~692.7 kcal/day with a high fat diet ranging from 29-45% calories from fat. This study detected no significant change in fasting ghrelin, however, postprandial ghrelin was suppressed to a greater extent following the oral fat tolerance test [39]. Finally, a long-term overfeeding study of 100-day in which twelve pairs of identical twins consumed an excess of 84,000 kilocalories (50% carbohydrates, 35% fat, and 15% protein) revealed a non-significant decrease in plasma ghrelin concentration [40]. Our current study using a typical North American diet (15% protein, 35% fat, and 50% carbohydrates) and a homogenous large sample size consisting of only young male university students, detected a significant increase in circulating acylated ghrelin after 7 days of overfeeding. The reason for the increased acylated ghrelin concentration after the positive energy challenge is not clear. In animal studies, the infusion of ghrelin has been shown to result in inhibition of beta-cell function and insulin secretion, but also an increase in insulin sensitivity [27, 48, 49]. Additionally in cross-sectional human studies, fasting ghrelin is negatively associated with insulin resistance [50]. It is therefore possible that the increased ghrelin secretion due to overfeeding was a counteractive response to the rising insulin resistance after the 7-day overfeeding, though this is purely speculative as multiple factors must be in play. However, the role of this increased response by ghrelin in long-term energy homeostasis, including the development of human obesity, warrants further study.

Although it has been reported that circulating ghrelin is higher in lean individuals than overweight and obese individuals [19-24], our cohort showed no significant difference in circulating acylated ghrelin among the three adiposity groups. However, when subjects were divided according to adiposity status, BMI was negatively correlated with acylated ghrelin concentration in both normal weight and overweight subjects. This is in line with the observation

that ghrelin may help maintain healthy body weight and BMI. The reason why such correlation was not observed in obese subjects is unclear. It is possible that the normal hormonal mechanism of ghrelin was dysfunctional in the obese state, as seen for many other hormones [51, 52]. This was further demonstrated by the differences in correlations found in overweight and obese subjects, between baseline acylated ghrelin and change in weight and BMI. Overweight subjects showed a negative relationship, while obese subjects showed a positive relationship, between fasting baseline acylated ghrelin and change in body weight and BMI. Thus higher baseline acylated ghrelin predicts low weight gain and BMI in the overweight group, but the opposite is true of the obese group. This may predispose to further weight gain in obese people.

Additionally, we sought to examine the association of acylated ghrelin with serum indices of lipid and glucose metabolism. The associations between ghrelin and circulating lipids/glucose seem to be affected by obesity status. We observed that only baseline acylated ghrelin concentration was positively associated with fasting glucose concentration in normal weight subjects. Additionally in obese subjects, acylated ghrelin was negatively related to circulating LDL cholesterol concentration. Why these relationships were found in specific adiposity groups is unknown. Evidently, more studies are warranted to further grasp the physiological association of ghrelin with glucose and lipid metabolism.

The present study is not without limitations. We only studied young men of the same ethnicity thus limiting its potential application to other ethnic, age, or female groups. Future large scale studies including females and a wider age range are needed to further understand the response of ghrelin to a positive energy challenge. Additionally, it must be noted that ghrelin concentrations are affected by meals [53]. However in our investigation, the relationship between

ghrelin and aforementioned physiological measurements were completed in a fasting state. Data of area under the curve after a standardized meal may provide additional useful information.

In summary, the response of acylated ghrelin to a short-term positive energy challenge was studied in 68 young healthy men. Serum acylated ghrelin was measured before and after a 7-day overfeeding challenge in 68 young men. Surprisingly, fasting ghrelin concentration was significantly increased in response to the positive energy challenge in the entire cohort. Based on our finding and the literature, we hypothesize that this increase may counteract the rising insulin resistance. At baseline, there were no significant differences in circulating acylated ghrelin concentration between normal weight, overweight, and obese men. However negative correlations were observed between ghrelin and BMI, in normal weight and overweight subjects. The baseline acylated ghrelin concentration correlated with change in weight and BMI in opposite directions in overweight and obese subjects. Thus future studies are warranted to understand the mechanistic pathway of ghrelin to further elucidate its role in energy homeostasis and human obesity.

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AUTHORS CONTRIBUTIONS

Analyzed the data: DW FC YY. Wrote the paper: DW. Performed statistical analysis: DW FC YY. Assisted with data collection: DW FC PA. Assisted with ghrelin measurements: ER.

Assisted with insulin measurements: SV. Responsible for the study design: GS. Responsible for the final content: GS. Responsible for the integrity of the data and the accuracy of the data analysis: GS. Assisted with the revisions of the manuscript: WZ ER SV GS. Read and approved the final manuscript: DW FC PA ER SV YY WZ GS.

TABLE 1

Physical and Biochemical Characteristics of Subjects at baseline and in response to 7-days of overfeeding[1]

	Entire Cohort (n = 68)		Normal Weight (n = 26)		Overweight (n= 14)		Obese (n=28)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Age	23.18 ± 0.38	NA	23.75 ± 0.72	NA	21.97 ± 0.83	NA	23.25 ± 0.49	NA
Height (cm)	179.11 ± 0.78	NA	179.04 ± 1.29	NA	179.62 ± 1.28	NA	178.91 ± 1.34	NA
Weight (kg)[4, 5]	82.18 ± 1.80	84.43 ± 1.85	72.86 ± 1.77	75.06 ± 1.84	77.81 ± 1.14	79.39 ± 1.14	93.01 ± 2.95	95.65 ± 3.03
BMI (kg/m^2)[4, 5]	25.64 ± 0.56	26.35 ± 0.58	22.72 ± 0.50	23.42 ± 0.53	24.13 ± 0.36	24.63 ± 0.39	29.10 ± 0.92	29.93 ± 0.95
Percent body fat[2, 6]	23.23 ± 1.03	23.49 ± 0.97	14.69 ± 0.66	15.48 ± 0.67	22.54 ± 0.22	22.82 ± 0.28	31.51 ± 0.95	31.26 ± 0.89
Percent trunk fat[2, 6]	26.08 ± 1.13	26.41 ± 1.06	16.59 ± 0.73	17.62 ± 0.75	25.39 ± 0.50	25.79 ± 0.59	35.22 ± 1.02	34.89 ± 0.96
Percent android fat[2, 5]	29.74 ± 1.34	30.46 ± 1.33	19.09 ± 0.89	20.01 ± 0.98	28.84 ± 0.68	29.46 ± 0.73	40.47 ± 1.38	41.06 ± 1.30
Percent gynoid fat	28.12 ± 0.99	28.36 ± 0.94	20.42 ± 0.96	20.94 ± 0.87	27.45 ± 0.48	28.23 ± 0.47	35.88 ± 0.89	35.58 ± 0.87
Total cholesterol (mmol/L)[5]	4.50 ± 0.10	4.73 ± 0.10	4.37 ± 0.18	4.67 ± 0.17	4.63 ± 0.24	4.73 ± 0.28	4.56 ± 0.14	4.79 ± 0.15
HDL cholesterol (mmol/L)[5]	1.30 ± 0.04	1.40 ± 0.03	1.38 ± 0.06	1.47 ± 0.05	1.39 ± 0.07	1.43 ± 0.07	1.19 ± 0.05	1.31 ± 0.05
LDL cholesterol (mmol/L)	2.72 ± 0.08	2.75 ± 0.08	2.59 ± 0.14	2.66 ± 0.13	2.82 ± 0.20	2.83 ± 0.24	2.79 ± 0.13	2.79 ± 0.11
Triglycerols (mmol/L)[4, 5]	1.09 ± 0.07	1.44 ± 0.17	0.89 ± 0.06	1.18 ± 0.16	0.92 ± 0.09	1.01 ± 0.14	1.37 ± 0.13	1.91 ± 0.36
Glucose (mmol/L)	5.11 ± 0.06	5.10 ± 0.06	4.97 ± 0.08	5.02 ± 0.10	5.03 ± 0.10	5.09 ± 0.15	5.28 ± 0.12	5.17 ± 0.10
Insulin (pmol/L)[3, 5]	70.82 ± 8.68	89.24 ± 8.13	43.34 ± 4.67	64.96 ± 4.65	69.51 ± 18.49	88.85 ± 23.07	97.00 ± 17.35	111.98 ± 14.57
HOMA-IR[3, 5]	2.47 ± 0.35	3.00 ± 0.30	1.40 ± 0.16	2.12 ± 0.17	2.36 ± 0.72	2.95 ± 0.79	3.51 ± 0.72	3.85 ± 0.56
HOMA-β[3, 5]	117.65 ± 9.20	163.29 ± 12.83	84.13 ± 7.69	128.08 ± 9.56	120.21 ± 19.80	175.91 ± 43.72	147.49 ± 17.13	189.67 ± 19.54
Serum ghrelin (ng/L)[5]	286.51 ± 52.30	348.65 ± 63.02	222.19 ± 41.60	226.14 ± 29.26	274.91 ± 92.44	284.14 ± 88.18	352.03 ± 112.42	494.66 ± 141.23

[1] All values are mean ± SE. Homeostasis model assessment of insulin resistance (HOMA-IR) and of B cell function (HOMA-β); NA, not applicable. Subjects were classifed based on adiposity recommendations by Bray as normal weight (8-20.9%), overweight (21-25.9%), or obese (>26%). Obesity status and overfeeding response were analyzed by 2-factor mixed-model ANOVA for repeated measures (IBM SPSS Statistics 19).

[2] Significant difference between normal weight, overweight, and obese subjects at baseline, $P < 0.05$ (one-factor ANOVA with Bonferroni correction).

[3] Significant difference between normal weight and obese subjects at baseline, $P < 0.05$ (one-factor ANOVA with Bonferroni correction).

[4] Significant difference between obese and normal weight and obese and overweight, at baseline, $P < 0.05$ (one-factor ANOVA with Bonferroni correction).

[5] Significant difference due to overfeeding, $P < 0.05$ (2-factor mixed-model ANOVA).

[6] Significant overfeeding x adiposity status interaction, $P < 0.05$ (2-factor mixed-model ANOVA with Bonferroni correction).

TABLE 2

Partial correlations of baseline variables related to baseline fasting serum ghrelin concentration.[a,b]

	All Subjects (n = 68)		Normal Weight (n = 26)		Overweight (n = 14)		Obese (n = 28)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Weight (kg)	-0.012	NS	-0.431	0.032	-0.234	NS	0.089	NS
BMI (kg/m^2)	-0.037	NS	-0.404	0.045	-0.667	0.013	0.067	NS
Percent body fat	-0.007	NS	-0.226	NS	-0.440	NS	-0.086	NS
Percent trunk fat	-0.034	NS	-0.277	NS	-0.425	NS	-0.130	NS
Percent android fat	-0.035	NS	-0.169	NS	-0.548	NS	-0.112	NS
Percent gynoid fat	0.070	NS	-0.096	NS	0.137	NS	0.122	NS
Total cholesterol	-0.138	NS	0.392	NS	-0.370	NS	-0.353	NS
HDL cholesterol	-0.148	NS	0.274	NS	-0.438	NS	-0.290	NS
LDL cholesterol	-0.178	NS	0.292	NS	-0.293	NS	-0.436	0.023
Triacylglycerols	0.145	NS	0.213	NS	-0.073	NS	0.172	NS
Glucose	-0.015	NS	0.473	0.017	-0.140	NS	-0.185	NS
Insulin	0.069	NS	0.187	NS	0.192	NS	-0.071	NS
HOMA-IR	0.061	NS	0.231	NS	0.152	NS	-0.092	NS
HOMA-β	0.097	NS	-0.034	NS	0.384	NS	0.036	NS

[a] Partial correlations controlled for age were used to screen for variables related to fasting ghrelin (P<0.05) (IBM SPSS Statistics 19).

[b] Homeostasis model assessment of insulin resistance (HOMA-IR) and of B cell function (HOMA-β); NS, non-significant. Subjects were classifed based on adiposity recommendations by Bray as normal weight (8-20.9%), overweight (21-25.9%), or obese (>26%).

TABLE 3

Partial correlations of change in variables related to baseline fasting serum ghrelin concentration.[a,b]

	All Subjects (n = 68)		Normal Weight (n = 26)		Overweight (n = 14)		Obese (n = 28)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Weight (kg)	0.217	NS	0.208	NS	-0.590	0.034	0.618	0.001
BMI (kg/m^2)	0.218	NS	0.227	NS	-0.599	0.031	0.616	0.001
Percent body fat	-0.017	NS	0.297	NS	-0.170	NS	-0.145	NS
Percent trunk fat	-0.042	NS	0.235	NS	-0.086	NS	-0.159	NS
Percent android fat	0.084	NS	0.246	NS	0.159	NS	-0.080	NS
Percent gynoid fat	-0.047	NS	0.017	NS	-0.298	NS	0.098	NS
Total cholesterol	0.022	NS	-0.056	NS	-0.043	NS	0.124	NS
HDL cholesterol	0.006	NS	-0.167	NS	-0.125	NS	0.185	NS
LDL cholesterol	0.041	NS	-0.134	NS	0.026	NS	0.202	NS
Triacylglycerols	-0.016	NS	0.233	NS	-0.108	NS	-0.103	NS
Glucose	-0.026	NS	-0.124	NS	-0.169	NS	0.106	NS
Insulin	-0.082	NS	0.044	NS	-0.178	NS	-0.135	NS
HOMA-IR	-0.080	NS	0.013	NS	-0.206	NS	-0.086	NS
HOMA-β	-0.061	NS	0.155	NS	-0.012	NS	-0.343	NS

[a] Partial correlations controlled for age were used to screen for variables related to fasting ghrelin (P<0.05) (IBM SPSS Statistics 19).

[b]Homeostasis model assessment of insulin resistance (HOMA-IR) and of B cell function (HOMA-β); NS, non-significant. Subjects were classifed based on adiposity recommendations by Bray as normal weight (8-20.9%), overweight (21-25.9%), or obese (>26%).

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CHAPTER 3: Circulating glucagon-like peptide-1 increases in response to short-term overfeeding in men

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ABSTRACT

Background: Glucagon-like Peptide-1 (GLP-1) is an incretin hormone secreted from the gastrointestinal tract that facilitates the glucose-dependent insulin response. Additionally, GLP-1 is thought to be involved in energy homeostasis. Currently little is known about GLP-1's responsiveness to an energy surplus, a fundamental cause of obesity and diabetes. Our objective was to examine the response of serum GLP-1 to short-term (7 day) overfeeding in young men.

Methods: Seventy-two young men from the Canadian province of Newfoundland were recruited for the study. For 7-days, the subjects consumed 70% more calories than required at baseline. Various measurements including: anthropometrics, body composition, markers of glucose/lipid metabolism and serum total GLP-1, were taken at a fasted state before (day 1) and after (day 8) the challenge. Paired t-test analyses were used to assess the change in variables after the overfeeding period. Additionally, the relationship between serum GLP-1 and the measured variables at baseline and change due to overfeeding were analyzed.

Results: Serum GLP-1 was significantly increased in all groups in response to the 7-day energy surplus, indicating the increase was independent of adiposity status. There was no significant difference in fasting GLP-1 at baseline between the normal weight and overweight/obese groups. At baseline, GLP-1 concentration negatively correlated with HDL-cholesterol and positively correlated with triacylglycerols and markers of insulin resistance in the overweight/obese group. Also GLP-1 was negatively correlated with change in percent gynoid fat in the overweight/obese subjects. Percent change in GLP-1 was negatively associated with percent change in gynoid fat in the normal weight group and positively associated with percent change in cholesterol in the overweight/obese group. Percentage change of circulating triacylglycerols was positively associated with percent change in GLP-1 in both adiposity groups.

Conclusion: Our findings showed that GLP-1 serum concentration is not a significant factor in determining obesity status. The increase of GLP-1 in all subjects regardless of obesity status, suggest GLP-1 serves as a protective role, counteracting energy surplus.

KEY WORDS

overfeeding, GLP-1, nutritional regulation, obesity, and diabetes

INTRODUCTION

Hormones secreted from the gastrointestinal (GI) tract play an important role as peripheral signals of energy homeostasis and are thought to be involved in the development of obesity and diabetes [1]. Glucagon-like peptide-1 (GLP-1) is a 30-amino acid peptide hormone secreted from the L-cells of the distal ileum, but is also present in the central nervous system [2]. GLP-1 is a product of the GCG gene and is formed due to tissue-specific post-translational modification of proglucagon. The hormone is released into circulation in response to food intake; the larger the meal size, the greater the GLP-1 response. [3, 4]. Therefore during the fasted state, GLP-1 is low (but still detectable) however the concentration rises postprandially. Active GLP-1 has a very short half-life of ~2 minutes as it is degraded by dipeptidyl peptidase-IV (DPP-IV) [5, 6].

GLP-1 binds to its receptors present in a variety of tissues and elicits a number of responses. For one, GLP-1 is designated an incretin hormone, facilitating the glucose-dependent release of insulin from the pancreatic beta-cells [7, 8]. Also it has been shown to decrease the secretion of glucagon in patients with type-I diabetes who have no beta-cell function but still exhibit the lowered plasma glucose via GLP-1 [9-11]. Other effects include, decreased appetite [12-14] and decreased gastric motility and secretion [15, 16].

Due to its many metabolic-related effects, GLP-1 has been implicated in many chronic metabolic diseases. For example, both its incretin effect and suppression of glucagon secretion action are disrupted in type-II diabetes [17]. It has also been shown that intravenous (IV) infusion of GLP-1 acts to lower blood glucose concentration in type-II diabetic patients [18, 19]. GLP-1 is also suggested to be involved in obesity as morbidly obese subjects show a decreased diurnal L-cell secretion [20]. Fasting GLP-1 has been found to be lower in diabetic and obese

diabetic patients as compared to healthy controls [21, 22] however, not all studies have consistent findings [23]. Additionally, the postprandial secretion of GLP-1 is inhibited in morbidly obese subjects, which is improved after weight loss [24, 25]. It has been found that GLP-1 infusions reduce food intake in normal weight and obese subjects, regardless of diabetic status [13, 14, 26-28].

Recognizing that obesity is a result of a chronic positive energy imbalance, it has been shown that modification of food intake, including overfeeding, influence various gut hormone concentrations [29-32]. However, most human studies on GLP-1 have been performed using a cross sectional study design which may not reflect biological and clinical relevance. The dynamic process of an energy surplus will provide insights of the role of GLP-1 in the development of obesity and diabetes [33-35].The objectives of the present study were therefore to investigate: a) the GLP-1 response to short-term overfeeding in young men; b) the difference in serum GLP-1 concentration in pre- and post- overfeeding between various adiposity groups; and c) the relationship of fasting GLP-1 with various obesity-related markers.

METHODS

Subjects

Seventy-two healthy men (age 19-29) from Newfoundland and Labrador, Canada, participated in the present study. All subjects were of at least 3rd generation Newfoundland descent and reported a stable body weight over the last six months. Participants had no serious endocrine, metabolic or cardiovascular diseases, nor were taking any medications affecting lipid/cholesterol metabolism. Informed and written consent were provided by each subject. Ethical approval was received from

the Human Investigations Committee for the Faculty of Medicine, Memorial University, St. John's, NL, Canada.

Overfeeding

Overfeeding was completed following a protocol previously described by us [31, 32, 36]. To assess the metabolic and endocrine effects of a short-term energy surplus, participants were overfed, for one week (7 days), 70% more calories than what they would normally consume. A 7-day overfeeding period was chosen to ensure changes in metabolic parameters. To estimate daily energy requirements, three 24-hour recall interviews (2 on weekdays, 1 on weekend) and a 30-day dietary inventory were administered to the subjects. A food recall kit containing standard portion sizes was used in a face-to-face interview, assessing the food intake of the previous 24-hours. An average of the questionnaires was utilized to determine daily caloric requirements. The positive energy challenge was consistent with the typical North American diet: 50% carbohydrates, 35% fat, and 15% protein. Throughout 7-days at time 0900, 1200, and 1700, subjects consumed meals with caloric and nutritional content calculated using Food Processor SQL (version 9.5.0.0; ESHA Research, Salem, OR). A laboratory member was present through the duration of all meals. Average caloric content was 2969 kcal pre-overfeeding and 5471 kcal during overfeeding [29]. While participating in the study, subjects were requested to refrain from consuming additional calorie-containing beverages, drinking alcohol, or taking drugs/medications.

Measured Variables

Various physical, anthropometric and biochemical markers were assessed after a 12-hr fasting period, before and after the 7-day overfeeding period. These are outlined below:

Anthropometric measurements

During all anthropometric measurements subjects wore a light standardized hospital gown. Total body weight was assessed on a platform scale balance (Health O Meter, IL) and height was measured using a fixed stadiometer, to the nearest 0.1 kilogram and centimeter, respectively. Body mass index (BMI) was calculated by dividing the participant's weight in kilograms by height in meters squared (kg/m^2). Both waist and hip circumference were evaluated using a measuring tape to the nearest 0.1 centimeter.

Body composition measurements

Body composition was assessed using dual-energy X-ray absorptiometry (DXA Lunar Prodigy; GE Medical Systems, Madison, WI). The scan can differentiate between fat, lean, and bone mass, and can therefore determine percent body fat (%BF), trunk fat (%TF), android fat (%AF), and gynoid fat (%GF) [37] (Method previously described by us [38]). Study subjects were classified as underweight, normal weight, overweight, and obese based on percentage body fat recommendations by Bray (for males aged 20-39: underweight= <8.0%, normal weight= 8-20.9%, overweight= 21-25.9%, or obese= >26.0%) [39].

Serum measurements

Venous blood was collected from subjects and after processing, serum was stored at -80°C. Serum total cholesterol, high-density lipoprotein cholesterol (HDL-c), triacylglycerols (TGs),

and glucose concentrations were determined by Synchron reagents using an Lx20 clinical chemistry analyzer (Beckman Coulter Inc., CA, USA). The concentration of serum insulin was measured utilizing the Immulite 2500 immunoassay analyzer (Siemens, Los Angeles, CA). The homeostasis model assessment (HOMA) was used to estimate indices of pancreatic β-cell function (HOMA-β: [20 x insulin (mU/L)/(glucose (mmol/L) - 3.5)]) and insulin resistance (HOMA-IR: [insulin (mU/L) x glucose (mmol/L)/22.5)]). The Friedwald equation (total cholesterol – HDL-c – TG/2.2) was used to calculate low-density lipoprotein cholesterol (LDL-c). Serum total GLP-1 was measured in duplicate using enzyme-linked immunosorbent assay (ELISA) kits (EMD Millipore, St. Charles, MO). The intra-assay variation ranged from 3.4% to 4.2%, and the inter-assay variation (n = 4) was 5.7%.

Statistical Analysis

All data are presented as mean ± S.E. unless stated otherwise. Data not normally distributed were log-transformed (concentrations of fasting: serum GLP-1, triacylglycerols, insulin, HOMA-IR and HOMA-β) where appropriate. As well, all data was analyzed using SPSS (Version 19) and statistical tests were two-sided with significance set at a P-value of 0.05. Because of the small sample size (n=3) underweight subjects were combined with normal weight subjects (total n=30). Additionally, as there were a small number of overweight individuals (n=14), these subjects were combined with obese subjects (total n=42).

The differences between various markers of adiposity, insulin resistance, and lipid metabolism, and fasting GLP-1 concentration before and after the overfeeding protocol, were assessed utilizing paired t-test analysis. Additionally a two-factor ANOVA (repeated measures) was used to examine the overfeeding-adiposity interaction of measured variables. One-way ANOVA (with

Bonferroni post hoc tests) was utilized to assess the differences of variables between adiposity groups at baseline.

Spearman correlation analysis was completed to assess the relationship between a) baseline GLP-1 serum concentration and aforementioned variables at baseline, b) baseline GLP-1 serum concentration and change in the variables; c) change in serum GLP-1 and change in the variables; and d) baseline variables and change in serum GLP-1. Additionally partial correlative analysis was completed controlling for potential confounding factors.

RESULTS

Pre- and Post- Overfeeding Descriptive Statistics

Biochemical and physical measurements prior-to and after the overfeeding challenge are presented in Table 1. Changes in anthropometrics, body composition and measures of glucose and lipid metabolism were previously described by us [31, 32, 40]; nevertheless, it was evident that the one-week overfeeding challenge increased body weight, adiposity, serum lipids, insulin and insulin resistance. After a one week energy surplus, circulating GLP-1 concentration rose in the cohort. The pre- and post- overfeeding fasting concentrations of GLP-1 were increased from 36.84±3.16 pmol/L at baseline to 42.39±3.18 pmol/L, after overfeeding. The average percent change in GLP-1 through the overfeeding study was 24.10±5.85 %. However based on 2-way ANOVA repeated measure analysis, there was no significant difference between normal weight and overweight/obese subjects for the increase in serum GLP-1 (P=0.590). Furthermore, there was no significant difference between the normal weight and overweight/obese group for fasting GLP-1 concentration at baseline (t-test: P=0.876).

Baseline correlations of GLP-1 with body composition and markers of lipid/glucose metabolism

Table 2 characterizes the baseline relationships between GLP-1 and the markers of adiposity, and lipid/glucose metabolism, utilizing Spearman correlation analysis. In the entire cohort, fasting GLP-1 concentration was negatively associated with HDL-c concentration and positively associated with triacylglycerol concentration. Also a positive relationship was evident between GLP-1 and the ratio of total cholesterol to HDL-c. When the cohort was grouped based on adiposity status (based on Bray recommendations), no relationships were found between GLP-1 concentration and the measured variables in the normal weight group. However, in the overweight/obese group, GLP-1 concentration remained significantly correlated with HDL-c, triacylglycerol concentration, and the ratio of total cholesterol to HDL-c. Interestingly, in the overweight/obese, a positive relationship was found between GLP-1 concentration and insulin, HOMA-IR, HOMA-β, and percent gynoid fat.

Due to the possible confounding effect of gynoid fat on HDL-cholesterol, triacylglycerols, and markers of insulin metabolism, partial correlations were performed controlling for gynoid fat and all analyses were repeated (data not shown). All of the aforementioned relationships remained significant except for the relationship between GLP-1 and HOMA-β in the overweight/obese group ($r = 0.300$, $P = 0.053$).

Correlations between baseline GLP-1 with percent change in body composition and markers of lipid/glucose metabolism

The correlative data between fasting baseline GLP-1 concentration and percent changes in the markers of adiposity, and lipid and glucose metabolism were also observed. In the entire cohort,

no relationships were present between circulating GLP-1 and changes in any of the measurements. However when subjects were grouped according to adiposity, a negative correlation was revealed between GLP-1 concentration and change in percent gynoid fat ($r = -0.349$, $P=0.025$) in the overweight/obese group.

Correlations between percent change in GLP-1 with percent change in body composition and markers of lipid/glucose metabolism

Presented in Table 3 is the correlative data between percent change in GLP-1 concentration and percent change in markers of adiposity, and markers of lipid/glucose metabolism. In the entire cohort, change in GLP-1 was significantly positively associated with percent change in HDL-c, and triacylglycerols. When split based on adiposity status, percent change in GLP-1 was positively correlated with percent change in triacylglycerols and negatively correlated with percent change in gynoid fat in the normal weight group. However, in the overweight/obese percent change in serum GLP-1 was significantly positively associated with percent change in total cholesterol and triacylglycerols.

Correlations between percent change in GLP-1 with baseline body composition and lipid/glucose metabolism variables

Additionally we wanted to assess the relationship between baseline variables and percent change in GLP-1 (Table 4). In the entire cohort, baseline weight and BMI were positively correlated with percent change in GLP-1 while baseline GLP-1 was negatively correlated with percent change in GLP-1. The same relationships were found significant in the normal weight group,

when the cohort was grouped based on adiposity. However, only the negative relationship with baseline GLP-1 remained significant in the overweight/obese group.

DISCUSSION

In the current investigation we examined the response of circulating GLP-1 to a 7-day energy surplus in 72 young men of Newfoundland descent. The most notable finding was that GLP-1 concentration significantly increased in response to the overfeeding challenge. The rise in GLP-1 concentration was independent of adiposity status as the increase of GLP-1 was present in normal weight and overweight/obese groups. GLP-1 has been shown to have beneficial effects as it facilitates the glucose-dependent insulin response, lowers glucagon secretion, and induces satiation [7-14]. Thus current literature suggests GLP-1 secretion would increase in response to a positive energy challenge, counteracting the response and potentially acting as a protective mechanism (cessation of appetite/regulation of insulin secretion). Though this may be the case, studies regarding circulating GLP-1 and overfeeding in humans are few in number, and vary largely in terms of overfeeding time, degree of overfeeding and macronutrient composition. A 3-day overfeeding study in which 21 subjects (15 males, 6 females) consumed 50% more calories than baseline requirements (energy breakdown: 20% protein, 30% fat, 50% carbohydrate) showed GLP-1 was unchanged over the duration of the study [35]. Similarly, Brons *et al*. overfed 26 healthy Danish young men 50% more calories than required (60% fat, 32.5% carbohydrates and 7.5% protein) utilizing a 5-day high fat diet and found no significant change in fasting circulating GLP-1 [33]. A small study on nine lean Caucasian males also found no significant difference in GLP-1 concentration after an overfeeding period which ceased when 5% of body weight was gained (average of 35 days, range of 28-43; composition: ~ 50%

carbohydrates, 35% fat, 15% protein) [34]. The excess calories were provided by a liquid drink which was used to bring total caloric intake to a value of 1.4 times the eucaloric diet. Evidently these studies differ in regards to subject homogeneity, length and amount of energy surplus. The negative results from all three studies could be due to the small sample size and/or shorter period of overfeeding (first two studies). The present investigation, utilizing a fairly homogenous sample population of young healthy men from the Newfoundland population, observed a significant increase in serum GLP-1 after a 7-day overfeeding challenge consistent with a typical North American diet (50% carbohydrates, 35% fat, and 15% protein). We suggest that the increase in GLP-1 was a homeostatic protective mechanism to offset the metabolic disturbance caused by the energy surplus.

Previous studies have suggested that obese individuals have a lower GLP-1 secretion as compared to lean individuals [20, 24, 25, 41]. In our cohort, we found no significant difference in fasting GLP-1 concentration between overweight/obese and normal weight subjects. In the entire cohort we found no significant relationship between baseline GLP-1 concentration and markers of adiposity including BMI and percent body fat. However, in the overweight/obese group we found baseline GLP-1 correlated with percent gynoid fat. In general, women are more likely to have greater gynoid fat distribution, and having this distribution is thought to oppose cardiovascular diseases through more efficient fat storage/lipoprotein lipase functionality [42, 43]. Additionally in the overweight/obese group baseline GLP-1 concentrations correlated with a negative change in percent gynoid fat. In other words, individuals with higher GLP-1 concentration had a smaller change in percent gynoid fat after the positive energy challenge; therefore the finding suggests higher baseline GLP-1 predicted a reduced gain in percent gynoid fat. Moreover, change in GLP-1 was negatively associated with a change in gynoid fat within the

normal weight group, again proposing a protective effect of GLP-1. Nevertheless, with lack of large-scale studies assessing GLP-1 and overfeeding we cannot fully elucidate the predictor ability of baseline/change in GLP-1 on change in metabolic variables.

In this study we also observed the relationships between GLP-1 and markers of lipid metabolism and insulin resistance. At baseline when controlling for percent gynoid fat, GLP-1 was positively correlated with triacylglycerols and markers of insulin resistance and negatively correlated with HDL cholesterol, in the overweight/obese group. Thus taken together, overweight/obese subjects with higher circulating baseline GLP-1 have a less favourable lipid profile (higher triacylglycerols, lower HDL cholesterol) and higher insulin resistance (increased HOMA-IR, HOMA-β, and insulin). This being said, studies have shown administration of GLP-1 receptor agonists to be associated with a beneficial change in lipid profile and insulin resistance [44-47]. Still however, a study by de Luis *et al*. [48] found that after biliopancreatic diversion surgery in morbidly obese patients, basal GLP-1 was negatively associated with HDL-c, consistent with our findings. Additionally it has been found that higher circulating GLP-1 in subjects with metabolic syndrome, are at greater risk for cardiovascular disease [49]. When we observed the relationship between percent change in GLP-1 and percent change in triacylglycerols, a positive association was found in both the normal weight and overweight/obese cohorts (also change in cholesterol was positively correlated with change in GLP-1 in the overweight/obese cohort). We theorize that the increased GLP-1 is trying to compensate for the increase in both triacylglycerols and total cholesterol. However, because our study is a forced overfeeding intervention (subjects ate 70% more calories than required a day) the metabolic disturbance caused by overfeeding potentially overwhelmed GLP-1's potential

effect. Nevertheless more studies are necessary to elucidate the role of GLP-1 in lipid/glucose metabolism.

A limitation of this investigation is that only young men (age 19-29) of Newfoundland descent were studied for one week; therefore similar studies are needed in females, and subjects of different age ranges and ethnic groups. Further large-scale overfeeding studies assessing such cohorts are warranted to fully elucidate the role of GLP-1 during a positive energy surplus. Additionally, only total human GLP-1 was measured rather than the suggested active form, GLP-1 (7-36 amide). Active GLP-1 has a very short half-life and is found in low concentrations, before it is degraded by DPP-IV, while total GLP-1 gives an indication of the secretion from intestinal L-cells. However, total GLP-1 has been shown to positively correlate with active GLP-1 (7-36amide) concentration [50]. Furthermore, although macronutrient composition was relatively constant, we did not account for the composition of fat (polyunsaturated, saturated, etc) or carbohydrate (complex vs. simple sugars).

CONCLUSION

Overall, our study investigated the response of fasting GLP-1 concentration to a 7-day overfeeding protocol in a total of 72 young men from the Canadian province of Newfoundland. In response to the short term energy surplus, circulating GLP-1 significantly increased in the entire cohort, regardless of adiposity. We suggest that the increased GLP-1 may act as a protective mechanism to counteract the positive energy challenge. Additionally at baseline, there was no significant difference in fasting GLP-1 concentration between the lean and overweight/obese groups. However at baseline, GLP-1 was positively correlated with triacylglycerols and markers of insulin resistance, and negatively associated with HDL-c in

overweight/obese individuals. Also in this group, baseline GLP-1 was negatively associated with percent change in percent gynoid fat. Percent change in GLP-1 was associated with percent change in specific variables of lipid metabolism (triacylglycerols, total cholesterol) in the overweight/obese group. Although the positive relationship between percent change in triacylglycerols and percent change in GLP-1 was present in the normal weight group, a negative relationship existed between percent change in gynoid fat and percent change in GLP-1. Our results suggest that GLP-1 can potentially serve as a protective factor in obesity and it is involved in lipid/glucose metabolism and fat distribution.

COMPETING INTERESTS

None of the authors had a personal or financial conflict of interest. All authors read and approved the final manuscript.

AUTHORS' CONTRIBUTIONS

The author' responsibilities were as follows: DW: wrote the paper. DW, FC and YY: analyzed the data and performed statistical analysis. DW, FC and PA: assisted with data collection. ER: assisted with GLP-1 measurements. SV assisted with insulin measurements. GS was responsible for the study design, final content, and takes responsibility for the integrity of the data and the accuracy of the data analysis. GS, JC, ER, SV, and YY: assisted with the revisions of the manuscript.

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TABLES

TABLE 1

Physical and Biochemical Characteristics of Subjects at baseline and in response to 7-days of overfeeding[a,b]

	Entire Cohort (n = 72)											
	Day 1			Day 8			*p-value*	Change (Δ) from baseline			Average Percent Change from baseline (%)		
Age	23.11	±	0.37	N/A			-	N/A			N/A		
Height (cm)	179.18	±	0.72	N/A			-	N/A			N/A		
Weight (kg)[b]	80.92	±	1.81	83.13	±	1.87	<0.001	2.21	±	0.16	2.74	±	0.20
BMI (kg/m^2)[b]	25.27	±	0.56	25.96	±	0.58	<0.001	0.69	±	0.05	2.74	±	0.20
Percent body fat	22.41	±	1.05	22.69	±	1.00	0.051	0.28	±	0.14	2.65	±	0.87
Percent trunk fat	25.15	±	1.17	25.52	±	1.10	0.088	0.38	±	0.21	3.25	±	1.10
Percent android fat[b]	28.64	±	1.38	29.44	±	1.35	<0.001	0.80	±	0.21	4.77	±	1.37
Percent gynoid fat	27.27	±	1.03	27.50	±	0.99	0.221	0.23	±	0.19	1.80	±	0.89
Total cholesterol (mmol/L)[b]	4.50	±	0.10	4.74	±	0.10	0.007	0.23	±	0.08	6.33	±	1.88
HDL cholesterol (mmol/L)[b]	1.31	±	0.03	1.41	±	0.03	<0.001	0.10	±	0.02	9.08	±	1.88
Total cholesterol:HDLc ratio[b]	3.58	±	0.10	3.47	±	0.10	0.020	-0.10	±	0.04	-2.11	±	1.12
LDL cholesterol (mmol/L)	2.72	±	0.08	2.75	±	0.08	0.639	0.03	±	0.06	2.88	±	2.17
Triacylglycerols (mmol/L)[b]	1.08	±	0.06	1.44	±	0.16	0.005	0.35	±	0.15	44.74	±	21.54
Glucose (mmol/L)	5.10	±	0.06	5.11	±	0.06	0.905	0.01	±	0.07	0.75	±	1.31
Insulin (pmol/L)[b]	68.54	±	8.27	86.84	±	7.78	<0.001	18.29	±	6.98	55.02	±	9.53
HOMA-IR[b]	2.38	±	0.34	2.92	±	0.29	<0.001	0.54	±	0.29	59.71	±	10.77
HOMA-β[b]	114.57	±	8.85	158.38	±	12.38	<0.001	43.80	±	9.30	50.60	±	8.01
Serum GLP-1 (pmol/L)[b]	36.84	±	3.16	42.39	±	3.18	<0.001	5.55	±	1.57	24.10	±	5.85

[a] Values are mean ± SE. Homeostasis model assessment of insulin resistance (HOMA-IR) and of B cell function (HOMA-β); GLP-1, Glucagon-like Peptide-1; N/A, not applicable.

[b] Significant difference present between pre- and post- overfeeding (paired t-test; SPSS 19.0).

TABLE 2

Spearman correlations of baseline variables related to baseline fasting serum GLP-1 concentration.[a,b]

	All Subjects (n = 72)		Normal Weight (n = 30)		Overweight+Obese (n = 42)	
	r	*P*	*r*	*P*	*r*	*P*
Weight (kg)	0.052	NS	-0.197	NS	0.175	NS
BMI (kg/m^2)	-0.006	NS	-0.218	NS	0.117	NS
Percent body fat	0.086	NS	-0.027	NS	0.186	NS
Percent trunk fat	0.098	NS	-0.059	NS	0.238	NS
Percent android Fat	0.079	NS	-0.075	NS	0.216	NS
Percent gynoid Fat	0.141	NS	-0.021	NS	0.328	0.036
Total cholesterol	0.103	NS	0.265	NS	-0.037	NS
HDL cholesterol	-0.357	0.002	0.141	NS	-0.582	<0.001
Total cholesterol:HDLc ratio	0.383	0.001	0.182	NS	0.489	0.001
LDL cholesterol	0.077	NS	0.286	NS	-0.065	NS
Triacylglycerols	0.412	<0.001	0.232	NS	0.526	<0.001
Glucose	0.157	NS	0.039	NS	0.191	NS
Insulin	0.216	NS	0.001	NS	0.394	0.010
HOMA-IR	0.207	NS	0.005	NS	0.375	0.014
HOMA-β	0.205	NS	-0.074	NS	0.353	0.022

[a] P<0.05 (IBM SPSS Statistics 19).

[b]Homeostasis model assessment of insulin resistance (HOMA-IR) and of β cell function (HOMA-β); NS, non-significant. Subjects were classifed based on adiposity recommendations by Bray as normal weight (8-20.9%), overweight (21-25.9%), or obese (>26%).

TABLE 3

Spearman correlations of changes in variables related to change in fasting serum GLP-1 concentration.[a,b]

	All Subjects (n = 72)		Normal Weight (n = 30)		Overweight+Obese (n = 42)	
	r	*P*	*r*	*P*	*r*	*P*
Weight (kg)	0.222	NS	0.354	NS	0.137	NS
BMI (kg/m^2)	0.223	NS	0.348	NS	0.142	NS
Percent body fat	0.122	NS	0.168	NS	0.087	NS
Percent trunk fat	0.126	NS	0.221	NS	0.021	NS
Percent android Fat	0.206	NS	0.286	NS	0.066	NS
Percent gynoid Fat	-0.061	NS	-0.384	0.036	0.243	NS
Total cholesterol	0.219	NS	0.117	NS	0.334	0.030
HDL cholesterol	0.258	0.029	0.321	NS	0.188	NS
Total cholesterol:HDLc ratio	-0.005	NS	-0.171	NS	0.191	NS
LDL cholesterol	0.007	NS	-0.085	NS	0.195	NS
Triacylglycerols	0.543	<0.001	0.573	0.001	0.533	<0.001
Glucose	0.131	NS	0.226	NS	0.099	NS
Insulin	0.218	NS	0.187	NS	0.271	NS
HOMA-IR	0.212	NS	0.198	NS	0.229	NS
HOMA-β	0.127	NS	0.024	NS	0.174	NS

[a] P<0.05 (IBM SPSS Statistics 19).

[b]Homeostasis model assessment of insulin resistance (HOMA-IR) and of β cell function (HOMA-β); NS, non-significant. Subjects were classifed based on adiposity recommendations by Bray as normal weight (8-20.9%), overweight (21-25.9%), or obese (>26%).

TABLE 4

Spearman correlations of baseline variables related to change in fasting serum GLP-1 concentration.[a,b]

	All Subjects (n = 72)		Normal Weight (n = 30)		Overweight+Obese (n = 42)	
	r	*P*	*r*	*P*	*r*	*P*
Weight (kg)	0.251	0.033	0.391	0.033	0.162	NS
BMI (kg/m^2)	0.295	0.012	0.446	0.013	0.188	NS
Percent body fat	0.130	NS	0.272	NS	0.109	NS
Percent trunk fat	0.139	NS	0.307	NS	0.104	NS
Percent android Fat	0.127	NS	0.278	NS	0.077	NS
Percent gynoid Fat	0.068	NS	0.239	NS	-0.074	NS
Total cholesterol	-0.112	NS	-0.255	NS	-0.030	NS
HDL cholesterol	-0.085	NS	-0.291	NS	0.095	NS
Total cholesterol:HDLc ratio	-0.012	NS	0.066	NS	-0.082	NS
LDL cholesterol	-0.118	NS	-0.179	NS	-0.115	NS
Triacylglycerols	-0.151	NS	-0.226	NS	-0.110	NS
Glucose	0.016	NS	-0.122	NS	0.124	NS
Insulin	0.135	NS	0.079	NS	0.074	NS
HOMA-IR	0.135	NS	0.059	NS	0.083	NS
HOMA-β	0.158	NS	0.248	NS	0.063	NS
GLP-1	-0.416	<0.001	-0.484	0.007	-0.396	0.009

[a] P<0.05 (IBM SPSS Statistics 19).

[b]Homeostasis model assessment of insulin resistance (HOMA-IR) and of β cell function (HOMA-β); NS, non-significant. Subjects were classifed based on adiposity recommendations by Bray as normal weight (8-20.9%), overweight (21-25.9%), or obese (>26%).

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CHAPTER 4: SUMMARY

The current global obesity epidemic puts more of the population at a greater risk of diabetes, cardiovascular complications, and certain forms of cancer. Although increased caloric intake and decreased energy expenditure are fundamental roots of increased adiposity, obesity is a multi-faceted condition and is therefore influenced by environmental and genetic factors. Moreover, various circulating peripheral hormones act as signals to the central nervous system (and other peripheral tissues) influencing energy homeostasis, metabolism, appetite and therefore, body composition. Hormones secreted from the GI tract, the largest endocrine organ in the body, are no exception as they are involved in energy homeostasis, influencing appetite, metabolism, and GI motility [1, 2]. For example ghrelin, secreted mainly from the fundus of the stomach before meals, has been shown to increase appetite in animal and humans [3-5]. Additionally, the gut hormone glucagon-like peptide-1 (GLP-1) has been described as an incretin hormone: helping to facilitate the glucose-dependent insulin response [6-8]. The hormone also decreases glucagon secretion, slows gastric secretions/motility and is suggested to increase satiety [9, 10]. Though studies have assessed the hormones ghrelin and GLP-1 in respect to human obesity, few studies exist assessing the change of gut hormones through a positive energy challenge. The positive energy balance, or energy surplus (calories consumed > calories expended) is the primary cause of weight gain and obesity, thus understanding metabolic and biochemical changes during such periods of overfeeding is of immense importance. Therefore our research goal was to investigate the response of two functionally related gut hormones, ghrelin and GLP-1 to a short-term overfeeding period involving young men. The specific research objectives were as follows: 1) to

observe the change of GLP-1 and ghrelin to a 7-day overfeeding intervention (70% more calories than required at baseline); 2) to assess the difference in circulating GLP-1 and ghrelin at pre and post-overfeeding between adiposity (normal weight, overweight, obese) groups; and 3) to examine the relationships of GLP-1 and ghrelin with body composition, physical and biochemical markers.

Seventy-two healthy young men aged 19-29 from Newfoundland were overfed 70% more calories than each required for one week. The diet consisted of 50% carbohydrates, 35% fat, and 15% protein which is typical of North America dietary consumption patterns. Aforementioned physical and biochemical measurements were taken at fasting before (morning Day 1) and after (morning Day 8) the overfeeding protocol. Serum ghrelin was measured using an EIA method, and GLP-1 was measured using an ELISA method, before and after the positive energy challenge.

As expected, circulating GLP-1 increased due to the overfeeding challenge. However to our surprise, circulating ghrelin also increased. After subjects were split into adiposity groups of normal weight, overweight and obese, there were no differences found for baseline ghrelin and GLP-1 between these groups. Additionally, there was no difference for the change in ghrelin and GLP-1 due to overfeeding between normal weight, overweight and obese cohorts. At baseline, circulating ghrelin was negatively associated with weight and BMI in the normal weight group, and negatively associated with BMI in the overweight group. Also at baseline, circulating GLP-1 was inversely correlated with HDL-cholesterol and positively correlated with triacylglycerols and

markers of insulin resistance in the overweight/obese group. Moreover, baseline ghrelin was negatively correlated with change in weight and BMI in the overweight group, and positively correlated with change in BMI in the obese group. Interestingly, baseline GLP-1 was negatively associated with change in percent gynoid fat in overweight/obese subjects. However, change in circulating GLP-1 was positively associated with percent change in triacylglycerol in normal weight and overweight/obese groups. When observing the normal weight group, percent change of GLP-1 was inversely correlated with percent change in gynoid fat. Furthermore in the overweight/obese subjects, percent change in GLP-1 was positively associated with percent change in total cholesterol.

The present study only investigated a homogenous population of young Newfoundland men aged 19-29. Future overfeeding studies observing larger-scale populations are needed to fully elucidate the role of ghrelin and GLP-1 during a positive energy challenge. Specifically populations including females and subjects of varying ethnic groups and age ranges are needed for further investigations. Also as mentioned, there are over 20 gut hormones secreted from the gastrointestinal tract [11]. However due to various limitations, only the functionally related hormones of ghrelin and GLP-1 were assessed in this study.

Overall, our results showed that circulating GLP-1 and ghrelin increased in a cohort of young Newfoundland men due to a 7-day positive energy challenge regardless of adiposity status. We propose the increase in GLP-1 is likely a protective role, counteracting the energy surplus. We also theorize that the rise in ghrelin could

potentially be attempting to offset the rising insulin resistance present during the overfeeding protocol. Hence the gut hormones ghrelin, and GLP-1 are involved in the regulation of body composition and lipid/glucose metabolism.

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