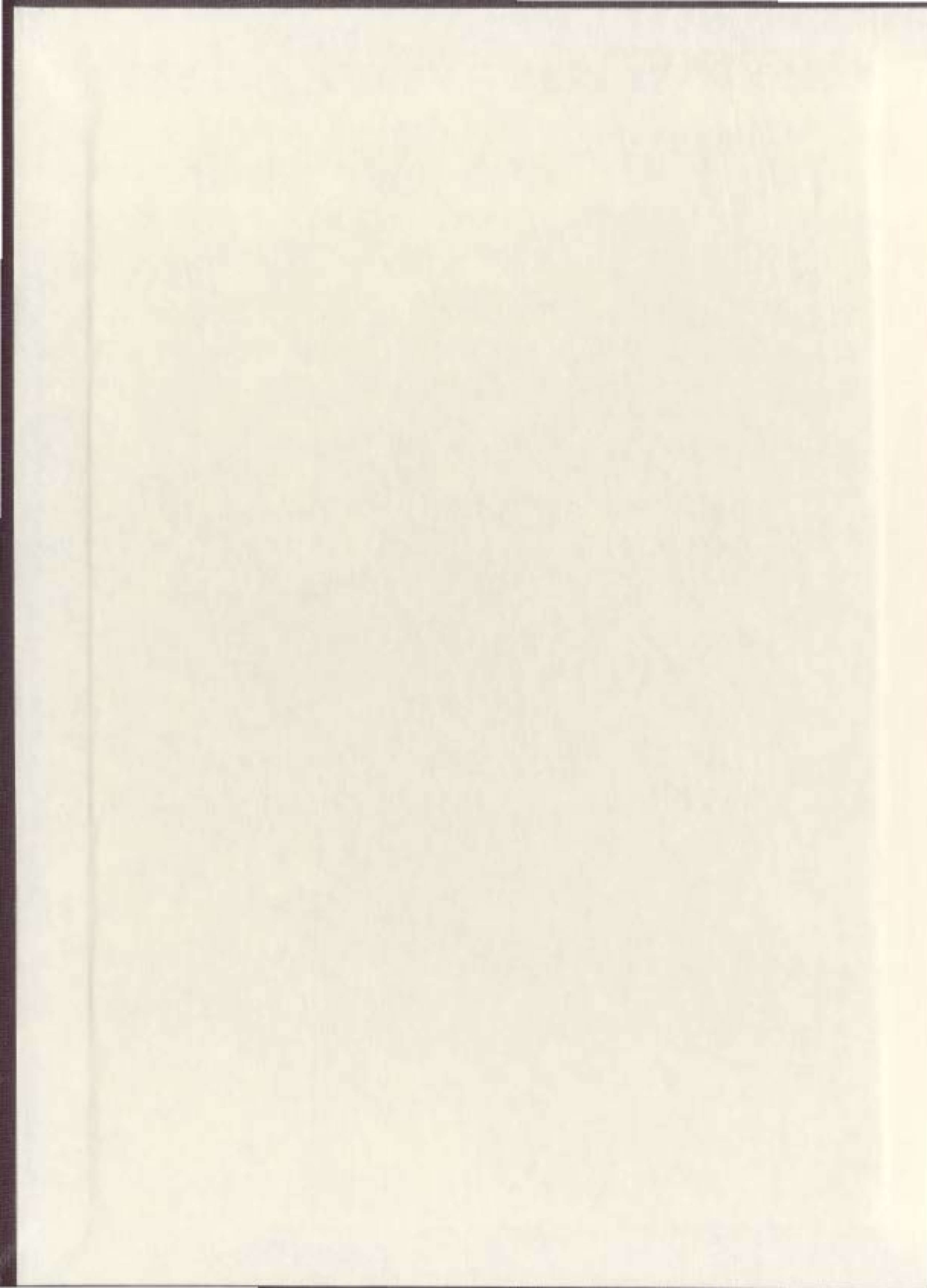
AN INVESTIGATION INTO THE UNDERLYING GENETIC DETERMINATES IN OBESITY USING THE CANDIDATE-GENE ASSOCIATION APPROACH AND MICROARRAY TECHNOLOGY

GLYNN R. MARTIN



Title Page

An investigation into the underlying genetic determinates in obesity using the candidate-gene association approach and microarray technology

by

Glynn R. Martin

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Abstract

Introduction: (1) Ghrelin is a peptide and has been suggested involved in energy metabolism. (2) Physical activity plays an important role in the regulation of body fat. Generally, the body composition of obese people tend to be resistant to negative energy balance. Little is known, however, with respect to the molecular mechanism and basis underlying the differences of responsiveness between obese and non-obese humans. **Methods:** This study consists of two parts: (1) Genetic association of 3 single nucleotide polymorphisms (SNPs) in the ghrelin precursor gene (*GHRL*) with obesity phenotypes in the NL population (1182 subjects); (2) A global gene expression study of abdominal subcutaneous adipose tissue (ASAT) and comparison between lean and obese young men (10 subjects) in response to a 7-day aerobic exercise protocol using whole genome mRNA microarrays. **Results**: No significant association of any of the variant sites and body compositions or serum lipids was found in allele, genotype and haplotype analyses. In microarray experiment, the discoveries include, and to our knowledge the first time reported the differential regulations: 1) 6 inflammatory-related genes (*SMPD3, CERK, ASAH2*, *ST3GAL5, IL1RII,* and *AGTR1*) within obese ASAT; 2) the genes in the lipolytic pathway (*PRKACA, SLC27A6, ADCY6, ADCYAP1, PPP1CB, DGAT2,* and *VLDLR*); 3) the genes in the protein tyrosine phosphatase (PTP) pathway (*PTPN11, PTPRD,* and *PTPN3*) related to insulin sensitivity; 4) *PPARA* and *PPRC1* that may favourably control energy metabolism in lean individuals, while not in obese. **Conclusion**: (1) The SNPs investigated within *GHRL* were not significantly associated with the variations of body composition and lipids in the NL population; (2) The genome-wide mRNA microarray

expression study revealed many major differences of ASAT between obese and non-obese at the molecular level, with respect to the regulation of inflammation, lipolysis and insulin sensitivity.

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First and foremost, I would like to thank Dr. Guang Sun for giving me an opportunity to work on such an interesting study. I appreciate the help and guidance he has given, as it has made my experience in the lab enjoyable. I would also like to thank Hongwei Zhang for all of her assistance in the lab, as well as other members in the lab for their advice and assistance. I would like to thank all volunteers who participated in the association study, and especially those in the exercise study. Without the willingness of these volunteers, both studies could not have been completed. I would also like to recognize all individuals who have contributed to data collection in the association study, and to Yang Zhang and Dax Rumsey for initially getting the exercise study off of the ground. Additionally, I would like to thank Drs. David Pace, Donald Fitzpatrick, and Wayne Gulliver for volunteering their time to perform the adipose tissue biopsies. Also, I would like to thank Lise Petrie for volunteering her time to perform VO_2 max tests on our exercise volunteers. Finally, I would like to recognize the Canadian Institute for Health Research (CIHR) for providing my funding through a CIHR Canada Graduate Masters Scholarship.

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List of Abbreviations

ACE............................angiotensin converting enzyme
ACO2..........................mitochondrial aconitase 2
ADCY6........................adenylate cyclase 6
ADCYAP1....................adenylate cyclase activating polypeptide 1
AGRP..........................agouti related protein
AGTL..........................adipose triglyceride lipase
AGTR1........................angiotensin II receptor, type 1
ANOVA.......................analysis of variance
APOAV....................... apolipoprotein A-V
APOE..........................apolipoprotein E
ASAH2........................N-acylsphingosine amidohydrolase 2
ATP............................adenosine triphosphate
BAT............................brown adipose tissue
%BF............................body fat percentage
BMI ....body mass index
C1P.............................ceramide-1-phosphate
CAPK..........................ceramide-activated protein kinase
CAPP..........................ceramide-activated protein phosphatase
CERK..........................ceramide kinase
CRP.............................C-reactive protein
DG..............................diglyceride
DGAT2........................diacylglycerol O-acyltransferase homolog 2/acyl-CoA:diacylglycerol acyltransferase 2
DXA............................dual energy X-ray absorptiometry
FDR............................false discovery rate
GH..............................growth hormone
GHRH.........................growth hormone releasing hormone
GHRL..........................ghrelin precursor gene
GHSR..........................growth hormone secretagogue receptor gene
GLUT4........................glucose transporter 4
GOTM.........................The Gene Ontology Tree Machine
HDL-C.........................high-density lipoprotein-cholesterol
HUGO.........................The Human Genome Organization
HSL............................hormone sensitive lipase
IL-1.............................interleukin-1
IL-6.............................interleukin-6
IL1R1..........................interleukin-1 receptor, type 1
JNK............................Jun kinase
LDL-C.........................low-density lipoprotein-cholesterol
%LF............................lower body (Legs) fat percentage
MAF...........................minor allele frequency

MAPK..........................mitogen-activated protein kinase
MGB...........................minor groove binder
NEFA..........................non-esterified fatty acids
NFQ...........................nonfluorescent quencher
NPY...........................neuropeptide Y
PCR...........................polymerase chain reaction
PKA...........................cAMP-dependent protein kinase
PKCζ..........................protein kinase Cζ
PP1...........................protein phosphatase 1
PPARγ2........................ peroxisome proliferator-activated receptor-gamma 2
PPARA.........................peroxisome proliferative activated receptor, alpha
PPARGC1A......................peroxisome proliferative activated receptor, gamma, co-activator 1 (also known as PGC1-alpha)
PPP1CB........................protein phosphatase 1, catalytic subunit, β isoform
PPRC1......................... peroxisome proliferative activated receptor, gamma, coactivator-related 1
PRKACA........................protein kinase, cAMP-dependent, catalytic, alpha
PTP...........................protein tyrosine phosphatase
PTP1B.........................protein tyrosine phosphatase 1 beta
PTPN3.........................protein tyrosine phosphatase, non-receptor type 3
PTPN11........................ protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1) (also known as SHP2)
PTPRD.........................protein tyrosine phosphatase, receptor type D
SDS........................... sequence detection system
SLC27A6....................... solute carrier family 27 (fatty acid transporter), member 6
SMPD3.........................sphingomyelin phosphodiesterase 3/neutral sphingomyelinase2
SNP...........................single nucleotide polymorphism
sPLS2.........................secretory phospholipase A2
ST3GAL5.......................ST3 β-galactoside α-2,3-sialyltransferase 5
SS............................somatostatin
TCA...........................tricarboxylic acid
%TF...........................trunk fat percentage
TG............................triglyceride
TNF-α.........................tumor necrosis factor
VLDLR.........................very low-density lipoprotein receptor
VO_2 max....................maximum oxygen consumption
WAT...........................white adipose tissue
WHO...........................World Health Organization

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Chapter 1: Introduction

1.1: The prevalence and consequences of obesity

Obesity, a condition of excessive body fat, is one of the most serious public health problems worldwide affecting millions of people (Zimmermann-Belsing and Feldt-Rasmussen, 2004). Canada, in particular, has recently experienced a major epidemic of obesity, with its prevalence more than doubling between 1985 and 1998 (Katzmarzyk, 2002). Moreover, Newfoundland and Labrador has one of the highest rates of obesity in Canada (Canadian Institute for Health Information, 2004). The state of being obese increases an individual's risk for adverse health problems, such as type II diabetes, cardiovascular disease, hypertension, stroke, dyslipidemia, osteoarthritis, and certain types of cancer (Burton *et al.*, 1985;Visscher and Seidell, 2001).

1.2: Definitions of obesity

Body composition is most commonly assessed using the body mass index (BMI), which is defined as weight, in kilograms, divided by the square of height, in meters (kg/m^2). According to the WHO (World Health Organization, 1995), overweight is defined as a BMI of over 25 kg/m^2, and obese as over 30 kg/m^2. However, it is the amount of body fat, rather than the amount of excess body weight, that determines the health risks of obesity (World Health Organization, 1998). BMI is not a direct measure of adiposity nor does it indicate body fat distribution. Furthermore, BMI does not differentiate people with different body sizes, thus, a person with a small frame may be

considered as having a healthy body composition when actually they have an unhealthy level of body fat. Consequently, obesity is more accurately defined according to body fat percentage. According to Bray (2003) obesity classification based on body fat percentage differs between ethnic groups and between age groups within these ethnic groups. Obesity for white females aged 20-39 years is classified as ≥ 39% body fat, for those aged 40-59 years, it is classified as ≥ 41%, and for 60-79 year olds, it is ≥ 43%. Obesity for white males aged 20-39 years is classified as ≥ 26% body fat, for those aged 40-59 years, it is classified as ≥ 29%, and for 60-79 year olds, it is ≥ 31% (Bray, 2003).

1.3: Etiology of obesity

The increased prevalence of obesity is largely due to the increased consumption of more energy-dense, nutrient-poor foods containing high levels of sugar and saturated fats in combination with decreased physical activity (World Health Organization, 2003). It has been known, however, for nearly 30 years that genetics plays a role in obesity (Feinleib *et al.*, 1977). It has also become apparent that obesity aggregates in families (Rice et al, 1999), and it is estimated that the heritability of fatness in Caucasian families is 46-60% (Katzmarzyk *et al.*, 2000). Animal studies have shown that the genetic backgrounds of different strains of rodents have a strong effect on the predisposition to obesity (Qiu *et al.*, 2001). Additionally, there are large variations in the extent to which an individual alters energy metabolism and gains or loses weight (Poehlman *et al.*, 1986;Bouchard *et al.*, 1990;Levine *et al.*, 1999).

Obesity that is caused by a single-gene mutation, such as the leptin and leptin receptor genes, are rare in humans and not likely responsible for the common form of

obesity (Comuzzie *et al.*, 2001). It is widely accepted that the common form of obesity is a complex disease that is influenced by both multiple genetic and environmental factors, as well as their interactions, which results in a significant degree of variation in expression across populations. As of October 2004, more than 600 genes, markers, and chromosomal regions have been associated or linked with obesity phenotypes (Perusse *et al.*, 2005). To better understand the pathogenesis of polygenic obesity, however, it is imperative to identify novel, and confirm suspected, underlying causative genes.

1.4: Approaches to identify obesity genes

A common approach employed to identify underlying genes of complex diseases, such as obesity, is through genetic association analysis. Association studies offer a powerful means of identifying genetic variants that influence susceptibility to complex diseases (Risch and Merikangas, 1996). In their simplest form, they compare the frequency of alleles or genotypes of a particular variant between disease cases and controls (Hirschhorn and Daly, 2005). Association studies have been successful in identifying many genetic risk factors for common diseases (Lohmueller *et al.*, 2003), such as apolipoprotein E (APOE) for the age of onset and risk of Alzheimer's disease (Strittmatter and Roses, 1996), peroxisome proliferator-activated receptor-gamma 2 (PPARγ2) for type 2 diabetes (Deeb *et al.*, 1998), and apolipoprotein A-V (APOAV) for hypertriglyceridemia (Pennacchio *et al.*, 2001). This approach, however, has been questioned due to non-replication of results as well as limitations in the ability to include all possible causative genes and polymorphisms (Tabor *et al.*, 2002). Explanations for

lack of reproducibility usually include small sample size, random error, poorly matched control groups, over-interpretation of data, and positive publication bias (Cardon and Bell, 2001). A good study design, however, can overcome all of these problems (Colhoun *et al.*, 2003).

Association studies are classified as either genome-wide or candidate-gene association studies (Hirschhorn and Daly, 2005). Genome-wide association studies employ genotyping a dense set of single nucleotide polymorphisms (SNPs) across the genome to determine causal genetic variants. This approach is a powerful method for identifying genes involved in common diseases, such as obesity, since no assumptions are made about their genomic location or their function. However, the major obstacles facing the genome-wide approach are the enormous associated cost and the issue of type one errors (false positives) when performing multiple tests. Candidate-gene studies, on the other hand, rely on generating hypotheses about, and identifying candidate genes that might have a role in, the etiology of the disease (Tabor *et al.*, 2002). This approach entails genotyping genetic variants, such as SNPs, in or near causative genes in a population and determining whether there is an association between those variants and the disease phenotype.

A more novel approach used to identify underlying causative genes in obesity, and other complex diseases, is microarray technology (Bell *et al.*, 2005). A commonly used platform for high-density microarrays utilizes robotic "spotting" of DNA molecules. These spotted arrays are commonly known as "cDNA microarrays", yet clones, PCR products, or oligonucleotides can all be spotted. Microarrays can provide simultaneous information on mRNA expression from thousands of genes in any tissue of interest, on a

single chip. Consequently, a more complete understanding of gene function, regulation, and the simultaneous interactions among thousands of genes is obtainable (Quackenbush, 2001). Through sophisticated analysis using bioinformatic tools, including gene ontology, cluster analysis, and systematic pathway analysis between diseased and healthy human tissues, we can narrow down the potential disease causing genes of the whole human genome to several hundred or even less based on their differential expression.

In the present study, both the candidate-gene association approach and microarray technology were employed to identify underlying causative genes of obesity, in an attempt to increase our knowledge of the mechanisms that underlie this condition.

1.5: Candidate-gene association approach – The ghrelin gene

Growth hormone (GH), which is secreted from the anterior pituitary, promotes protein building in all cells, increases use of fatty acids for energy, and reduces use of carbohydrates (Mosby's Medical Encyclopedia, 1997). GH release is stimulated by hypothalamic growth hormone releasing hormone (GHRH) and is inhibited by somatostatin (SS). Recent studies, however, have indicated that another mechanism exists for the regulation of GH release. An endogenous ligand, known as ghrelin, was shown to have specificity for the growth hormone secretagogue receptor (GHSR) subtype 1a (Howard *et al.*, 1996;Kojima *et al.*, 1999), a G-protein-coupled receptor, which stimulates the release of GH (Takaya *et al.*, 2000;Peino *et al.*, 2000;Arvat *et al.*, 2000). SNPs and haplotypes within the *GHSR* gene are linked and associated with obesity in humans (Baessler *et al.*, 2005). The ghrelin receptor, as demonstrated by *in situ*

hybridization analyses, is expressed in the hypothalamus, pituitary, and hippocampus (Guan *et al.*, 1997).

The human ghrelin gene (**Figure 1.1**), also known as the ghrelin precursor gene (*GHRL*), is encoded by 4 exons spanning 4.1 kb of genomic DNA chromosome 3p26-25 (Wajnrajch *et al.*, 2000). The mature ghrelin product arises following modification of preproghrelin, which consists of 117 amino acids. Amino acids 1-23 and 52-117 of preproghrelin are cleaved leaving the 28-amino acid ghrelin peptide, which is encoded by parts of exons 1 and 2. Furthermore, ghrelin has a post-translational serine-3 residue n-octanoylation, which is essential for its activity (**Figure 1.2**).

The major source of circulating ghrelin is from the stomach (Kojima *et al.*, 1999), where ghrelin is produced in X/A-like cells, a distinct cell type found in the submucosal layer (Date *et al.*, 2000). Ghrelin, however, is also expressed in other tissues such as the hypothalamus, pituitary, small and large intestines, lung, placenta, and kidney (Kojima *et al.*, 1999;Mori *et al.*, 2000;Korbonits *et al.*, 2001;Ariyasu *et al.*, 2001;Kojima *et al.*, 2001a).

Recent studies have shown that ghrelin specifically stimulates GH release (Kojima *et al.*, 1999;Peino *et al.*, 2000) and that ghrelin is more potent for GH release than GHRH, on a per mole basis (Takaya *et al.*, 2000). Aside from this role, however, ghrelin has also been implicated in food intake and control of energy balance. It has been demonstrated that intravenous ghrelin infusion in humans stimulates food intake (Wren *et al.*, 2001), and that central or peripheral administration of ghrelin in rodents stimulates food intake, reduces fat utilization and increases body weight (Wren *et al.*, 2000;Tschop *et al.*, 2000;Shintani *et al.*, 2001;Nakazato *et al.*, 2001). Studies have also demonstrated that

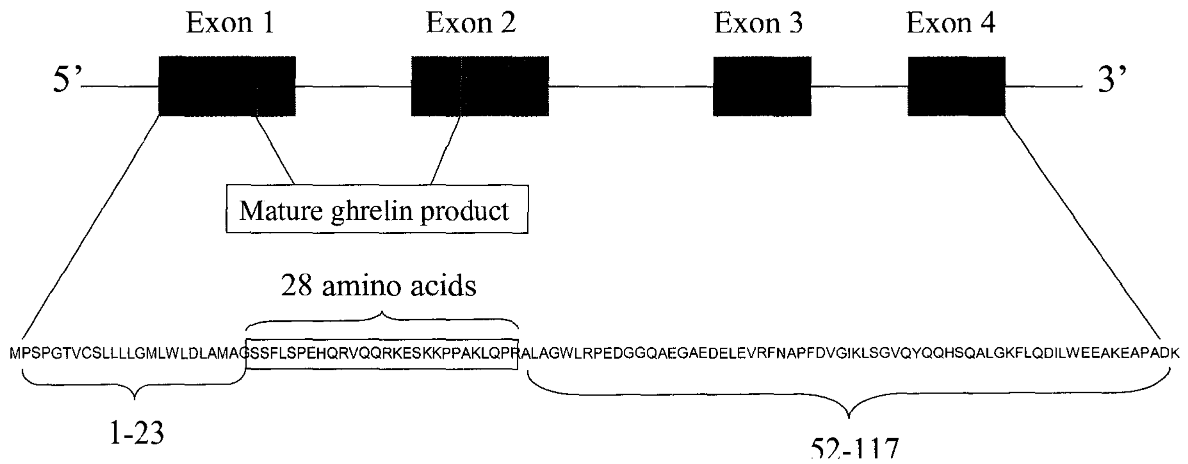

Figure 1.1 Schematic diagram of the 4 exons of the human ghrelin gene showing the encoded 117 amino acid sequence of preproghrelin, along with the sequence of mature ghrelin peptide highlighted in yellow.

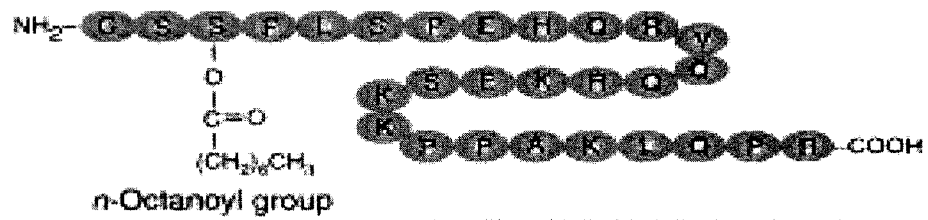

Figure 1.2 The structure of the 28-amino acid mature ghrelin peptide, with its Ser3 residue modified by n-octanoic acid (Kojima *et al.*, 2001b).

part of ghrelin's orexigenic effect stems from its stimulatory action on genes encoding the appetite stimulators agouti-related protein (AGRP) and neuropeptide Y (NPY), which are found mainly in the arcuate nucleus of the hypothalamus (Kamegai *et al.*, 2000;Nakazato *et al.*, 2001).

Based on previous literature indicating ghrelin's strong orexigenic action, it would seem plausible that elevated levels of ghrelin might be a contributing factor to obesity in humans. Moreover, the hypothesis that ghrelin plays a physiological role in meal initiation in humans has been demonstrated by the rise of circulating ghrelin levels with fasting and its decrease after food intake in humans (Cummings *et al.*, 2001). Yet, contrary to this hypothesis, it has been reported that plasma ghrelin levels are decreased in obese Caucasians as compared with lean Caucasians (Tschop *et al.*, 2001), and that weight loss increases circulating levels of ghrelin in humans (Hansen *et al.*, 2002). Thus, recent evident suggests that the regulation of the entire ghrelin system is altered in obesity (Marzullo *et al.*, 2004). It should be noted, however, that current methods can only measure total ghrelin levels, that is, ghrelin's active, n-octanoylated form cannot be distinguished from its inactive form.

Since the gene encoding ghrelin and its overall genomic structure (Wajnrajch *et al.*, 2000) has previously been characterized, it is possible to perform genomic screening of the *GHRL* gene within certain populations. Genetic polymorphisms within the *GHRL* gene, even if they do not lead to an amino acid change or they are not in a coding region, may still affect gene function by altering the stability, splicing, or localization of the mRNA (Cartegni *et al.*, 2002). Thus, in effect, the expression of the ghrelin protein may be altered, which may, in turn, alter GH secretion and/or control of food intake and

energy balance, and thus have importance in the etiology of obesity. The *GHRL* gene appears in the 2004 human obesity gene map (Perusse *et al.*, 2005), however, the roles of genetic variations within this gene on body compositions and serum lipids are still not clear in humans. Our study investigated three SNPs within the *GHRL* gene to determine their relationship with body fat percentage (%BF), trunk fat percentage (%TF), lower body (legs) fat percentage (%LF), BMI, and serum lipids in a healthy population.

1.6: Microarray technology approach – Global gene expression responses to acute aerobic exercise

When studying the underlying causative genes of obesity, it is of importance to study human tissue that plays a major role in energy homeostasis. Adipose tissue is an active participant in whole body energy homeostasis (Rajala and Scherer, 2003), and functional adipocytes are critical in physiological and pathophysiological conditions (Nadler and Attie, 2001).

1.6.1: Tissue of interest: adipose

Adipose tissue is the largest energy reservoir in the body (Fruhbeck *et al.*, 2001). It is found in mammals in two forms: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT makes up the majority of adipose tissue in the body (Albright and Stern, 1998), and it is the main focus here.

The primary role of adipocytes is to synthesize and store lipids when nutrients are abundant, and release fatty acids when nutrients are needed (Spiegelman and Flier, 1996). Excessive adipose tissue, however, accumulates when caloric intake continues to exceed

expenditure. Under these conditions, adipocytes swell, to as much as 6 times their minimum size, and then begin to multiply, from 40 billion (average adult) to 100 billion (Underwood and Adler, 2004). It is also known that excessive adipose tissue increases one's risk for numerous medical conditions such as coronary artery disease, hypertension, dyslipidemias, type II diabetes, and cancer (Rajala and Scherer, 2003).

Adipose tissue is a heterogeneous organ, whereby it is composed of mature adipocytes as well as several other cell types in the stromavascular fraction, which include preadipocytes, endothelial cells, and macrophages (Clement *et al.*, 2004). The biological function of WAT is not simply the storage of lipids, as once believed. The tissue is now recognized as a highly dynamic organ, with involvement in metabolic regulation and physiological homeostasis (Trayhurn and Beattie, 2001). The paradigm shift occurred upon the discovery of leptin, a cytokine-like hormone secreted from adipocytes that established adipocytes as endocrine cells (Zhang *et al.*, 1994). Since then, numerous other adipokines, or protein signals and factors synthesized and secreted by adipocytes, have been discovered. The classification of these adipokines by functional role include appetite and energy balance, immunity, insulin sensitivity, angiogenesis, inflammation and acute-phase response, blood pressure, lipid metabolism, and haemostasis (Trayhurn and Wood, 2004).

There are various approaches to studying obesity. One of these approaches is to introduce an intervention that influences energy metabolism within the human body, thus influencing the expression and regulation of genes, and possibly providing insight into the mechanisms that underlie obesity. Exercise, for instance, exerts an immediate and

substantial increase in energy expenditure, which causes the body to establish adaptive mechanisms to meet the fuel demands of the muscle (Pagano *et al.*, 1999).

1.6.2: Exercise as an intervention

During physical activity, to meet the fuel demand of the body, excess non-esterified fatty acids (NEFA) are released through an increase in lipolysis of the major triglyceride (TG) store within the body, the adipose tissue (Stich and Berlan, 2004). Once NEFA are released, they initially enter the interstitial space, and then ultimately into circulation where they are delivered into the skeletal muscle, and then the mitochondria before being oxidized. The entry of fatty acids into the skeletal muscle, however, is a complex process involving a series of protein carriers, such as plasma membrane fatty acid-binding protein, fatty acid translocase, and fatty acid transport protein, to facilitate fatty acid entry into the cells and solubility within the aqueous cytosol (Horowitz, 2003).

The different adipose tissue depots in the body have varying lipolytic activity (Martin and Jensen, 1991). During exercise, for instance, most fatty acids delivered to the systemic circulation are derived from subcutaneous adipose tissue (Horowitz, 2003). However, it is the subcutaneous adipose tissue of the upper body, rather than lower body, that has the greater lipolytic rate during exercise (Arner *et al.*, 1990). Consequently, the majority of the plasma fatty acids available to the working muscle most likely are derived from abdominal subcutaneous fat (Horowitz, 2003). Furthermore, the lipolytic response to exercise differs between obese and lean men. It was demonstrated that the lipolytic response to exercise decreases with increasing adiposity, which limits the availability of plasma fatty acids as a fuel during exercise for obese men, in comparison with lean men

(Mittendorfer *et al.*, 2004). However, the underlying molecular and genetic mechanisms responsible for this difference between lean and obese men remain unknown. The responses, especially the possible differences between obese and non-obese individuals, to all known and possibly unknown adipokines to exercise are of great interest due to their interactions with other tissues, organs, as well as the central nervous system.

Exercise is also of great importance when studying obesity, since it has been shown that regular aerobic physical activity contributes to the cure of metabolic complications associated with this condition (Richterova *et al.*, 2004). Physical activity may also modify the effect of genes on the regulation of body weight (Meirhaeghe *et al.*, 1999), however, little is known about the global genetic differences between lean or obese individuals. The present study investigated the differential global mRNA expression profiles of abdominal subcutaneous adipose tissue of lean and obese males, in response to short-term aerobic exercise, using long oligonucleotide whole human genome microarrays. We decided to use long oligonucleotide microarrays because these arrays are known to have a superior ability to distinguish closely related sequences, and in fact a single long oligonucleotide is suitable to detect single-copy genes in human cells (Hughes *et al.*, 2001).

Chapter 2: Materials and Methods

2a: Candidate-gene association study

2a.1: Subjects

Subjects were recruited from the province of Newfoundland and Labrador (NL), Canada, by means of poster distribution and person-to-person contact. Subjects who met the following criteria were eligible to participate in the study: (1) 19 to 60 years of age, (2) at least third generation Newfoundlander, (3) healthy, without any serious metabolic, cardiovascular or endocrine diseases, and (4) not pregnant at the time of the study. One thousand one hundred and eighty two subjects participated in the study. All subjects provided written consent and completed questionnaires pertaining to their demographic background, health status, and family history. The Research Ethics Board of the Faculty of Medicine, Memorial University of Newfoundland, approved the study.

2a.2: Study Design

This study was a candidate-gene association study on a large NL based cohort utilizing a combination of case-control and haplotype analyses. All measurements and blood samplings were performed in the morning following a 12-hour fast.

2a.2.1: Blood samples

For plasma isolation, blood was collected in evacuated tubes containing K_3EDTA as an anti-coagulant. Tubes were immediately centrifuged at 4 °C for 15 minutes at 1300g. Plasma was removed and stored at -80 °C until further analysis. For serum isolation, blood was collected in tubes containing a clot activator. Tubes were allowed to sit at room temperature for 20 minutes, followed by centrifugation at 25 °C for 10 minutes at 3500 rpm. Serum was removed and stored at -80 °C until further analysis.

2a.2.2: Body composition measurements

Subjects were weighed, and their heights were measured, wearing standardized light gowns, without shoes, on a platform manual scale balance (Health o meter Inc., Bridgeview, IL, USA). Whole-body composition measurements, including %BF, %TF, %LF, lean body mass, and bone mineral densities were determined using DXA Lunar Prodigy (GE Medical Systems, Madison, WI, USA). Measurements were performed on subjects, following the removal of all accessories containing metal, while lying in a supine position. Software version 4.0 was used for analysis. Classification for lean and obese were as follows: obese for females aged 20-39 years was classified as ≥ 39% body fat (lean as < 33%) and aged 40-59 years was classified as ≥ 41% (lean as < 35%); obese for males aged 20-39 years was classified as ≥ 26% body fat (lean as < 21%) and aged 40-59 years was classified as ≥ 29% (lean as < 23%) (Bray, 2003).

2a.2.3: Biochemical measurements

Serum concentrations of glucose, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TGs) were measured using Synchron reagents performed on an Lx20 (Beckman Coulter, Inc., CA, USA) by Dr. Edward Randell in the discipline of Laboratory Medicine. Serum insulin levels were measured on an Immulite immunoassay analyzer (DPC, CA, USA). Low-density lipoprotein-cholesterol (LDL-C) was determined using the following equation: [total cholesterol – HDL-C – (TGs/ 2.2)], and risk factor was determined using the equation: (total cholesterol/HDL-C).

2a.3: Genomic DNA extraction and genotyping

Genomic DNA was extracted from whole blood (5ml) using the Wizard® Genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. This kit is designed for the isolation of DNA from white blood cells. Briefly, the protocol was comprised of four main steps. Firstly, red blood cells were lysed in the Cell Lysis Solution and removed. Secondly, white blood cells and their nuclei were lysed in the Nuclei Lysis Solution. Thirdly, cellular proteins were removed by a salt precipitation step, leaving genomic DNA in solution. Finally, the genomic DNA was concentrated and desalted by an isopropanol precipitation.

The three SNPs in the *GHRL* gene that were investigated were an A/G polymorphism (public ID, dbSNP: rs35684; public location: chromosome 3 - 10,301,686) found in exon 1 (mis-sense mutation), a C/T intronic polymorphism (public ID, dbSNP: rs2075356; public location: chromosome 3 - 10,303,809) located intron 2, and an A/G intergenic polymorphism (public ID, dbSNP: rs26311; public location: chromosome 3 -

10,307,926) found near the 3' untranslated region (UTR) (**Figure 2.1**). Since the ghrelin gene is small, three SNPs were sufficient to cover any signal across the gene. The SNPs were not chosen at random, they were chosen based on the following priority hierarchy: 1) minor allele frequency ≥ 0.05; 2) located in exon region; 3) located in exon-intron splice region; 4) located in intron; 5) located in the intergenic region.

Genotyping was performed using the TaqMan® validated SNP Genotyping Assays (Applied Biosystems, CA, USA) according to the manufacturer's protocol. Each assay required polymerase chain reaction (PCR) amplification and allelic discrimination plate read and analysis. PCR amplification required four components: 1) purified genomic DNA (20ng). 2) 20X SNP Genotyping Assay Mix (0.75μl), which contained sequence-specific forward and reverse primers to amplify the desired SNP and two TaqMan® minor groove binder (MGB) probes. Each probe had a reporter dye attached

to its 5' end and a nonfluorescent quencher (NFQ) to its 3' end (**Figure 2.2**). One probe was labelled with VIC dye and detected the Allele 1 sequence and the other probe was labelled with FAM dye and detected the Allele 2 sequence. 3) 2X TaqMan® Universal PCR Master Mix, No AmpErase® Uracil-N-Glycosylase (UNG) (7.5μl), which contained AmpliTaq Gold® DNA polymerase and other components required for the PCR reaction, such as deoxynucleotides and magnesium chloride. 4) DNAse, RNAse Free, distilled water (2.75μl). During the PCR reaction, which was performed on an Eppendorf Mastercycler [five steps: 1) 2 minutes at 50°C, 2) 10 minutes at 95 °C, 3) 15 seconds at 95 °C, 4) 1 minute at 60 °C, 5) 40 cycles of steps 3-4] each probe bound to a complementary sequence between the forward and reverse primers, and the proximity of

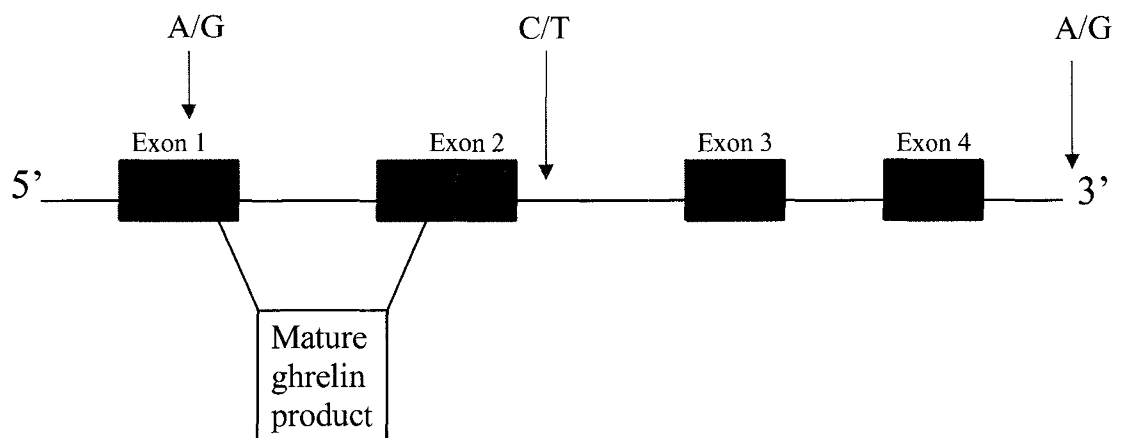

Figure 2.1 Approximate locations of the 3 SNPs investigated within the *GHRL* gene.

Figure 2.2 Illustration of the TaqMan minor groove binder probe with a reporter dye attached to its 5’ end and a nonfluorescent quencher (NFQ) attached to its 3’ end (adapted from ABI Assays-on-Demand Gene Expression Products Protocol).

the reporter dye to the quencher resulted in suppression of the reporter fluorescence. The AmpliTaq Gold® DNA polymerase only cleaved probes that were hybridized to the target, thereby separating the reporter dye from the quencher, resulting in an increase in fluorescence by the reporter. Following PCR amplification, allelic discrimination and analysis was performed using an ABI Prism 7000® sequence detection system (SDS). Briefly, the SDS software calculated the fluorescence measurements from the plate read and created a plot of the signals. If there was a substantial increase in the VIC dye fluorescence only, it indicated that the sample was homozygous for allele 1. If there was a substantial increase in the FAM dye fluorescence only, it indicated that the sample was homozygous for allele 2. And if there was a substantial increase in both fluorescent signals, it indicated heterozygosity for allele 1 and allele 2 (**Figure 2.3**).

To evaluate the quality of genotyping, 5% of the samples were randomly selected and re-genotyped. A total of 3,723 SNP genotyping assays (3 SNPs × 1,182 subjects + 5% verification) were completed.

2a.4: Statistical analysis

All statistical analyses were performed using SPSS software (version 11.5 for Windows), with significance set at $p<0.05$. Hardy-Weinberg equilibrium was tested using chi-square analysis. Chi-square analysis was also used to assess the allele differences between obese and non-obese subjects for each of the SNPs investigated. One-way genotype groups of SNPs and haplotype groups (combinations of the 3 SNPs ANOVA, corrected with Bonferroni *t*-test, was used to assess differences among investigated) with body composition (%BF, %TF, %LF, and BMI) in males and females. Analyses were

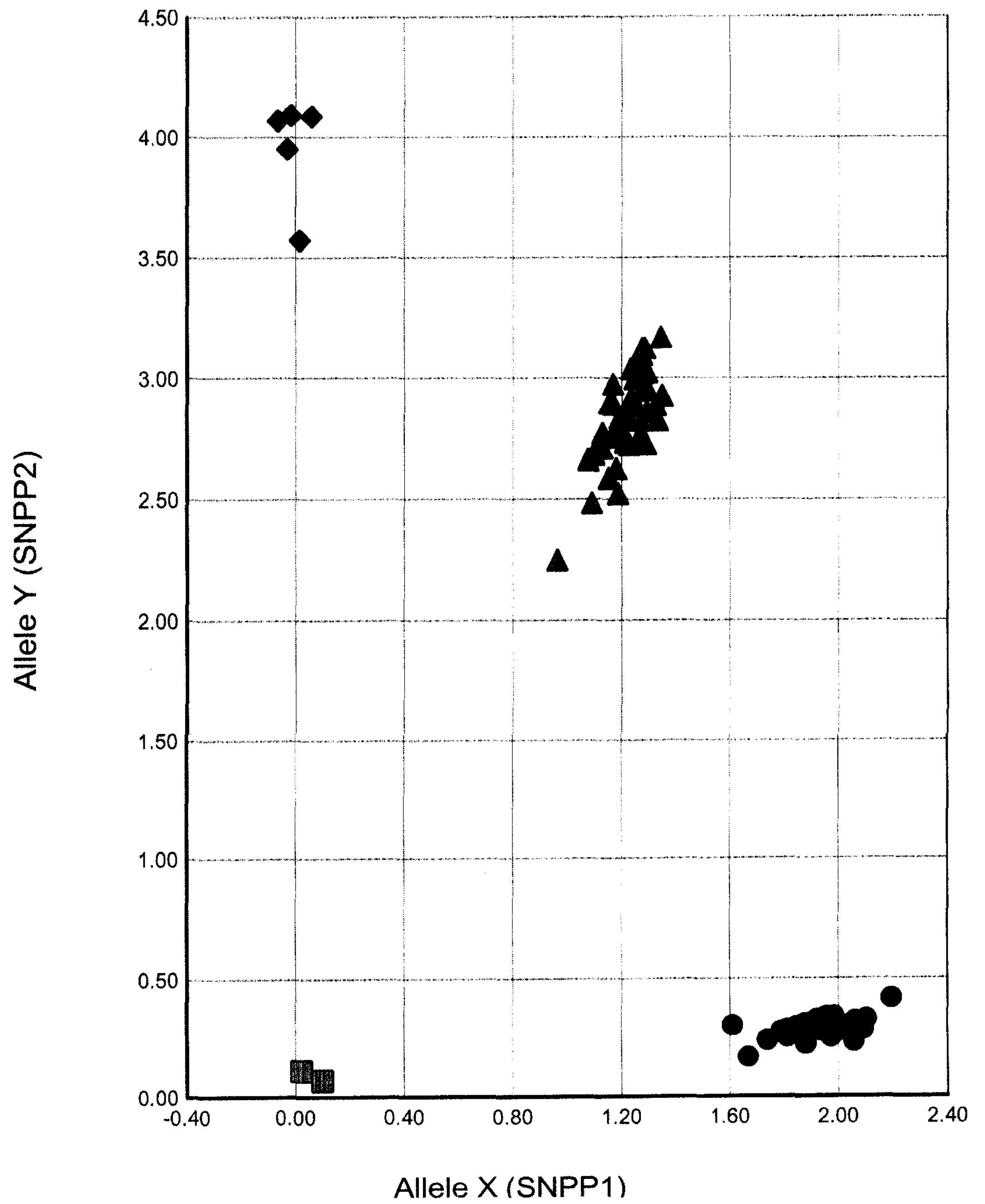

Figure 2.3 An example of an allelic discrimination showing 4 distinct groups: grey squares refer to non-template controls; blue diamonds refer to homozygosity for allele Y; green triangles refer to heterozygosity; red circles refer to homozygosity for allele X.

repeated after adjusting %BF, %TF, %LF, and BMI for and age. One-way ANOVA, corrected with Bonferroni *t*-test, was also used to assess differences among genotype groups and haplotype groups of SNPs with serum lipid parameters in males and females. Due to the skewed distributions, the serum lipid parameters were transformed using $\log_{10}$ prior to data analysis. Similarly, analyses were repeated after adjusting serum lipid parameters for age and %BF.

2b: Genome-wide expression study of adipose tissue in response to short-term aerobic exercise using microarray technology

2b.1: Subjects

Subjects were recruited from NL, Canada, by means of poster distribution and person-to-person contact. Subjects who met the following criteria were eligible to participate in the study: (1) male, (2) 20 to 27 years of age, (3) at least third generation Newfoundlander, (4) healthy, without any serious metabolic, cardiovascular or endocrine diseases, (5) not on medication for lipid metabolism, and (6) either classified, according to percent body fat, as lean ($< 21\%$) or as obese ($\geq 26\%$) (Bray, 2003). All subjects provided written consent, and the Research Ethics Board of the Faculty of Medicine, Memorial University of Newfoundland, approved the study.

2b.2: Study Design

This study employed a longitudinal design. All measurements (except VO_2 max), blood samplings, and adipose tissue samplings were performed twice: prior to exercise and the day following the exercise week (8th day).

2b.2.1: Blood samples

Refer to section 2a.2.1

2b.2.2: Body composition measurements

Refer to section 2a.3.1.

2b.2.3: Biochemical measurements

Refer to section 2a.3.2.

2b.2.4: Maximum oxygen consumption (VO_2 max) measurements

VO_2 max is the maximum volume of oxygen in millimetres consumed during exercise per kilogram of body weight per minute (ml/kg/min). The rational behind the determination of VO_2 max is that an individual's ability to undergo exercise for periods of time depends on their capacity to transport oxygen from the air into the mitochondria (Astrand, 1976). Oxygen consumption is linearly related to energy expenditure, which provides a good indication of one's aerobic capacity. In our study, VO_2 max was determined using the SensorMedics MMC Horizon System (Version 11.20) using a standard five-stage treadmill protocol. The five stages of the protocol were as follows: 1) 0-2 minutes at a pace of 5 km/h, with a 0 vertical incline; 2) 2-5 minutes at a pace of 8 km/h, with a 0 vertical incline; 3) 5-7 minutes at a pace of 10.5 km/h, with a 0 vertical incline; 4) 7-9 minutes at a pace of 10.5 km/h, with a 2.0 vertical incline; 5) every 2 minutes thereafter, vertical grade was increased by 1.0, while pace remained at 10.5 km/h. Test was terminated after subjects were exhausted or when they reached their theoretical maximum heart rate (220 minus their age).

2b.3: Exercise protocol

Subjects trained for 50 minutes, after reaching a heart rate that corresponded to 60% of their VO_2 max, for 7 consecutive days using a treadmill. Subjects were not restricted to a particular diet; they were to maintain their regular diet.

2b.4: Adipose tissue isolation from subjects

The "superwet liposuction technique", performed by surgeons, was used to obtain subcutaneous adipose tissue from the sub-umbilicus region of subjects before and the day after the exercise period, in the morning following a 12-hour fast. Briefly, the protocol entailed 5 steps: 1) anaesthetizing the sub-umbilicus region, using a combination of 10 cc of Xylocaine with adrenalin (1:1000), and 50 ml of 0.25% Marcaine in 250 cc of N/S; 2) inserting a cannula, fitted with a syringe to create negative pressure, into the subcutaneous fat; 3) removal of ~4 grams of fat; 4) draining of fluid from fat; 5) immediately flash freezing fat sample in liquid nitrogen. All samples were stored in liquid nitrogen until further analysis.

2b.5: Total RNA isolation from adipose tissue

Total RNA was extracted from adipose tissue (500 mg) using the RNeasy® Lipid Tissue Midi Kit (Qiagen, CA, USA) according to the manufacturer's protocol. This kit is designed for the optimal lysis of adipose tissue and the purification of high-quality total RNA molecules that are greater than 200 nucleotides in length. Thus, since RNAs less than 200 nucleotides are excluded, such as 5.8S rRNA, 5S rRNA, and tRNAs, this protocol allows for the enrichment of mRNA. Briefly, the protocol comprised six main steps. First, the adipose tissue was homogenized, using a rotor-stator homogenizer (Brinkmann Instruments, NY, USA), in QIAzol Lysis Reagent, which is a monophasic solution of phenol and guanidine thiocyanate that is designed to facilitate lysis of fatty tissues and inhibit RNases. Second, chloroform was used to separate the homogenate into aqueous and organic phases. RNA resided in the upper, aqueous phase, while DNA

remained in the interphase, and proteins remained in either the interphase or the organic phase. Third, the aqueous phase was extracted, and ethanol was added to allow appropriate binding conditions. Fourth, the sample was loaded onto the RNeasy Midi Spin Column. Total RNA adhered to the membrane, while phenol and other contaminants were washed away. Fifth, an on column DNase digestion was performed to remove possible DNA contamination. Lastly, high-quality RNA was eluted off the membrane using RNase-free water. RNA concentration and purity were determined using the Eppendorf BioPhotometer.

2b.6: Reference RNA

Universal Human Reference RNA (Stratagene, CA, USA) was used to serve as a reference for the microarray experiments. It contained equal, pooled quantities of total RNA from 10 different human cell lines, since RNA species differ in abundance between cell lines. The 10 human cell lines were as follows: 1) Adenocarcinoma, mammary gland; 2) Hepatoblastoma, liver; 3) Adenocarcinoma, cervix; 4) Embryonal carcinoma, testis; 5) Glioblastoma, brain; 6) Melanoma; 7) Liposarcoma; 8) Histiocytic lymphoma; macrophage; histocyte; 9) Lymphoblastic leukemia, T lymphoblast; 10) Plasmacytoma; myeloma; B lymphocyte.

2b.7: Assessment of total RNA integrity and purity

RNA integrity and purity was assessed prior to microarray assays using the RNA 6000 nanoassay on an Agilent Bioanalyzer 2100 Automated Analysis System (Agilent Technologies, CA, USA). Briefly, this technology uses electrokinetic forces to drive the

RNA sample through micro-channels, comprised of a sieving polymer and fluorescence dye, to perform the electrophoretic separations (**Figure 2.4**). The components were then detected by their fluorescence and translated into electropherograms, as well as gel-like images. Refer to Appendix 1 for the electropherograms and gel-like images of all RNA samples used.

2b.8: Microarray assays

Agilent's *in situ* synthesized 60-mer oligonucleotide microarrays, containing 44,290 genes and transcripts probes, printed on one single 1" x 3" glass slide, were used (Agilent Technologies, CA, USA). The protocol comprised 3 major components: (1) linear amplification (fluorescent cRNA synthesis), (2) hybridization and washing, and (3) scanning and quantifying the arrays.

2b.8.1: Linear amplification: Fluorescent cRNA synthesis

Agilent's Low RNA Input Fluorescent Linear Amplification kit was used to generate 100-fold fluorescent complimentary RNA. This procedure involved labelling the total RNA sample (300 ng) with either cyanine 3 (cy3) or cyanine 5 (cy5) (Perkin-Elmer, MA, USA) and the reference RNA sample with the opposite dye. To control for the differences between cy3 and cy5 signals, 2 arrays were competed per sample allowing for a dye swap. Briefly, a primer, containing poly dT and a T7 polymerase promoter, was annealed to the poly A RNA. Reverse transcription was then used to synthesize the first and second strands of cDNA. Next, cRNA was synthesized using T7 RNA polymerase, which also incorporated cy3 or cy5 labelled CTP (**Figure 2.5**).

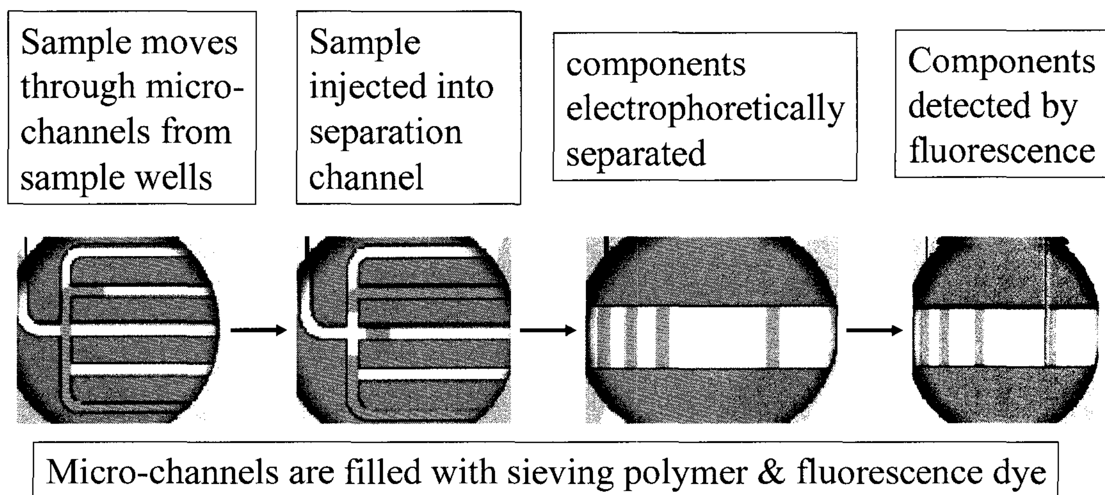

Figure 2.4 A schematic diagram showing the electrophoretic separation of an RNA sample within the micro-channels of the polymer chip (adapted from Agilent Technologies Lab-on-a-Chip Products Protocol).

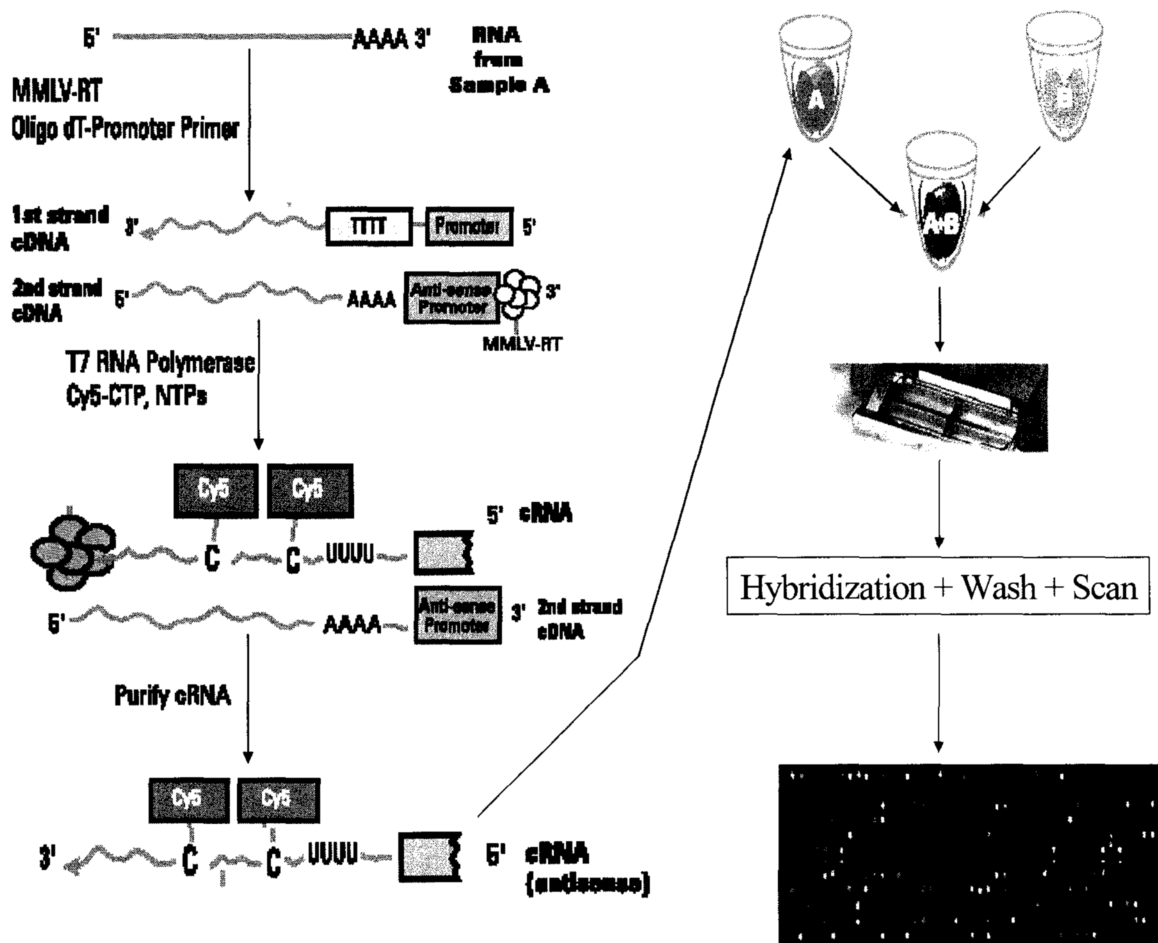

Figure 2.5 A schematic overview of Agilent's microarray protocol, including linear amplification (fluorescent cRNA synthesis), hybridization and washing, and scanning of the arrays (adapted from Agilent Technologies Low RNA Input Fluorescent Linear Amplification Kit Protocol).

2b.8.2: Hybridization and washing

Labelled sample (0.75 μg) and reference RNA (0.75 μg), along with control targets, were combined and then added to the gasket slide to which the microarray was placed on, creating a "sandwich slide" pair. The "sandwich slide" pair was then hybridized at 60°C for 17 hours at 4 rotations per minute in an Agilent hybridization oven (**Figure 2.5**). Following hybridization, each "sandwich slide" pair was disassembled and washed. The wash solution was a stabilization and drying solution that contained an ozone scavenging compound that was dissolved in acetonitrile.

2b.9.3: Scanning and quantifying the microarrays

Each microarray was scanned using ScanArray Express Microarray Scanner (Packard BioScience). One laser was set at 550 nm to excite cy3 and the other was set at 650 nm to excite cy5, while the laser power was set to 80% to avoid overexposure of features (**Figure 2.5**). The microarray images were quantified to obtain raw intensity measurements using the ImaGene software, version 6.0 (BioDiscovery, Inc.), using default parameters.

2b.9: Statistical analysis

Raw intensity measurements for each microarray underwent further data processing for subsequent data analysis using GeneSight Lite version 6.0 (BioDiscovery, Inc., CA, USA). The following parameters were used: (1) correction for background – background pixel intensity was automatically averaged over the local background and

subtracted from the original intensity value; (2) ratio of sample-to-reference – a ratio of the intensity value of the experimental sample with intensity value of Universal Human Reference RNA was taken; (3) omit multiple flagged spots – spots were automatically flagged and omitted if any spot had a mean signal that was not at least twice that of the background mean, if any spot was low-expressed or missing, or if any spot had poor quality (such as abnormal shape or donut shape); (4) $\log_2$ shift – all remaining values were transformed into log (base 2) scale; (5) normalization – all remaining values were normalized by dividing by the Z-score to eliminate differences in intensities between equal experiments due to external conditions. A mean value was calculated for each pair of processed intensity values (two arrays per sample), and was used in subsequent analyses.

The following criteria were used to determine any differentially expressed genes in pre-exercise samples when compared to post-exercise samples (pre/post) of lean and obese subjects: (1) 3 of the 5 samples must be in the same direction by 1.5 fold; (2) average of 5 samples must be 1.5 fold in that particular direction. These criteria ensured that a gene or transcript was not considered significant based on an extreme outlier. A fold change of 1.5 was chosen based on previous microarray literature. Only genes that met the above criteria were used for further analysis. Student paired *t*-tests were then performed on remaining genes, where $p \leq 0.01$ was taken to be statistically significant.

To help decipher which of the remaining genes were of interest, gene ontology analysis was completed. There are three categories of gene ontology: (1) biological process – refers to a biological objective to which the gene or gene product contributes; (2) molecular function – refers to the biochemical activity of a gene product; (3) cellular

component – refers to the place in the cell where a gene product is active (Ashburner *et al.*, 2000). The Gene Ontology Tree Machine (GOTM), which is a web-based software (http://genereg.ornl.gov/~gotm) for interpreting microarray data using gene ontology hierarchies, was used to statistically identify gene ontology terms with enriched, that is over-represented, genes from our list of differentially expressed genes. The software was able to identify over-represented genes by comparing our list of differentially expressed genes, with defined HUGO names, with the list of all genes, with defined HUGO names, present on each of the whole human genome microarrays. This type of analysis was completed separately for up- and down-regulated genes of lean and obese subjects.

Chapter 3: Results

3.1a: Candidate-gene association study

The physical and biochemical characteristics of the subjects are shown in **Table 3.1**. There were 1182 eligible subjects (946 females and 236 males). On average, the mean age of males was about 3 years less than females. Males were also taller, heavier and had significantly larger BMI than females. However, when DXA was used to evaluate adiposity percentages, males had about 12% less body fat, 8% less trunk fat, and 3% less lower body fat than females. The mean levels of fasting serum glucose, insulin, and triglycerides were significantly higher in males than females, while total cholesterol and HDL-C were significantly lower. Furthermore, risk factor for cardiovascular disease, as calculated by total cholesterol divided by HDL-C, was significantly higher in males than females.

The minor allele frequencies (MAFs) of the 3 SNPs investigated are presented in **Table 3.2**. The frequencies for alleles A and G of the SNP located in exon 1 were 0.73 and 0.27, frequencies for alleles C and T of the intron 2 SNP were 0.11 and 0.89, and frequencies for alleles A and G of the SNP located in the 3' intergenic region were 0.88 and 0.12, respectively. All SNPs investigated were in Hardy-Weinberg equilibrium according to Chi-Square analysis.

The genotype distributions of the 3 SNPs investigated in lean and obese males and females, based on %BF, are presented in **Table 3.3**. In males, the MAFs of the lean and obese groups for the SNP located in exon 1 were 0.19 and 0.26, the SNP located in

Table 3.1 Physical and biochemical characteristics of subjects in candidate-gene association study

	Females (n = 946)	Males (n = 236)
Age (yr)	43.0 ± 9.9 (19-62)[a]	39.8 ± 12.7 (19-61)
Weight (kg)	69.2 ± 13.8 (45.4-156.8)[a]	85.0 ± 14.3 (53.9-149.4)
Height (cm)	162.1 ± 5.8 (135.0-185)[a]	175.3 ± 6.7 (157-198)
BMI (kg/m^2)	26.3 ± 5.1 (17.0-54.3)[a]	27.7 ± 4.6 (18.6-50.4)
%BF[1]	37.3 ± 7.1 (4.6-60)[a]	25.6 ± 7.5 (6.0-48.0)
%TF[2]	38.4 ± 8.2 (3.9-64.3)[a]	30.3 ± 8.7 (4.7-53.1)
%LF[3]	35.6 ± 8.7 (5.6-57.2)[a]	32.5 ± 10.9 (6.8-50.7)
Total Cholesterol (mmol/L)	5.24 ± 1.0 (2.25-9.98)[b]	5.08 ± 1.09 (2.82-9.22)
LDL-C[4] (mmol/L)	3.11 ± 0.91 (1.02-7.61)	3.14 ± 0.92 (1.31-5.69)
HDL-C[5] (mmol/L)	1.58 ± 0.36 (0.79-3.25)[a]	1.26 ± 0.25 (0.65-2.21)
Glucose (mmol/L)	5.00 ± 0.83 (3.0-16.3)[a]	5.26 ± 1.02 (2.2-14.4)
Insulin (pmol/L)	73.7 ± 46.9 (15.4-720)[b]	78.9 ± 52.0 (17.1-363)
TGs[6] (mmol/L)	1.18 ± 0.74 (0.27-5.88)[a]	1.50 ± 1.02 (0.31-5.05)
Risk factor[7]	3.46 ± 0.93 (1.56-8.41)[a]	4.17 ± 1.08 (2.27-7.72)

Values are expressed as mean ± standard deviation (min. – max.); %BF[1] = body fat percentage; %TF[2] = trunk fat percentage; %LF[3] = lower body fat percentage; LDL-C[4] = low-density lipoprotein-cholesterol; HDL-C[5] = high-density lipoprotein-cholesterol; TGs[6] = triglycerides; risk factor[7] = total cholesterol/HDL-C. [a]p value < 0.001, [b]p value < 0.05 in the comparison between males and females using Student's *t*-test.

Table 3.2 Minor allele frequencies of SNPs in the *GHRL* gene

SNPs	[ξ]Reported for Caucasians	NL population
Exon 1 (A/G; rs35684)	0.18	0.27
Intron 2 (C/T; rs2075356)	0.08	0.89
Intergenic (A/G; rs26311)	0.07	0.12

All SNPs are in Hardy-Weinberg Equilibrium; [ξ]MyScience SNP genotyping search website (http://myscience.appliedbiosystems.com).

Table 3.3 Genotype distributions (percentages in parentheses) of three SNPs within *GHRL* in lean and obese (based on %BF) males and females

	Exon 1 SNP			Intron 2 SNP			Intergenic SNP		
	AA	AG	GG	CC	CT	TT	AA	AG	GG
Males*									
Lean (n=74-75)	47 (62.7)	26 (34.7)	1 (1.3)	1 (1.3)	12 (16.0)	62 (82.7)	60 (80.0)	14 (18.7)	1 (1.3)
Obese (n=94)	54 (57.4)	32 (34.0)	8 (8.5)	0 (0.0)	16 (17.0)	78 (83.0)	74 (78.7)	20 (21.3)	0 (0.0)
χ^2	2.069			0.070			0.000		
p	0.15			0.80			0.99		
Females$^{\varphi}$									
Lean (n=310-311)	174 (56.1)	115 (37.1)	21 (6.8)	5 (1.4)	64 (19.6)	242 (79.1)	240 (79.5)	68 (18.9)	3 (1.4)
Obese (n=329-330)	177 (53.6)	125 (37.9)	28 (8.5)	8 (2.4)	69 (21.0)	252 (76.6)	246 (74.5)	78 (23.6)	6 (1.8)
χ^2	0.727			0.306			0.868		
p	0.39			0.58			0.35		

*Males: lean = <21% (20-39 yrs) and <23% (40-59 yrs); obese = ≥26% (20-39 yrs) and ≥29% (40-59 yrs); $^{\varphi}$Females: lean = <33% (20-39 yrs) and <35% (40-59 yrs); obese = ≥39% (20-39 yrs) and ≥41% (40-59 yrs); Significance assessed using χ^2 test between lean and obese groups for each SNP.

intron 2 were 0.92 and 0.914, and the SNP located in the 3' intergenic region were 0.11 and 0.11, respectively. According to chi-squared analysis, there was no significant difference of allele frequencies between lean and obese groups, based on %BF, for any SNP investigated. The MAFs of the lean and obese females for the SNP located in exon 1 were 0.25 and 0.27, the SNP located in intron 2 were 0.88 and 0.87, and the SNP located in the 3' intergenic region were 0.12 and 0.14, respectively. According to chi-squared analysis, there was no significant difference of allele frequencies between lean and obese groups, based on %BF, for any SNP investigated. Similarly, there was no significant difference of allele frequencies between lean and obese groups, based %TF **(Table 3.4)**, %LF **(Table 3.5)**, or BMI **(Table 3.6)**, for males or females for any SNP investigated.

The genotype effect of the 3 SNPs investigated in males and females on body composition are presented in **Table 3.7**. Using one-way ANOVA, corrected by Bonferroni *t*-test, there was no significant difference among the 3 genotype groups for %BF, %TF, %LF, or BMI for the 3 SNPs investigated in males or females. Although statistically not significant, a trend appeared to exist toward higher body fat and the GG genotype of Exon 1 in males. Furthermore, because of possible confounding factors on body composition, all analyses were repeated after adjusting for age using linear regression. Similar results were obtained for all SNPs investigated in males and females.

The genotype effect of the 3 SNPs investigated in males and females on serum lipid parameters are presented in **Table 3.8**. Using one-way ANOVA, corrected by Bonferroni *t*-test, there was no significant difference among the 3 genotype groups for

Table 3.4 Genotype distributions (percentages in parentheses) of three SNPs within *GHRL* in lean and obese (based on %TF) males and females

	Exon 1 SNP			Intron 2 SNP			Intergenic SNP		
	AA	AG	GG	CC	CT	TT	AA	AG	GG
Males*									
Lean (n=41)	25 (61.0)	15 (36.6)	1 (2.4)	1 (2.4)	8 (19.5)	32 (78.0)	30 (73.2)	10 (24.4)	1 (2.4)
Obese (n=151-152)	85 (56.3)	54 (35.8)	12 (7.9)	0 (0.0)	22 (14.5)	130 (85.5)	126 (82.9)	26 (17.1)	0 (0.0)
χ^2	0.900			2.088			2.691		
p	0.34			0.15			0.10		
Females$^{\varphi}$									
Lean (n=277)	156 (56.3)	101 (36.5)	20 (7.2)	4 (1.4)	55 (19.9)	218 (78.7)	216 (78.0)	58 (20.9)	3 (1.1)
Obese (n=421-422)	224 (53.1)	166 (39.3)	32 (7.6)	8 (1.9)	91 (21.6)	322 (76.5)	313 (74.2)	101 (23.9)	8 (1.9)
χ^2	0.556			0.558			1.584		
p	0.46			0.46			0.21		

*Males: lean = <21% (20-39 yrs) and <23% (40-59 yrs); obese = ≥26% (20-39 yrs) and ≥29% (40-59 yrs); $^{\varphi}$Females: lean = <33% (20-39 yrs) and <35% (40-59 yrs); obese = ≥39% (20-39 yrs) and ≥41% (40-59 yrs); Significance assessed using χ^2 test between lean and obese groups for each SNP.

Table 3.5 Genotype distributions (percentages in parentheses) of three SNPs within *GHRL* in lean and obese (based on %LF) males and females

	Exon 1 SNP			Intron 2 SNP			Intergenic SNP		
	AA	AG	GG	CC	CT	TT	AA	AG	GG
Males*									
Lean (n=54)	37 (68.5)	14 (25.9)	3 (5.5)	1 (1.9)	10 (18.5)	43 (79.6)	41 (75.9)	11 (20.4)	2 (3.7)
Obese (n=160-161)	86 (53.8)	63 (39.4)	11 (6.9)	0 (0.0)	20 (12.4)	141 (87.6)	133 (82.6)	28 (17.4)	0 (0.0)
χ^2	2.822			2.819			2.424		
p	0.09			0.09			0.12		
Females$^{\varphi}$									
Lean (n=362-363)	191 (52.8)	139 (38.4)	32 (8.8)	6 (1.7)	77 (21.3)	279 (77.1)	274 (75.5)	84 (23.1)	5 (1.4)
Obese (n=290-291)	146 (50.2)	121 (41.6)	24 (8.2)	7 (2.4)	50 (17.2)	233 (80.3)	221 (76.2)	62 (21.4)	7 (2.4)
χ^2	0.158			0.492			0.007		
p	0.69			0.48			0.93		

*Males: lean = <21% (20-39 yrs) and <23% (40-59 yrs); obese = ≥26% (20-39 yrs) and ≥29% (40-59 yrs); $^{\varphi}$Females: lean = <33% (20-39 yrs) and <35% (40-59 yrs); obese = ≥39% (20-39 yrs) and ≥41% (40-59 yrs); Significance assessed using χ^2 test between lean and obese groups for each SNP.

Table 3.6 Genotype distributions (percentages in parentheses) of three SNPs within *GHRL* in lean and obese (based on BMI) males and females

	Exon 1 SNP			Intron 2 SNP			Intergenic SNP		
	AA	AG	GG	CC	CT	TT	AA	AG	GG
Males*									
Lean (n=62)	39 (62.9)	20 (32.3)	3 (4.8)	1 (1.6)	13 (21.0)	48 (77.4)	44 (71.0)	16 (25.8)	2 (3.2)
Obese (n=57)	30 (52.6)	23 (40.4)	4 (7.0)	0 (0.0)	10 (17.5)	47 (82.5)	46 (80.7)	11 (19.3)	0 (0.0)
χ^2	1.264			0.698			2.201		
p	0.26			0.40			0.14		
Females*									
Lean (n=432-433)	238 (55.1)	161 (37.3)	33 (7.6)	8 (1.8)	83 (19.2)	342 (79.0)	339 (78.3)	86 (19.9)	8 (1.8)
Obese (n=169)	87 (51.5)	68 (40.2)	14 (8.3)	2 (1.2)	36 (21.3)	131 (77.5)	124 (73.4)	43 (25.4)	2 (1.2)
χ^2	0.561			0.039			1.014		
p	0.45			0.84			0.31		

*Males and Females: lean = $\leq$24.9; obese = $\geq$30%; Significance assessed using χ^2 test between lean and obese groups for each SNP.

Table 3.7 Genotype effect of three SNPs within *GHRL* on %BF, %TF, %LF, and BMI in males and females

	Exon 1 SNP				Intron 2 SNP				Intergenic SNP			
	AA	AG	GG	p	CC*	CT	TT	p	AA	AG	GG*	p
Males												
n	137	82	14		1	37	196		189	43	2	
%BF	25.3	25.8	28.0	ns		25.1	25.8	ns	25.8	24.9		ns
%TF	30.2	30.3	33.5	ns		29.8	30.6	ns	30.7	29.5		ns
%LF	31.2	34.3	34.7	ns		29.7	33.1	ns	33.2	29.3		ns
BMI	27.4	28.1	28.2	ns		27.0	27.8	ns	27.8	27.3		ns
Females												
n	495	372	74		17	193	731		715	212	15	
%BF	37.2	37.5	37.3	ns	38.3	37.3	37.3	ns	37.2	37.6	38.5	ns
%TF	38.3	38.6	38.6	ns	39.7	38.5	38.3	ns	38.2	38.9	40.4	ns
%LF	35.6	35.7	35.1	ns	35.6	35.5	37.6	ns	35.6	35.6	38.0	ns
BMI	26.2	26.5	26.3	ns	25.7	26.6	26.3	ns	26.2	26.8	25.7	ns

%BF, %TF, %LF, and BMI are expressed as mean values. Significance was calculated using one-way ANOVA, corrected by Bonferroni *t*-test; ns = not significant; *where n < 5, genotype group was combined with heterozygous group.

Table 3.8 Genotype effect of three SNPs within *GHRL* on serum lipid parameters (log_{10} transformed) in males and females

	Exon 1 SNP				Intron 2 SNP				Intergenic SNP			
	AA	AG	GG	p	CC*	CT	TT	p	AA	AG	GG*	p
Males												
n	137	82	14		1	37	196		189	43	2	
Total Chol	0.699	0.690	0.711	ns		0.695	0.696	ns	0.696	0.698		ns
LDL-C	0.488	0.464	0.497	ns		0.467	0.482	ns	0.483	0.465		ns
HDL-C	0.096	0.088	0.076	ns		0.096	0.092	ns	0.089	0.103		ns
Glucose	0.717	0.715	0.700	ns		0.705	0.717	ns	0.717	0.708		ns
Insulin	1.807	1.850	1.890	ns		1.831	1.826	ns	1.825	1.834		ns
TGs	0.076	0.103	0.151	ns		0.113	0.085	ns	0.084	0.110		ns
Risk factor	0.606	0.603	0.634	ns		0.111	0.112	ns	0.608	0.597		ns
Females												
n	495	372	74		17	193	731		715	212	15	
Total Chol	0.710	0.711	0.716	ns	0.717	0.714	0.710	ns	0.711	0.714	0.708	ns
LDL-C	0.473	0.485	0.479	ns	0.491	0.480	0.477	ns	0.477	0.480	0.479	ns
HDL-C	0.189	0.187	0.193	ns	0.188	0.186	0.189	ns	0.190	0.180	0.211	ns
Glucose	0.695	0.695	0.696	ns	0.706	0.699	0.694	ns	0.693	0.703	0.701	ns
Insulin	1.780	1.783	1.756	ns	1.738	1.801	1.782	ns	0.178	0.181	0.176	ns
TGs	0.017	-0.001	0.006	ns	0.009	0.014	0.007	ns	0.007	0.016	-0.023	ns
Risk factor	0.522	0.527	0.522	ns	0.527	0.531	0.522	ns	0.521	0.534	0.527	ns

Serum lipid parameters are expressed as mean values. Significance was calculated using one-way ANOVA, corrected by Bonferroni *t*-test; ns = not significant; *where n < 5, genotype group was combined with heterozygous group.

total cholesterol, LDL-C, HDL-C, glucose, insulin, TGs, or risk factor for the 3 SNPs investigated in males or females. However, in males for the Exon 1 SNP, the calculated antilog10 mean values for HDL-C indicated about 1.25 mmol/L for AA, 1.22 mmol/L for AG, and 1.19 mmol/L for GG. The calculated antilog10 mean values for TG were 1.19 mmol/L for AA, 1.27 mmol/L for AG, and 1.42 mmol/L for GG. These values match well with the expected trend if there were an association of the G allele and obesity, that is, a trend with the number of G alleles with the greatest difference in the GG genotype group. Obese individuals, as expected, showed lower HDL-C and higher TG levels. Once again, all analyses were repeated after adjusting for age and %BF, possible confounding factors, using linear regression. Similar results were obtained for all SNPs investigated in males and females.

The most common haplotypes (n > 50), or the combinations of the 3 SNPs investigated within GHRL, in the NL cohort are presented in **Table 3.9**. The effect of the haplotypes on body compositions in males and females are presented in **Table 3.10**. Using one-way ANOVA, corrected by Bonferroni *t*-test, there was no significant difference among the 5 common haplotype groups on %BF, %TF, %LF, or BMI in males or females. Furthermore, similar results were obtained after all analyses were repeated upon adjusting for age in both males and females.

The haplotype effect of the most common haplotypes on serum lipid parameters in males and females are presented in **Table 3.11**. Using one-way ANOVA, corrected by Bonferroni *t*-test, there was no significant difference among the among the 5 common haplotype groups for total cholesterol, LDL-C, HDL-C, glucose, insulin, TGs, or risk factor in males or females. Also, similar results were obtained after all analyses were

Table 3.9 Common haplotypes within the *GHRL* gene in the NL population

Haplotype*	n	Males	Females
2-0-2	423	104	319
2-1-1	146	21	125
1-0-2	371	66	305
1-1-1	50	11	39
0-0-2	74	13	61

*Order of haplotype: SNP in Exon 1 – SNP in intronic region – SNP in intergenic region;

0 = homozygous for minor allele; 1 = heterozygous; 2 = homozygous for major allele.

Table 3.10 Haplotype effect of three SNPs within *GHRL* on %BF, %TF, %LF, and BMI in males and females

	Haplotype					
	0-0-2	1-0-2	1-1-1	2-0-2	2-1-1	p
Males						
n	13	66	11	104	21	
%BF	27.8	25.7	26.1	25.7	24.9	ns
%TF	33.4	30.3	30.5	30.6	30.1	ns
%LF	38.5	34.7	36.5	36.0	30.7	ns
BMI	28.7	28.1	27.9	27.6	26.9	ns
Females						
n	61	305	39	319	125	
%BF	37.2	37.8	35.0	36.6	38.2	ns
%TF	38.4	38.9	36.5	37.6	39.5	ns
%LF	34.8	35.3	34.0	34.7	35.0	ns
BMI	26.3	26.5	26.1	25.9	26.8	ns

Significance was calculated using one-way ANOVA, corrected by Bonferroni *t*-test; ns = not significant.

Table 3.11 Haplotype effect of three SNPs within *GHRL* on serum lipid parameters (log_{10} transformed) in males and females

	Haplotype					
	0-0-2	1-0-2	1-1-1	2-0-2	2-1-1	p
Males						
n	13	66	11	104	21	
Total chol.	0.718	0.699	0.646	0.691	0.718	ns
LDL-C	0.517	0.481	0.371	0.481	0.501	ns
HDL-C	0.083	0.090	0.056	0.091	0.112	ns
Glucose	0.710	0.717	0.709	0.719	0.703	ns
Insulin	1.901	1.847	1.827	1.800	1.841	ns
TGs	0.125	0.107	0.155	0.067	0.123	ns
Risk Factor	0.634	0.609	0.597	0.604	0.607	ns
Females						
n	61	305	39	319	125	
Total chol.	0.721	0.714	0.699	0.706	0.722	ns
LDL-C	0.491	0.489	0.464	0.465	0.492	ns
HDL-C	0.201	0.190	0.165	0.189	0.191	ns
Glucose	0.696	0.692	0.719	0.693	0.695	ns
Insulin	1.762	1.778	1.831	1.787	1.807	ns
TGs	-0.004	0.000	0.006	0.013	0.011	ns
Risk Factor	0.522	0.527	0.527	0.515	0.535	ns

Significance was calculated using one-way ANOVA, corrected by Bonferroni *t*-test; ns = not significant.

repeated upon adjusting for the confounding factors age and %BF in both males and females.

3.1b: Genome-wide expression study of adipose tissue in response to short-term aerobic exercise using microarray technology

The physical and biochemical characteristics of lean and obese subjects prior to exercise are presented in **Table 3.12.** On average, the mean age of lean males was 0.8 years younger than obese males, while the average height for lean males was about 5 cm greater than obese males. Age and height was not significantly different between lean and obese groups. Lean males had significantly less body mass (~22kg), BMI (~9kg/m^2), body fat (~20%), trunk fat (~23%), and lower body (legs) fat (~17%). The mean levels of fasting serum total cholesterol, LDL-C, triglycerides, and risk factor for cardiovascular disease, as calculated by total cholesterol divided by HDL-C, were also significantly lower in lean males.

The change in the physical and biochemical characteristics of lean and obese subjects following 7 days of aerobic exercise are presented in **Table 3.13**. According to paired *t*-test analyses (before and after exercise values), we report that obese individuals significantly decreased their %TF ($p < 0.05$). As well, in obese individuals, we observed a decrease in %BF and TG levels, although not quite significant ($p = 0.07$ for both). In lean individuals, in response to 7 days of aerobic exercise at the same intensity level, we did not observe the decreases in %TF, %BF, or TG levels as we did with the obese subjects. However, we did observe a decrease in %LF in lean subjects, although not quite significant ($p = 0.08$). All other post-exercise values of physical characteristics and biochemical measurements for both lean and obese subjects did not differ significantly from the pre-exercise values.

Table 3.12 Physical and biochemical characteristics of subjects in the microarray exercise study (pre-exercise values)

	Lean (n = 5)	Obese (n = 5)
Age (yr)	22.8 ± 1.6 (21-25)	23.6 ± 2.6 (22-28)
Weight (kg)	70.4 ± 7.8 (64.2-82.0)[b]	92.0 ± 21.4 (74.6-123.0)
Height (cm)	180.6 ± 6.1 (174-190)	174.7 ± 6.0 (165-182)
BMI (kg/m^2)	21.6 ± 2.8 (17.9-25.6)[b]	30.7 ± 9.9 (22.5-45.0)
%BF[1]	12.0 ± 5.0 (7.6-19.8)[a]	32.2 ± 6.9 (26.6-42.2)
%TF[2]	12.9 ± 5.8 (7.1-21.7)[a]	35.8 ± 4.7 (32.7-42.6)
%LF[3]	13.0 ± 5.1 (9.0-20.9)[a]	30.1 ± 6.4 (22.3-37.9)
Total Cholesterol (mmol/L)	3.21 ± 0.32 (2.87-3.62)[a]	4.62 ± 0.63 (3.88-5.61)
LDL-C[4] (mmol/L)	1.75 ± 0.35 (1.29-2.13)[b]	2.54 ± 0.59 (1.59-3.15)
HDL-C[5] (mmol/L)	1.21 ± 0.07 (1.13-1.29)	1.18 ± 0.24 (0.93-1.46)
TGs[6] (mmol/L)	0.54 ± 0.09 (0.43-0.66)[a]	0.93 ± 0.10 (0.79-1.07)
Risk factor[7]	2.66 ± 0.27 (2.24-2.86)[b]	3.82 ± 0.71 (3.22-4.83)

Values are expressed as mean ± standard deviation (min. – max.); %BF[1] = body fat percentage; %TF[2] = trunk fat percentage; %LF[3] = lower body fat percentage; LDL-C[4] = low-density lipoprotein-cholesterol; HDL-C[5] = high-density lipoprotein-cholesterol; TGs[6] = triglycerides; risk factor[7] = total cholesterol/HDL-C. [a]p value < 0.01, [b]p value < 0.05 in the comparison between lean and obese males using Student's *t*-test.

Table 3.13 The change in the physical and biochemical characteristics of subjects in the microarray exercise study (post-exercise values – pre-exercise values)

	Lean (n = 5)	p	Obese (n = 5)	p
Weight (kg)	-0.18 ± 0.63 (-1.0-0.6)	0.56	-0.90 ± 1.0 (-2.6- -0.1)	0.11
BMI (kg/m^2)	-0.05 ± 0.19 (-0.3-0.2)	0.58	-0.30 ± 0.32 (-.9- -0.03)	0.11
%BF[1]	-0.80 ± 0.62 (-0.7-0.7)	0.79	-1.08 ± 0.96 (-2.6-0.0)	0.07
%TF[2]	0.22 ± 0.73 (-0.5-1.3)	0.54	-1.08 ± 0.84 (-2.1-0.0)	**<0.05**
%LF[3]	-0.62 ± 0.61 (-1.3-0.3)	0.08	-0.44 ± 0.66 (-1.3-0.4)	0.21
Total Chol. (mmol/L)	0.06 ± 0.19 (-0.1-0.4)	0.50	0.06 ± 0.57 (-0.6-0.7)	0.82
LDL-C[4] (mmol/L)	-0.06 ± 0.22 (-0.2-0.3)	0.60	-0.05 ± 0.67 (-0.9-0.71)	0.87
HDL-C[5] (mmol/L)	0.08 ± 0.10 (-0.02-0.2)	0.15	0.10 ± 0.13 (-0.01-0.28)	0.23
TGs[6] (mmol/L)	0.08 ± 0.23 (-0.2-0.4)	0.50	-0.23 ± 0.21 (-0.41-0.04)	0.07
Risk factor[7]	-0.11 ± 0.17 (-0.4-0.04)	0.22	-0.11 ± 0.62 (-0.6-0.8)	0.74

Values are expressed as mean ± standard deviation (min. – max.); Statistical significance assessed using paired *t*-test between pre- and post-exercise values for lean and obese separately. %BF[1] = body fat percentage; %TF[2] = trunk fat percentage; %LF[3] = lower body fat percentage; LDL-C[4] = low-density lipoprotein-cholesterol; HDL-C[5] = high-density lipoprotein-cholesterol; TGs[6] = triglycerides; risk factor[7] = total cholesterol/HDL-C.

Upon data processing of raw intensity measurements for each microarray, the number of genes and transcripts of interest for both lean and obese samples greatly decreased. After introducing our 5 parameters ((1) correction for background, (2) ratio of sample-to-reference, (3) omit multiple flagged spots, (4) $\log_2$ shift, and (5) normalization), the average number of genes and transcripts remaining for the 10 lean samples (5 pre and 5 post exercise) was 21,198, while 21,867 remained for the 10 obese samples. Subsequently, after introducing our criteria to determine any differentially expressed genes in pre-exercise samples when compared to post-exercise samples (3 of the 5 samples must be in the same direction by 1.5 fold and the average of 5 samples must be 1.5 fold in that particular direction), 6,627 genes remained as differentially expressed for lean samples, while 8,156 remained for obese samples. Finally, comparing pre-exercise sample intensity values to post-exercise values of the remaining genes using student-paired *t*-tests, 235 genes were statistically significant (46 up-regulated and 189 down-regulated) in lean subjects, while 279 were significant (90 up-regulated and 189 down-regulated) in obese subjects. In lean subjects, of the 235 differentially expressed genes, 182 (77%) had defined HUGO names **(Table 3.14)**, while 199 (71%) had defined HUGO names in obese subjects **(Table 3.15)**. The false discovery rate (FDR), which provides the expected percent of false positives, was calculated after the consideration of duplication of genes and transcripts on the arrays, as well as some function associations between genes. The following formula was used: ((# of comparison x level of significance)/2/# of significant genes). The FDR, using $p \leq 0.01$ as the level of significance, was 14.6% for obese samples and 14.1% for lean samples. It should be pointed out, however, that the calculated FDR for lean and obese is the maximum

Table 3.14 Significantly down- and up-regulated genes, classified by biological process, of lean subjects in response to short-term exercise.

Gene symbol	Gene name	Fold change*	p value
Regulation of transcription			
ZNF582	zinc finger protein 582	0.5394	0.0117
ZNF444	zinc finger protein 444	5.4609	0.0136
ZNF136	zinc finger protein 136 (clone pHZ-20)	3.9637	0.0043
ZNF277	zinc finger protein (C2H2 type) 277	2.5750	0.0056
ZNF295	zinc finger protein 295	5.2743	0.0014
ZNF419	zinc finger protein 419	1.5869	0.0037
IRX5	iroquois homeobox protein 5	3.1788	0.0023
CNOT7	CCR4-NOT transcription complex, subunit 7	1.6756	0.0119
HNRPD	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1 37kDa)	2.7444	0.0024
CRSP9	cofactor required for Sp1 transcriptional activation, subunit 9, 33kDa	2.0479	0.0055
KIAA0543	KIAA0543 protein	3.8393	0.0060
MLL5	myeloid/lymphoid or mixed-lineage leukemia 5 (trithorax homolog, Drosophila)	1.9891	0.0148
RELA	v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65 (avian)	1.7897	0.0024
MED28	mediator of RNA polymerase II transcription, subunit 28 homolog (yeast)	5.5106	0.0007
FOXP2	forkhead box P2	15.1966	0.0146
Apoptosis			
BCAP31	B-cell receptor-associated protein 31	5.0099	0.0067
HD	huntingtin (Huntington disease)	4.5855	0.0031
BCL2L2	BCL2-like 2	2.9707	0.0020
Metabolism			
SLC6A6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	0.5823	0.0111
VNN3	vanin 3	0.4342	0.0041

ACO2	aconitase 2, mitochondrial	1.9984	0.0025
MAN2B1	mannosidase, alpha, class 2B, member 1	3.2674	0.0140
DDEFL1	development and differentiation enhancing factor-like 1	3.5499	0.0059
PUM2	pumilio homolog 2 (Drosophila)	14.4063	0.0116
TDG	thymine-DNA glycosylase	2.3615	0.0086
ENPP4	ectonucleotide pyrophosphatase/ phosphodiesterase 4 (putative function)	3.3667	0.0114
VLDLR	very low density lipoprotein receptor	3.5715	0.0056

Lipid metabolism

PPARA	peroxisome proliferative activated receptor, alpha	0.2626	0.0014
PLCL1	phospholipase C-like 1	0.0347	0.0016

Glycogen metabolism

PPP1CB	protein phosphatase 1, catalytic subunit, beta isoform	3.6976	0.0033

Triacylglycerol biosynthesis

DGAT2	diacylglycerol O-acyltransferase homolog 2 (mouse)	0.3131	0.0143

Electron transport

ACOXL	acyl-Coenzyme A oxidase-like	0.4633	0.0086
COX11	COX11 homolog, cytochrome c oxidase assembly protein (yeast)	7.1352	0.0040
COX7A2L	cytochrome c oxidase subunit VIIa polypeptide 2 like	8.1928	0.0055
NDUFS7	NADH dehydrogenase (ubiquinone) Fe-S protein 7, 20kDa (NADH-coenzyme Q reductase)	2.1335	0.0131
NQO2	NAD(P)H dehydrogenase, quinone 2	1.7998	0.0048

Transport

SCL41A1	solute carrier family 41, member 1	0.1772	0.0036

LTBP2	latent transforming growth factor beta binding protein 2	0.0994	0.0059
LRP1B	low density lipoprotein-related protein 1B (deleted in tumors)	0.5083	0.0053
RAB4A	RAB4A, member RAS oncogene family	2.2779	0.0067
RILP	Rab interacting lysosomal protein	2.3935	0.0023
RBP5	retinol binding protein 5, cellular	2.7949	0.0120
MARCO	macrophage receptor with collagenous structure	18.9251	0.0068
ATP6V0E	ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e	6.2983	0.0068
VPS18	vacuolar protein sorting protein 18	2.3520	0.0080
SPATA13	spermatogenesis associated 13	4.3103	0.0124
SLC9A10	solute carrier family 9, isoform 10	18.8188	0.0087
ATP1A4	ATPase, Na+/K+ transporting, alpha 4 polypeptide	13.4470	0.0100
DJ971N18.2	hypothetical protein DJ971N18.2	1.8839	0.0031
C6orf85	chromosome 6 open reading frame 85	3.3291	0.0125

Signal transduction/Signaling pathway

RSU1	Ras suppressor protein 1	0.0720	0.0131
ADCYAP1	adenylate cyclase activating polypeptide 1 (pituitary)	0.5976	0.0070
TAS2R39	taste receptor, type 2, member 39	0.5248	0.0102
ADCY6	adenylate cyclase 6	4.6358	0.0136
GPR114	G protein-coupled receptor 114	3.8409	0.0093
RHOQ	ras homolog gene family, member Q	1.7825	0.0053
GPR23	G protein-coupled receptor 23	8.9842	0.0013
ITGAL	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)	7.2962	0.0093
ASB1	ankyrin repeat and SOCS box-containing 1	2.4934	0.0006
BCR	breakpoint cluster region	2.1912	0.0076
PLEKHM1	pleckstrin homology domain containing, family M (with RUN domain) member 1	2.8836	0.0057

Protein ubiquitination/Ubiquitin cycle

UBE1	ubiquitin-activating enzyme E1 (A1S9T and BN75 temperature sensitivity complementing)	0.5931	0.0025
RNF13	ring finger protein 13	2.5864	0.0145
LNX2	ligand of numb-protein X 2	2.3361	0.0128
TRIM33	tripartite motif-containing 33	2.0976	0.0027
UBE2L3	ubiquitin-conjugating enzyme E2L 3	3.7103	0.0108
FBXO31	F-box protein 31	3.3846	0.0091

DZIP3	zinc finger DAZ interacting protein 3	4.3924	0.0122

Protein biosynthesis

MRPL48	mitochondrial ribosomal protein L48	0.3718	0.0046
AARSL	alanyl-tRNA synthetase like	2.4349	0.0093

Protein amino acid phosphorylation/dephosphorylation

NEK7	NIMA (never in mitosis gene a)-related kinase 7	2.1979	0.0086
WNK4	WNK lysine deficient protein kinase 4	3.1255	0.0107
PPM2C	protein phosphatase 2C, magnesium-dependent, catalytic subunit	4.6163	0.0061
PTPN3	protein tyrosine phosphatase, non-receptor type 3	1.9053	0.0131
PTPRD	protein tyrosine phosphatase, receptor type, D	9.5312	0.0036
PTPN11	protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1)	2.8648	0.0135
DCAMKL1	doublecortin and CaM kinase-like 1	5.2023	0.0127

Protein folding

FIGN	fidgetin	0.1756	0.0099
DCPS	decapping enzyme, scavenger	1.5727	0.0070
BAG3	BCL2-associated athanogene 3	1.9559	0.0147
SEC63	SEC63-like (S. cerevisiae)	2.0669	0.0041

Cell proliferation

CDK5RAP3	CDK5 regulatory subunit associated protein 3	1.8158	0.0006
EVI5	ecotropic viral integration site 5	2.2237	0.0145
SKP2	S-phase kinase-associated protein 2 (p45)	2.4496	0.0051

Regulation of cell cycle

LTBP2	latent transforming growth factor beta binding protein 2	0.0994	0.0059
CCND2	cyclin D2	3.2020	0.0088

MCM2	MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae)	2.1924	0.0101
PHB	prohibitin	1.7153	0.0040

Miscellaneous

BOLL	bol, boule-like (Drosophila)	0.1625	0.0060
EXOSC2	exosome component 2	0.3446	0.0012
MAP2	microtubule-associated protein 2	0.0626	0.0058
SPRR2A	small proline-rich protein 2A	0.6369	0.0100
EMD	emerin (Emery-Dreifuss muscular dystrophy)	2.8855	0.0022
SECTM1	secreted and transmembrane 1	1.9046	0.0108
PHPT1	phosphohistidine phosphatase 1	4.1273	0.0008
BACE2	beta-site APP-cleaving enzyme 2	3.8050	0.0062
FLJ11016	hypothetical protein FLJ11016	4.0256	0.0052
RSAD1	radical S-adenosyl methionine domain containing 1	1.9727	0.0009
ARP3BETA	actin-related protein 3-beta	1.8156	0.0025
VAPA	VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa	2.5508	0.0118
HIST1H2AA	histone 1, H2aa	1.6043	0.0039

Biological process unknown

C11orf21	chromosome 11 open reading frame 21	0.2923	0.0028
SLC41A1	solute carrier family 41, member 1	0.1772	0.0036
FLJ22639	hypothetical protein FLJ22639	0.2401	0.0055
LOC374946	hypothetical gene supported by AK075558; BC021286	0.6390	0.0070
LOC57821	hypothetical protein LOC57821	0.3656	0.0072
MGC15763	hypothetical protein BC008322	0.2782	0.0090
C21orf128	chromosome 21 open reading frame 128	0.3822	0.0111
TSSC4	tumor suppressing subtransferable candidate 4	0.2760	0.0125
C6orf166	chromosome 6 open reading frame 166	0.6383	0.0127
PNPLA5	patatin-like phospholipase domain containing 5	0.4493	0.0056
GPHA2	glycoprotein hormone alpha 2	0.5797	0.0066
PHC2	polyhomeotic-like 2 (Drosophila)	0.3501	0.0039
UNC84A	unc-84 homolog A (C. elegans)	0.1983	0.0079
RANBP2L1	RAN binding protein 2-like 1	3.9948	0.0059
LENG4	leukocyte receptor cluster (LRC) member 4	2.8539	0.0149
CALU	calumenin	2.2629	0.0131
ACTR1B	ARP1 actin-related protein 1 homolog B, centractin beta (yeast)	6.6772	0.0103
OLFM4	olfactomedin 4	9.9232	0.0113
TDRKH	tudor and KH domain containing	129.9912	0.0073

DDX52	DEAD (Asp-Glu-Ala-Asp) box polypeptide 52	2.4363	0.0066
TTC18	tetratricopeptide repeat domain 18	34.2659	0.0096
PPRC1	peroxisome proliferative activated receptor, gamma, coactivator-related 1	2.2269	0.0128
KIAA0853	KIAA0853	2.3708	0.0131
HS6ST3	heparan sulfate 6-O-sulfotransferase 3	2.8146	0.0050
GALNTL4	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-like 4	7.4970	0.0090
TTC15	tetratricopeptide repeat domain 15	3.6655	0.0023
NUDT12	nudix (nucleoside diphosphate linked moiety X)-type motif 12	5.5836	0.0089
C9orf140	chromosome 9 open reading frame 140	3.2908	0.0115
SUV420H1	suppressor of variegation 4-20 homolog 1 (Drosophila)	5.3170	0.0118
PCNXL2	pecanex-like 2 (Drosophila)	2.6497	0.0109
SLITRK6	SLIT and NTRK-like family, member 6	3.8098	0.0083
FTO	fatso	2.1995	0.0001
KIAA1155	KIAA1155 protein	2.8633	0.0003
FLJ39441	hypothetical protein FLJ39441	65.3742	0.0011
HBA2	hemoglobin, alpha 2	1.8548	0.0015
MGC14839	similar to RIKEN cDNA 2310030G06 gene	3.1291	0.0016
TDH	L-threonine dehydrogenase	5.4626	0.0020
MGC3234	hypothetical protein MGC3234	2.2071	0.0022
KIAA0754	KIAA0754 protein	2.6722	0.0023
FLJ90024	fasting-inducible integral membrane protein TM6P1	3.0180	0.0024
[DKFZ p586M1819]	putative lysophosphatidic acid acyltransferase	4.3404	0.0080
ARMCX6	armadillo repeat containing, X-linked 6	3.4875	0.0025
RP11-19J3.3	similar to hypothetical protein	2.5574	0.0031
IFI44L	interferon-induced protein 44-like	1.7523	0.0032
ZNF419	zinc finger protein 419	1.5869	0.0037
IL8RBP	interleukin 8 receptor, beta pseudogene	2.2217	0.0041
TTLL2	tubulin tyrosine ligase-like family, member 2	18.6815	0.0050
FLJ21019	hypothetical protein FLJ21019	1.7953	0.0050
DCL-1	type I transmembrane C-type lectin receptor DCL-1	5.0830	0.0050
PSIP1	PC4 and SFRS1 interacting protein 1	2.4778	0.0052
KIAA1957	KIAA1957	3.5739	0.0052
LOC340281	hypothetical protein LOC340281	3.5030	0.0053
[DKFZP547 E1010]	DKFZP547E1010 protein	2.2962	0.0054
MGC27165	hypothetical protein MGC27165	2.5221	0.0059
LOC284889	hypothetical protein LOC284889	2.4647	0.0061
[DKFZp762 K222]	hypothetical protein DKFZp762K222	4.7026	0.0066

[DKFZp761 E198]	hypothetical protein DKFZp761E198	47.9190	0.0067
LOC63920	transposon-derived Buster3 transposase-like	11.5783	0.0068
C9orf105	chromosome 9 open reading frame 105	12.6042	0.0071
T1A-2	lung type-I cell membrane-associated glycoprotein	1.9100	0.0073
C10orf24	chromosome 10 open reading frame 24	1.8704	0.0074
TCFL4	transcription factor-like 4	4.4176	0.0074
LOC401967	similar to KIAA1693 protein	3.6775	0.0078
VPS18	vacuolar protein sorting protein 18	2.3520	0.0080
[DKFZp586 M1819]	putative lysophosphatidic acid acyltransferase	4.3404	0.0080
PLEKHA6	pleckstrin homology domain containing, family A member 6	5.2061	0.0082
ZD52F10	dermokine	3.9068	0.0093
PNMA5	paraneoplastic antigen like 5	2.4504	0.0100
MOBK1B	MOB1, Mps One Binder kinase activator-like 1B (yeast)	2.9382	0.0101
C6orf10	chromosome 6 open reading frame 10	3.4673	0.0102
LOC92482	hypothetical protein LOC92482	1.9243	0.0105
LOC126755	hypothetical protein LOC126755	9.2021	0.0116
[DKFZp762 C1112]	hypothetical protein DKFZp762C1112	2.4060	0.0116
CBWD2	COBW domain containing 2	4.7765	0.0118
TA-PP2C	T-cell activation protein phosphatase 2C	3.6550	0.0126
MGC34805	hypothetical protein MGC34805	2.1248	0.0128
LOC285749	hypothetical protein LOC285749	5.2981	0.0130
MGC32124	hypothetical protein MGC32124	6.0151	0.0132
NCDN	neurochondrin	3.3893	0.0138
FLJ40201	hypothetical protein FLJ40201	2.9315	0.0139
LOC163782	hypothetical protein LOC163782	11.4807	0.0142
MGC12760	hypothetical protein MGC12760	2.4207	0.0144
FAM26C	family with sequence similarity 26, member C	2.4931	0.0146
FLJ43860	FLJ43860 protein	6.9144	0.0146
LOC285908	hypothetical protein LOC285908	2.6054	0.0144
FNDC3A	fibronectin type III domain containing 3A	3.2624	0.0149

*Fold change indicates pre-exercise intensity values divided by post-exercise values. Classification based on GOTM software (http://genereg.ornl.gov.gotm). Only genes with identified HUGO names are included.

Table 3.15 Significantly down- and up-regulated genes, classified by biological process, of obese subjects in response to short-term exercise.

HUGO name	Description	Fold change*	p value
Regulation of transcription			
RBPSUH	recombining binding protein suppressor of hairless (Drosophila)	0.2260	0.0040
ZFPM2	zinc finger protein, multitype 2	0.4756	0.0011
SSX5	synovial sarcoma, X breakpoint 5	0.3969	0.0042
HOXB9	homeo box B9	4.2387	0.0007
SOX3	SRY (sex determining region Y)-box 3	3.4097	0.0108
ZBTB12	zinc finger and BTB domain containing 12	11.7818	0.0019
ZNF85	zinc finger protein 85 (HPF4, HTF1)	5.2315	0.0067
ZNF146	zinc finger protein 146	548.5253	0.0071
ZNF586	zinc finger protein 586	9.4432	0.0062
ASCC3	activating signal cointegrator 1 complex subunit 3	7.9331	0.0084
HES2	hairy and enhancer of split 2 (Drosophila)	9.1263	0.0054
Proteolysis and peptidolysis			
PCSK1	proprotein convertase subtilisin/kexin type 1	0.4478	0.0046
MSTP9	macrophage stimulating, pseudogene 9	0.0618	0.0094
CPB1	carboxypeptidase B1 (tissue)	0.1005	0.0038
F9	coagulation factor IX (plasma thromboplastic component, Christmas disease, hemophilia B)	0.3673	0.0065
UBD	ubiquitin D	2.6744	0.0120
MMP19	matrix metalloproteinase 19	2.5244	0.0141
RNPEP	arginyl aminopeptidase (aminopeptidase B)	16.0016	0.0018
ADAM33	a disintegrin and metalloproteinase domain 33	10.0491	0.0078
Apoptosis			
PLAGL1	pleiomorphic adenoma gene-like 1	0.6193	0.0022
PHLDA2	pleckstrin homology-like domain, family A, member 2	2.5743	0.0117

Immune response

IGHM	immunoglobulin heavy constant mu	0.6742	0.0077
CST7	cystatin F (leukocystatin)	0.1476	0.0061
CD96	CD96 antigen	2.6522	0.0127
LILRA2	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	11.4194	0.0129
LTB	lymphotoxin beta (TNF superfamily, member 3)	1.5402	0.0123
BPI	bactericidal/permeability-increasing protein	9.3217	0.0129

Inflammatory response

IL1R1	interleukin 1 receptor, type I	0.5342	0.0085
ITCH	itchy homolog E3 ubiquitin protein ligase (mouse)	0.5677	0.0082
S100A12	S100 calcium binding protein A12 (calgranulin C)	4.0037	0.0140

Metabolism

ST3GAL5	ST3 beta-galactoside alpha-2,3-sialyltransferase 5	0.5338	0.0071
CERK	ceramide kinase	0.5205	0.0003
ITIH5	inter-alpha (globulin) inhibitor H5	0.3362	0.0120
AKR7A3	aldo-keto reductase family 7, member A3 (aflatoxin aldehyde reductase)	1.9419	0.0046
GK	glycerol kinase	4.6494	0.0083
SMPD3	sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II)	11.7743	0.0118
CA5BL	carbonic anhydrase VB-like	4.7331	0.0036
AGPAT5	1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon)	2.3159	0.0088
ASAH2	N-acylsphingosine amidohydrolase (non-lysosomal ceramidase) 2	2.3289	0.0006

Lipid metabolism

SLC27A6	solute carrier family 27 (fatty acid transporter), member 6	0.5183	0.0119
ALDH3A2	aldehyde dehydrogenase 3 family, member A2	3.2094	0.0099

Phospholipid biosynthesis

GPAM	glycerol-3-phosphate acyltransferase, mitochondrial	0.4116	0.0036

Transport

KCNH7	potassium voltage-gated channel, subfamily H (eag-related), member 7	0.4359	0.0145
ATCAY	ataxia, cerebellar, Cayman type (caytaxin)	0.2881	0.0004
SLC8A1	solute carrier family 8 (sodium/calcium exchanger), member 1	0.6177	0.0105
ARF3	ADP-ribosylation factor 3	0.4129	0.0112
SLC4A5	solute carrier family 4, sodium bicarbonate cotransporter, member 5,	0.5821	0.0009
SLC39A4	solute carrier family 39 (zinc transporter), member 4	6.6898	0.0110
SCAMP2	secretory carrier membrane protein 2	6.3755	0.0095
COPG2	coatomer protein complex, subunit gamma 2	2.2355	0.0081
HFE	hemochromatosis	23.1734	0.0136
ITPR3	inositol 1,4,5-triphosphate receptor, type 3	2.2531	0.0126
KCNJ13	potassium inwardly-rectifying channel, subfamily J,member 13	43.6760	0.0052
KCNK2	potassium channel, subfamily K, member 2	5.8440	0.0122
SLC16A3	solute carrier family 16 (monocarboxylic acid transporters), member 3	1.9997	0.0064
SYTL4	synaptotagmin-like 4 (granuphilin-a)	13.4468	0.0028
ARL5	ADP-ribosylation factor-like 5	2.2058	0.0017
NRAP	nebulin-related anchoring protein	2.8601	0.0027

Lipid transport

APOL6	apolipoprotein L, 6	0.1809	0.0005

Signal transduction/Signaling pathway

GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4	0.6248	0.0070
AGTR1	angiotensin II receptor, type 1	0.2100	0.0005
CHRNA5	cholinergic receptor, nicotinic, alpha polypeptide 5	0.5776	0.0024

OR6A2	olfactory receptor, family 6, subfamily A, member 2	0.3355	0.0140
EPOR	erythropoietin receptor	2.9297	0.0032
PIK4CA	phosphatidylinositol 4-kinase, catalytic, alpha polypeptide	1.9830	0.0080
DEPDC1	DEP domain containing 1	2.1030	0.0124
GPR132	G protein-coupled receptor 132	22.6237	0.0098
HTR4	5-hydroxytryptamine (serotonin) receptor 4	2.1086	0.0010
RAB27A	RAB27A, member RAS oncogene family	48.9776	0.0044

DNA repair/Base-excision repair/DNA damage response

DMC1	DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast)	0.3759	0.0051
FANCF	Fanconi anemia, complementation group F	1.9348	0.0010
APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1	4.9311	0.0010
GTSE1	G-2 and S-phase expressed 1	6.2095	0.0045

Defense response

NCF4	neutrophil cytosolic factor 4, 40kDaP	2.6028	0.0101

DNA methylation

DISC1	disrupted in schizophrenia 1	0.3868	0.0016
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	7.3024	0.0010

Chromatin Modification

EP400	E1A binding protein p400	2.1558	0.0099
SMARCC1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1	4.8370	0.0122

Development

LMO2	LIM domain only 2 (rhombotin-like 1)	1.9804	0.0085
NUMB	numb homolog (Drosophila)	2.5043	0.0085
PLXNA2	plexin A2	3.7048	0.0113

Protein ubiquitination/Ubiquitin cycle

C20orf18	chromosome 20 open reading frame 18	1.5315	0.0101
C17orf27	chromosome 17 open reading frame 27	4.2055	0.0051
RNF5	ring finger protein 5	3.0652	0.0046
CUL3	cullin 3	6.4005	0.0063
FBXO44	F-box protein 44	1.5814	0.0021

Protein repair

SEPX1	selenoprotein X, 1	0.6052	0.0086

Protein biosynthesis

FNBP1	formin binding protein 1	1.9508	0.0062

Protein amino acid phosphorylation

EPHA8	EPH receptor A8	0.4522	0.0109
PRKACA	protein kinase, cAMP-dependent, catalytic, alpha	6.2556	0.0075

Complement activation

C4BPA	complement component 4 binding protein, alpha	2.5218	0.0136

Cell cycle

ASPM	asp (abnormal spindle)-like, microcephaly associated (Drosophila)	12.5706	0.0106
BIN1	bridging integrator 1	2.5330	0.0047
BRDT	bromodomain, testis-specific	2.6835	0.0135
TCF19	transcription factor 19 (SC1)	3.0190	0.0001
MAD1L1	MAD1 mitotic arrest deficient-like 1 (yeast)	2.4644	0.0104
KNTC1	kinetochore associated 1	3.1984	0.0005

Cell adhesion

ARHGAP5	Rho GTPase activating protein 5	3.2735	0.0029
CYR61	cysteine-rich, angiogenic inducer, 61	5.0410	0.0114

Miscellaneous

LSM6	LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae) phosphatase 1	0.6058	0.0034
TXNDC2	thioredoxin domain containing 2 (spermatozoa)	0.3333	0.0063
S100A11	S100 calcium binding protein A11 (calgizzarin)	0.5571	0.0107
FKBP1B	FK506 binding protein 1B, 12.6 kDa	3.0337	0.0103
CPS1	carbamoyl-phosphate synthetase 1, mitochondrial	2.2007	0.0133
IFRD1	interferon-related developmental regulator 1	3.6688	0.0140
MMD2	monocyte to macrophage differentiation-associated 2	2.9010	0.0132
MYH10	myosin, heavy polypeptide 10, non-muscle	3.7555	0.0135
FRAS1	Fraser syndrome 1	8.9096	0.0067
HIST1H2BN	histone 1, H2bn	10.7272	0.0072
HIST1H3H	histone 1, H3h	2.9266	0.0103
HARS2	histidyl-tRNA synthetase 2	799.5183	0.0120

Biological process unknown

FLJ10647	hypothetical protein FLJ10647	0.5556	4.34E-05
CST3	cystatin C (amyloid angiopathy and cerebral hemorrhage)	0.3867	0.0005
LOC51145	erythrocyte transmembrane protein	0.4922	0.0008
KIR2DS4	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 4	0.5542	0.0009
FLJ11017	hypothetical protein FLJ11017	0.4463	0.0011
FLJ20712	hypothetical protein FLJ20712	0.3826	0.0016
FLJ30313	hypothetical protein FLJ30313	0.6121	0.0018
DDI2	DNA-damage inducible protein 2	0.1900	0.0020
MGC41945	hypothetical protein MGC41945	0.4552	0.0050
FLJ90680	FLJ90680 protein	0.3901	0.0056
C9orf9	chromosome 9 open reading frame 9	0.5910	0.0061
ADHFE1	alcohol dehydrogenase, iron containing, 1	0.5841	0.0070
FLJ22795	hypothetical protein FLJ22795	0.6537	0.0071
LOC339290	hypothetical protein LOC339290	0.5866	0.0074
ZNF552	zinc finger protein 552	0.4226	0.0075
KIAA1539	KIAA1539	0.4662	0.0083
RUFY2	RUN and FYVE domain containing 2	0.4475	0.0090
KIAA0420	KIAA0420 gene product	0.0938	0.0101
KIAA1666	KIAA1666 protein	0.5293	0.0108
LOC55565	hypothetical protein LOC55565	0.5013	0.0123
SYNJ2	synaptojanin 2	0.5414	0.0123

LOC57146	promethin	0.4374	0.0123
PKHD1	polycystic kidney and hepatic disease 1	0.3666	0.0131
R29124_1	carcinoembryonic antigen-related cell adhesion molecule (autosomal recessive)	0.2862	0.0149
CTDSP1	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small	0.3988	0.0112
FANCD2	Fanconi anemia, complementation group D2	3.1373	0.0110
SURF4	surfeit 4	1.9436	0.0089
C5orf18	chromosome 5 open reading frame 18	4.2410	0.0016
TRIM16	tripartite motif-containing 16	2.3662	0.0040
CKMT1	creatine kinase, mitochondrial 1 (ubiquitous)	2.1000	0.0059
C20orf179	chromosome 20 open reading frame 179	2.5898	0.0020
DPPA2	developmental pluripotency associated 2	50.2380	0.0125
TTC21A	tetratricopeptide repeat domain 21A	5.9986	0.0106
ZCWCC1	zinc finger, CW type with coiled-coil domain 1	3.2077	0.0003
CLDN15	claudin 15	8.4307	0.0147
C20orf22	chromosome 20 open reading frame 22	10.3375	0.0120
DHX35	DEAH (Asp-Glu-Ala-His) box polypeptide 35	2.3807	0.0140
C10orf81	chromosome 10 open reading frame 81	2.5719	0.0057
ZNF237	zinc finger protein 237	15.9584	0.0059
PSCDBP	pleckstrin homology, Sec7 and coiled-coil domains, binding protein	2.3044	0.0078
ASTN2	astrotactin 2	1.8333	0.0101
HIST2H3C	histone 2, H3c	3.5299	0.0003
DERP6	S-phase 2 protein	2.1299	0.0008
LNK	lymphocyte adaptor protein	2.6924	0.0012
LOC92305	hypothetical protein BC009331	6.0684	0.0015
MGC16028	MGC16028 similar to RIKEN cDNA 1700019E19 gene	4.4320	0.0017
SECP43	tRNA selenocysteine associated protein	24.8545	0.0017
ANKRD29	ankyrin repeat domain 29	56.6436	0.0019
KIAA0746	KIAA0746 protein	3.1993	0.0022
[DKFZp566 C0424]	putative MAPK activating protein PM20, PM21	6.9359	0.0022
[DKFZp762 I137]	hypothetical protein DKFZp762I137	2.6880	0.0024
FLJ43654	FLJ43654 protein	1.7922	0.0025
KIAA1799	KIAA1799 protein	1.7388	0.0030
HHIP	hedgehog interacting protein	4092.3492	0.0038
PSD3	pleckstrin and Sec7 domain containing 3	2.5929	0.0039
NRG4	neuregulin 4	3.2214	0.0043
FLJ20323	hypothetical protein FLJ20323	4.0887	0.0047
FAM33A	family with sequence similarity 33, member A	2.9785	0.0053
PTGES2	prostaglandin E synthase 2	2.6718	0.0054
RAMP	RA-regulated nuclear matrix-associated protein	2.9757	0.0055
KIAA0913	KIAA0913	5.6352	0.0056

FLJ12529	pre-mRNA cleavage factor I, 59 kDa subunit	3.0348	0.0057
KIAA1755	KIAA1755 protein	4.2521	0.0061
[DKFZP586 H2123]	regeneration associated muscle protease	5.7173	0.0064
HSU79274	protein predicted by clone 23733	3.4482	0.0072
HIT-40	zinc finger protein HIT-40	6.7390	0.0073
LOC284021	hypothetical protein LOC284021	3.1845	0.0075
OBSCN	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	5.5655	0.0076
MGC45491	hypothetical protein MGC45491	8.3547	0.0077
CSS3	chondroitin sulfate synthase 3	5.1775	0.0078
XIST	X (inactive)-specific transcript	18.2111	0.0083
ODC-p	ornithine decarboxylase-like	3.0672	0.0095
LOC349408	hypothetical protein LOC349408	2.4370	0.0097
PSD	pleckstrin and Sec7 domain containing	7.2301	0.0101
FLJ42953	FLJ42953 protein	2.9393	0.0103
KIAA0753	KIAA0753 gene product	2.4950	0.0103
MGC27277	hypothetical protein MGC27277	4.4653	0.0104
FLJ31164	hypothetical protein FLJ31164	2.4956	0.0108
MAC30	hypothetical protein MAC30	20.4965	0.0109
FLJ21736	esterase 31	2.9579	0.0112
LOC284454	hypothetical protein LOC284454	168.3068	0.0118
MGC34821	hypothetical protein MGC34821	2.5772	0.0119
CDCA2	cell division cycle associated 2	9.7505	0.0125
GPR55	G protein-coupled receptor 55	5.7368	0.0126
Ells1	hypothetical protein Ells1	3.2652	0.0129
AF1Q	ALL1-fused gene from chromosome 1q	3.1241	0.0134
CSGlcA-T	chondroitin sulfate glucuronyltransferase	3.2605	0.0134

*Fold change indicates pre-exercise intensity values divided by post-exercise values.

Classification based on GOTM software (http://genereg.ornl.gov.gotm). Only genes with identified HUGO names are included.

possible FDR. Many genes are functionally closely associated with each other, and thus they are not theoretically independent. Consequently, the differentially expressed genes can be categorized into a number of functional groups. Thus, taking this factor into consideration, the FDR would be less that 5% for both lean and obese samples.

In the subcutaneous adipose tissue of lean samples, in response to 7 days of aerobic exercise, there was a significant ($p < 0.01$) enrichment of down-regulated genes in 13 gene ontology categories **(Figure 3.1)**, such as dephosphorylation, macromolecule metabolism, negative regulation of metabolism, magnesium ion binding, phosphoprotein phosphatase activity, and primary active transporter activity to name a few. There was no significant enrichment of up-regulated genes in gene ontology categories for the lean samples. In the subcutaneous adipose tissue of obese samples, in response to 7 days of aerobic exercise at the same intensity level, there was a significant enrichment of down-regulated genes in 5 gene ontology categories (**Figure 3.2**), according to gene ontology analysis, such as membrane lipid metabolism, mitotic and cell cycle checkpoint, and transition metal, especially zinc, ion binding in obese samples. As well, there was a significant enrichment of up-regulated genes in 10 gene ontology categories for obese samples (**Figure 3.3**), such as membrane lipid metabolism, sphingolipid metabolism, ceramide metabolism, serine-type endopeptidase activity, and protease and endopeptidase inhibitor activity to name a few.

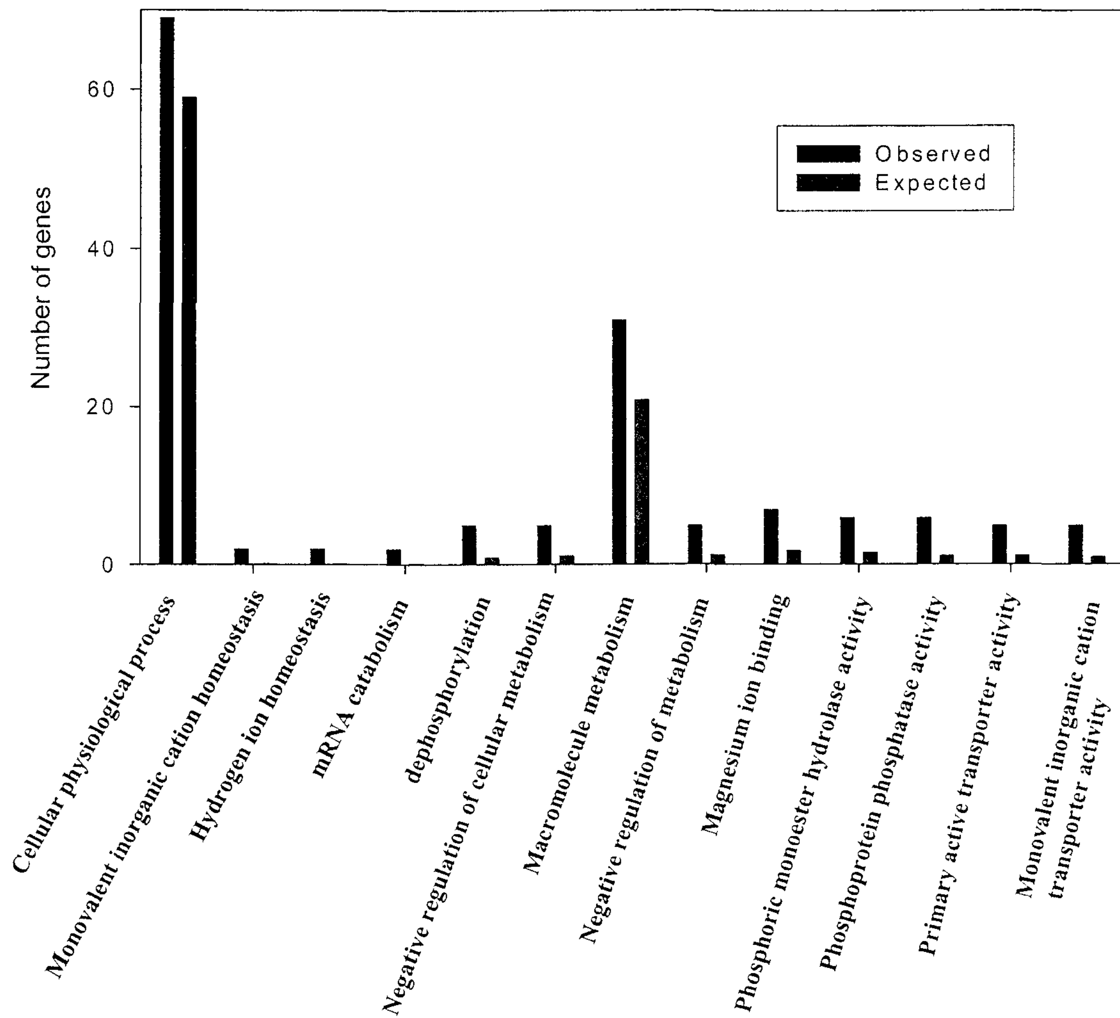

Figure 3.1 Enrichment of down-regulated genes in 13 gene ontology categories (in biological process and molecular function) of lean subjects in response to short-term aerobic exercise ($p < 0.01$). Genes may appear in more than one ontology category. Significance assessed using the Gene Ontology Tree Machine (http://genereg.ornl.gov/~gotm).

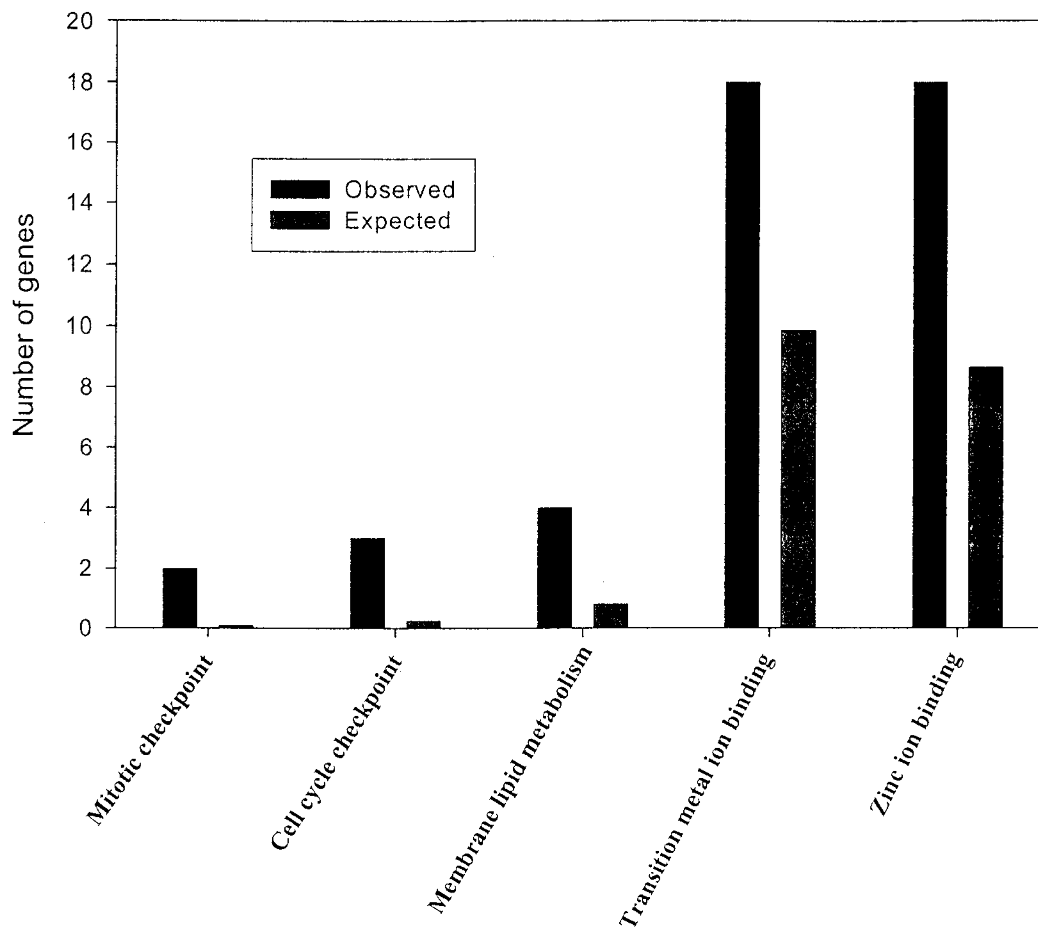

Figure 3.2 Enrichment of down-regulated genes in 5 gene ontology categories (in biological process and molecular function) of obese subjects in response to short-term aerobic exercise ($p < 0.01$). Genes may appear in more than one ontology category. Significance assessed using the Gene Ontology Tree Machine (http://genereg.ornl.gov/~gotm).

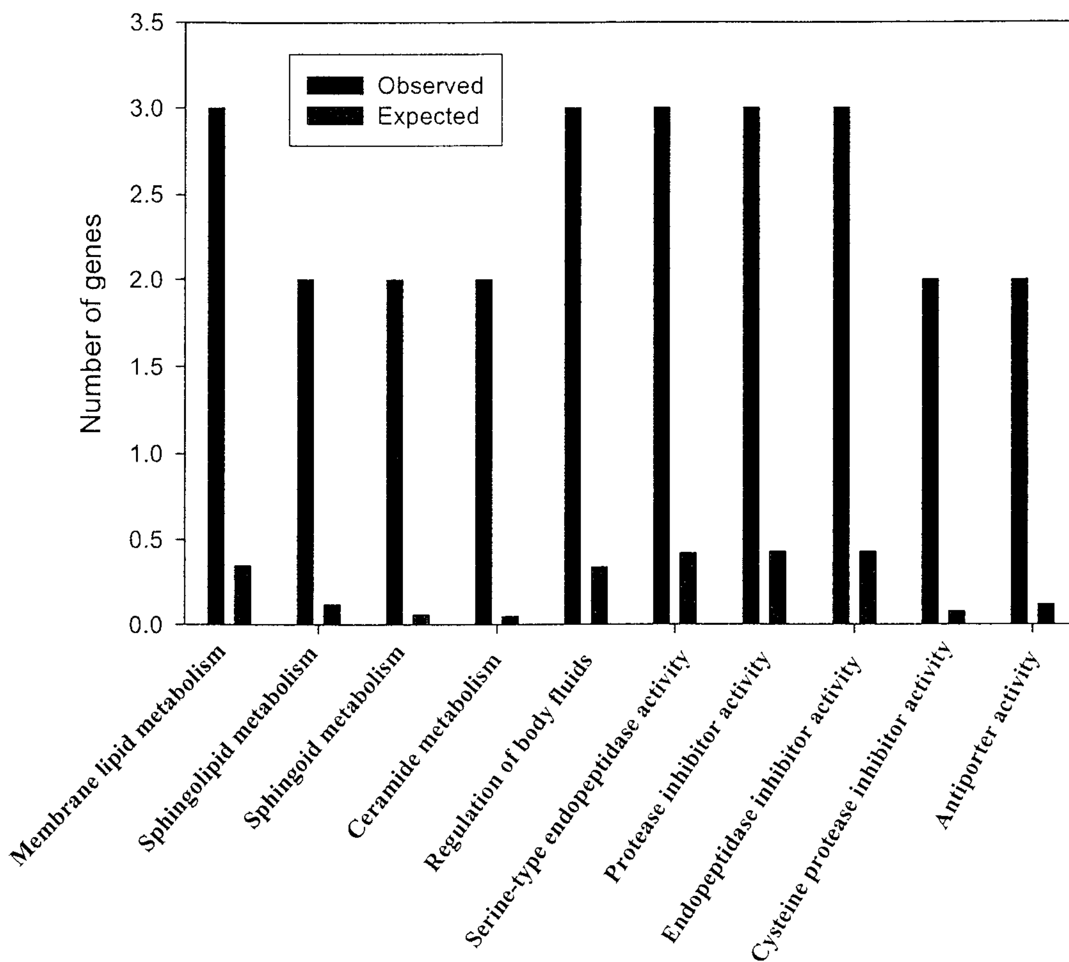

Figure 3.3 Enrichment of up-regulated genes in 10 gene ontology categories (in biological process and molecular function) of obese subjects in response to short-term aerobic exercise ($p < 0.01$). Genes may appear in more than one ontology category. Significance assessed using the Gene Ontology Tree Machine (http://genereg.ornl.gov/~gotm).

In the abdominal subcutaneous adipose tissue lean subjects, in response to aerobic exercise, 53 potential genes, without defined HUGO names, were differentially expressed (14 up regulated and 39 down regulated) **(Table 3.16)**. Eighty potential genes, without defined HUGO names, were differentially expressed (31 up regulated and 49 down regulated) in obese subjects **(Table 3.17)**.

Table 3.16 Significantly down- and up-regulated genes, without HUGO names, in lean subjects in response to short-term exercise

Systematic name	Description	Fold change	p value
Down-regulated			
AK057476	Homo sapiens cDNA FLJ32914 fis, clone TESTI2006409	2.5268	0.0001
A_23_P209527	Unknown	3.4973	0.0016
A_24_P58607	Unknown	2.0678	0.0018
THC2207101	ALU6_HUMAN (P39193) Alu subfamily SP sequence contamination warning entry, partial (18%)	2.0360	0.0024
ENST00000355343	Seven transmembrane helix receptor [Source:Uniprot/SPTREMBL; Acc:Q8NH82]	3.1860	0.0025
AF289590	Homo sapiens clone pp7583 unknown mRNA	11.5804	0.0027
NP109438	copper transporting P-type ATPase, ATP7B [human, Wilson disease patient, Peptide Partial, 38 aa]	5.4189	0.0033
AL522024	Homo sapiens NEUROBLASTOMA COT 10-NORMALIZED Homo sapiens cDNA clone CS0DB007YI07 3-PRIME, mRNA sequence	10.1536	0.0036
ENST00000314984	Seven transmembrane helix receptor [Source:Uniprot/SPTREMBL; Acc:Q8NH35]	3.7717	0.0047
AK096567	Homo sapiens cDNA FLJ39248 fis, clone OCBBF2008586	18.5505	0.0051
AK000038	Homo sapiens cDNA FLJ20031 fis, clone ADSU02180	4.0084	0.0051
AV739766	CB Homo sapiens cDNA clone CBDAGB02 5', mRNA sequence	4.8664	0.0054
THC2169084	Q99P30 Coenzyme A diphosphatase, partial (64%)	2.7922	0.0056
AK023328	Homo sapiens cDNA FLJ13266 fis, clone OVARC1000960	3.8714	0.0060
AL834124	Homo sapiens mRNA; cDNA DKFZp547C162 (from clone DKFZp547C162)	2.0906	0.0068
A_24_P298099	Unknown	2.8402	0.0077
A_32_P8806	Unknown	1.6836	0.0079
A_24_P84112	Unknown	2.0843	0.0079
THC2184722	Unknown	7.7821	0.0082

BM678922	UI-E-EO0-ahx-b-23-0-UI.s1 UI-E-EO0 Homo sapiens cDNA clone UI-E-EO0-ahx-b-23-0-UI 3', mRNA sequence	3.1434	0.0086
BC015836	Homo sapiens, clone IMAGE: 4293240, mRNA	2.2674	0.0087
AY358732	Homo sapiens clone DNA135173 KVVM3106 (UNQ3106) mRNA, complete cds	6.7423	0.0089
THC2141315	Unknown	3.3237	0.0094
THC2038739	Unknown	6.0296	0.0102
THC2198832	Q6IFN5 Olfactory receptor OR19-14, partial (71%)	2.0429	0.0102
A_23_P128897	Unknown	3.8107	0.0110
A_32_P78285	Unknown	3.3296	0.0110
A_24_P325533	Unknown	12.3972	0.0117
BI062366	IL3-UT0117-160301-502-D08 UT0117 Homo sapiens cDNA, mRNA sequence	12.4723	0.0119
THC2096232	Q7Z6F8 MGC18079, partial (7%)	5.1552	0.0125
A_32_P215745	Unknown	2.6438	0.0133
A_24_P349539	Unknown	1.7014	0.0133
AF279782	Homo sapiens clone P1 NTera2D1 teratocarcinoma mRNA	3.0588	0.0135
AI190733	qd61a01.x1 Soares_testis_NHT Homo sapiens cDNA clone IMAGE: 1733928 3', mRNA sequence	2.3098	0.0138
BG612665	602641001F1 NIH_MGC_61 Homo sapiens cDNA clone IMAGE: 4771873 5', mRNA sequence	5.3988	0.0142
A_24_P307075	Unknown	2.2367	0.0142
A_32_P228601	Unknown	1.8444	0.0143
A_23_P13232	Unknown	4.0263	0.0147
A_24_P281683	Unknown	2.1222	0.0149

Up-regulated

AK023038	Homo sapiens cDNA FLJ12976 fis, clone NT2RP2006258	0.6633	0.0005
THC2101189	Unknown	0.2889	0.0007
BM725480	UI-E-EJ0-aie-p-22-0-UI.r1 UI-E-EJ0 Homo sapiens cDNA clone UI-E-EJ0-aie-p-22-0-UI 5', mRNA sequence	0.2803	0.0012
CD630738	CD630738 56066364H1 FLP Homo sapiens cDNA, mRNA sequence	0.4062	0.0014

THC2164534	ALU1_HUMAN (P39188) Alu subfamily J sequence contamination warning entry, partial (12%)	0.3526	0.0017
AK023773	Homo sapiens cDNA FLJ13711 fis, clone PLACE2000379	0.5698	0.0029
THC2181597	Unknown	0.3074	0.0042
U44029	Human cathepsin B mRNA, 5' UTR variant	0.2075	0.0047
AK024241	Homo sapiens cDNA FLJ14179 fis, clone NT2RP2003668	0.3980	0.0059
A_32_P18300	Unknown	0.3677	0.0071
THC2089776	Unknown	0.2350	0.0078
THC2055711	Unknown	0.3948	0.0103
THC2141633	Q7NI46 (Q7NI46) Cytochrome c550, partial (8%)	0.2834	0.0134
A_23_P317548	Unknown	0.4220	0.0149

*Fold change indicates pre-exercise intensity values divided by post-exercise values.

Table 3.17 Significantly down- and up-regulated genes, without HUGO names, in obese subjects in response to short-term exercise

Systematic name	Description	Fold change	p value
Down-regulated			
K03192	Human cytochrome P-450 mRNA, partial	1.8421	0.0004
A_24_P929413	Unknown	4.7903	0.0006
A_32_P31206	Unknown	9.0128	0.0007
THC2203406	Unknown	3.7121	0.0009
A_24_P195454	Unknown	7.6128	0.0011
A_24_P101181	Unknown	4.5782	0.0014
THC2123705	Unknown	1.8557	0.0015
AW964144	EST376217 MAGE resequences, MAGH Homo sapiens cDNA, mRNA Sequence	3.3960	0.0017
THC2210842	Unknown	15.0467	0.0019
ENST00000321887	Unknown	2.5422	0.0024
AK022793	Homo sapiens cDNA FLJ12731 fis, clone NT2RP2000108	5.2731	0.0025
CD388102	AGENCOURT_14302060 NIH_ MGC_173 Homo sapiens cDNA 5', mRNA sequence	80.4062	0.0026
A_32_P105893	Unknown	2.7567	0.0029
THC2199235	GPS2_HUMAN (Q13227) G protein pathway suppressor 2 (GPS2 protein), partial (52%)	2.9029	0.0029
ENST00000308384	MHC class II HLA-SX-alpha gene. (Fragment). [Source:Uniprot/ SPTREMBL;Acc:Q30181]	2.9316	0.0029
BM665043	UI-E-CQ1-aev-p-07-0-UI.s1 UI-E-CQ1 Homo sapiens cDNA clone UI-E-CQ1-aev-p-07-0-UI 3', mRNA sequence	4.1893	0.0033
A_23_P134704	Unknown	2.5086	0.0037
BU618438	BU618438 UI-H-FH1-bfg-c-03-0-UI. s1 NCI_CGAP_FH1 Homo sapiens cDNA clone UI-H-FH1-bfg-c-03-0-UI 3', mRNA sequence	7.7169	0.0038
CR611166	full-length cDNA clone CS0CAP007YF02 of Thymus of Homo sapiens (human)	3.5653	0.0040
BI261159	BI261159 602968272F1 NIH_MGC_ 12 Homo sapiens cDNA clone IMAGE: 5107840 5', mRNA sequence	5.7604	0.0047

AL359605	Homo sapiens mRNA; cDNA DKFZp547G036 (from clone DKFZp547G036)	33.4759	0.0048
A_24_P383751	Unknown	20.3587	0.0054
BQ632351	BQ632351 il25h07.x1 HR85 islet Homo sapiens cDNA clone IMAGE: 6031332 3' similar to TR:Q9Z2X1 Q9Z2X1 RIBONUCLEOPROTEIN F. ;, mRNA sequence	3.2538	0.0057
AW178774	AW178774 PM0-HT0123-310899-001-b08 HT0123 Homo sapiens cDNA, mRNA sequence	3.3274	0.0059
ENST00000344214	Unknown	3.9833	0.0060
BC002886	Homo sapiens cDNA clone MGC: 11241 IMAGE:3939857, complete cds	4.0206	0.0063
AF075119	Homo sapiens full length insert cDNA ZD26E01	35.4745	0.0069
THC2054435	ALU1_HUMAN (P39188) Alu subfamily J sequence contamination warning entry, partial (13%)	3.2213	0.0079
A_23_P28927	Unknown	4.1034	0.0090
THC2177003	Unknown	2.4535	0.0090
A_24_P307126	Unknown	9.1521	0.0093
BC011136	Homo sapiens, clone IMAGE: 3448872, mRNA, partial cds	2.8523	0.0098
THC2166062	Unknown	12.7600	0.0099
THC2210983	ALU1_HUMAN (P39188) Alu subfamily J sequence contamination warning entry, partial (7%)	1.8061	0.0102
A_24_P268474	Unknown	4.6119	0.0103
A_24_P268474	Unknown	4.6119	0.0103
AK022865	Homo sapiens cDNA FLJ12803 fis, clone NT2RP2002172	1.9371	0.0105
AK124847	Homo sapiens cDNA FLJ42857 fis, clone BRHIP2009340	10.7109	0.0105
THC2164534	ALU1_HUMAN (P39188) Alu subfamily J sequence contamination warning entry, partial (12%)	2.0865	0.0108
AF130063	Homo sapiens clone FLB7723 PRO2055 mRNA, complete cds	39.7652	0.0109
THC2178569	IL10_HUMAN (P22301) Interleukin-10 precursor (IL-10) (Cytokine synthesis inhibitory factor) (CSIF), partial (7%)	5.2657	0.0111
AK098257	Homo sapiens cDNA FLJ40938 fis, clone UTERU2007639	2.8171	0.0113
ENST00000328398	Homo sapiens cDNA FLJ25433 fis, clone TST06506	4.1934	0.0116

BX397553	Homo sapiens PLACENTA COT 25-NORMALIZED Homo sapiens cDNA clone CS0DI039YG01 3-PRIME, mRNA sequence	91.8707	0.0124
A_32_P212820	Unknown	1.6892	0.0124
THC2131693	Unknown	4.6878	0.0126
THC2098249	SET_HUMAN (Q01105) SET protein (Phosphatase 2A inhibitor I2PP2A) (I-2PP2A) (Template activating factor I) (TAF-I) (HLA-DR associated protein II) (PHAPII) (Inhibitor of granzyme A-activated DNase) (IGAAD), partial (66%)	7.8556	0.0132
A_24_P920699	Unknown	30.0948	0.0141
THC2136793	Unknown	4.0488	0.0145
A_24_P255865	Unknown	2.4373	0.0147

Up-regulated

THC2120948	Unknown	0.1772	0.0003
THC2122677	Q6P1A9 Ribosomal protein L7a, partial (11%)	0.3429	0.0003
CR610892	full-length cDNA clone CS0DD006YA06 of Neuroblastoma Cot 50-normalized of Homo sapiens (human)	0.5257	0.0012
M84605	Human putative opioid receptor mRNA, complete cds	0.3857	0.0017
A_24_P33403	Unknown	0.5920	0.0018
THC2230645	Unknown	0.6478	0.0021
AK025388	Homo sapiens cDNA: FLJ21735 fis, clone COLF3350	0.4567	0.0026
BC066916	Homo sapiens cDNA clone IMAGE: 4838452, partial cds	0.4099	0.0027
THC2096438	GP20_HUMAN (Q99678) Probable G protein-coupled receptor GPR20, complete	0.4451	0.0027
THC2138308	Unknown	0.5225	0.0029
BX091213	Soares_multiple_sclerosis_2NbHMSP Homo sapiens cDNA clone IMAGp998H14631 ; IMAGE:284605, mRNA sequence	0.2751	0.0031
AK092378	Homo sapiens cDNA FLJ35059 fis, clone OCBBF2018827	0.3937	0.0031
A_24_P917971	Unknown	0.3446	0.0044

AF229804	Homo sapiens endozepine-like protein type 2 mutant mRNA, complete cds	0.2297	0.0046
ENST00000354689	Myosin-reactive immunoglobulin heavy chain variable region (Fragment). [Source:Uniprot/ SPTREMBL;Acc:Q9UL88]	0.2961	0.0047
A_23_P89529	Unknown	0.3428	0.0050
THC2097125	1207289A reverse transcriptase related protein. {Homo sapiens;}, partial (3%)	0.2921	0.0050
BC063702	Homo sapiens cDNA clone IMAGE: 4538703, partial cds	0.4259	0.0058
N34499	N34499 yy55g04.s1 Soares_multiple_ sclerosis_2NbHMSP Homo sapiens cDNA clone IMAGE:277494 3' similar to contains Alu repetitive element; contains element MER9 repetitive element;, mRNA sequence	0.3576	0.0059
THC2063896	Unknown	0.1180	0.0063
BX108468	Soares_testis_NHT Homo sapiens cDNA clone IMAGp998J144405, mRNA sequence	0.4796	0.0067
AK057923	Homo sapiens cDNA FLJ25194 fis, clone REC04095	0.3382	0.0074
BC043523	Homo sapiens, clone IMAGE:5166551, mRNA	0.4064	0.0087
A_24_P413286	Unknown	0.2731	0.0089
BI490920	BI490920 df01a10.w1 Morton Fetal Cochlea Homo sapiens cDNA clone IMAGE:2482074 3', mRNA sequence	0.2011	0.0093
THC2186083	Unknown	0.6317	0.0093
A_24_P84719	Unknown	0.4875	0.0102
A_24_P930482	Unknown	0.1375	0.0110
AK024371	Homo sapiens cDNA FLJ14309 fis, clone PLACE3000221	0.5410	0.0112
A_32_P101019	Unknown	0.1235	0.0121
THC2214096	Unknown	0.2751	0.0140

*Fold change indicates pre-exercise intensity values divided by post-exercise values.

Chapter 4: Discussion

4a: Candidate-gene association study

Ghrelin has been recognized for its involvement in the coordination of energy balance and weight regulation, and while its dysregulation may be important in the etiology of obesity (Eisenstein and Greenberg, 2003), the roles of genetic variations in this gene on obesity in humans are still not clear. In the present study, we have investigated the association between three SNPs in the ghrelin precursor gene with body composition and serum lipids in a large population. The results of the current study do not support a significant role for genetic variations within the ghrelin precursor gene in the regulation of body composition or serum lipid profiles in the Newfoundland population.

In the present study, the genetic association analyses were performed from three aspects. First, we determined if a difference in allele frequencies existed between lean and obese groups, while accounting for confounding factors, such as gender and age. Since it is the amount of body fat, rather than the amount of excess body weight, that determines the health risks of obesity (World Health Organization, 1998), we classified our cohort according to adiposity (%BF, %LF, and %TF) using DXA, which is one of the best methods for the determination of body composition (Prior *et al.*, 1997). Also, as a comparison, we classified our subjects according to BMI, which is known to provide an estimation of adiposity. The sole use of BMI, a field method, in analyses will introduce unreliability into reported findings. In the present study, for instance, we found a large

difference when classifying our subjects as obese and non-obese according to BMI and %BF using DXA. When females were classified according to %BF, 49% of subjects were considered lean and 51% were obese, while according to BMI, 72% were classified as lean and only 28% as obese. A very similar misclassification also occurred for males as well, thus indicating that BMI is not a reliable classification method of body composition for analyses in association studies of this type. Upon analysis of our results, we found no significant differences of allele frequencies for any SNP investigated between the lean and obese groups when classified according to %BF, %TF, %LF, or BMI.

The second type of analysis investigated whether a genotype effect existed; that is, whether a certain genotype was associated with significantly different body composition (%BF, %TF, %LF, and BMI) or serum lipid parameter. Although not statistically significant, a trend was observed in males whereby the GG genotype of Exon 1 had higher body fat and as well the G allele appeared to associate with higher levels of TGs and lower levels HDL-C. The polymorphism locate in Exon 1 would have the greatest "potential" of affect functioning ghrelin levels. This polymorphism results in an amino acid substitution in the pre- or pro- portion of the ghrelin peptide and thus may potentially affect further processing, such as proteolytic cleavage or acylation in this case. It is interesting, despite not being statistically significant (possibly related to sample size), that males homozygotes for the GG genotype tended to have a greater likelihood of being obese and a poorer lipid profile. Lastly, in addition to allele and genotype associations, we also investigated haplotype associations, since it has been suggest that the determination of haplotypes, or combinations of SNPs, that are in linkage disequilibrium

may offer more power to detect associations than simply measuring individual SNPs (Drysdale *et al.*, 2000). In agreement with our other results, we found that no significant difference existed between the different haplotype groups for body composition or for any serum lipid parameter.

The current status of genetic association studies on *GHRL* has indicated that SNP 346G→A, which leads to the replacement of arginine with glutamine at amino acid 51 of the preproghrelin sequence (amino acid 28 of the mature peptide), was associated with obesity in the Swedish population (Ukkola *et al.*, 2001). They discovered that the arg51gln mutation, in heterozygote form, was found in 6 of the 96 severe obese female subjects, but not among the 96 non-obese female control subjects ($p<0.05$). The association between the arg51gln polymorphism and obesity, however, could not be confirmed by this group using a larger cohort (Ukkola *et al.*, 2002) or by another group using a German cohort (Hinney *et al.*, 2002). In the present study, it should be noted that the three SNPs investigated did not correspond to amino acid alterations within the mature ghrelin protein. However, a previous study has shown that children carrying the SNP 408C→A, which leads to the substitution of leucine for methionine at amino acid 72 of the preproghrelin sequence, had significantly higher BMI and an earlier age of obesity onset compared to those children carrying only the wildtype allele (Korbonits *et al.*, 2002).

In summary, since no significant difference existed between the allele, genotype, or haplotype groups for any SNP investigated for body composition (after accounting for gender and age) or for any serum lipid parameter (after accounting for gender, age, and %BF), the results suggest no relationship exists. Thus, the results of the present study do

not support a significant role for genetic variations within the ghrelin precursor gene in the regulation of body compositions and serum lipid profiles in the NL population. Furthermore, since candidate gene association studies have been questioned because of non-replication of results, in part due to small sample size and over-interpretation of data, our study demonstrated a comprehensive study design whereby these factors were minimized.

4b: Changes in adipose tissue gene expression in response to short-term aerobic exercise

The most common and detrimental metabolic diseases affecting over 50% of the adult population are obesity and its associated metabolic pathologies (Wellen and Hotamisligil, 2003). The present study investigated the differential global mRNA expression profiles of abdominal subcutaneous adipose tissue of lean and obese males, in response to a 7-day aerobic exercise protocol (60% VO_2 max), using long oligonucleotide whole human genome microarrays. All individuals in the present study were males and within a narrow age range in an effort to reduce the effects of gender and age on the global mRNA expression patterns.

We also investigated the effect of aerobic exercise on body composition and biochemical parameters in lean and obese men. We found that the response to short-term aerobic exercise differed in lean and obese subjects. Obese subjects, for instance, significantly decreased their percentage of trunk fat after the exercise protocol. Furthermore, triglyceride levels also showed a similar decreasing trend ($p = 0.07$). Lean individuals, on the other hand, did not experience similar changes, despite being exposed to the same intensity exercise regimen. Other studies have also reported similar results whereby obese individuals experienced a significant reduction in abdominal fat in response to aerobic exercise ($\leq$ 70% VO_2 max) without a corresponding change in BMI (Ross *et al.*, 2000;Ross *et al.*, 2004). Another study reported that, following a prolonged exercise intervention, the bodyweight of obese children significantly decreased, while the normal- weight control group actually gained weight, possibly due to an increase in lean

body mass (Hayashi *et al.*, 1987). Responses to exercise interventions, however, often vary considerably between individuals, and previous research has indicated that the response to exercise may be significantly affected by genetic components (Bouchard *et al.*, 1999). Consequently, the main goal of the present study was to investigate the molecular genetic basis of metabolic routes within abdominal subcutaneous adipose tissue of obese and lean men in response to exercise through the analysis of global mRNA expression patterns. It should be realized, however, that not all differentially expressed genes result in a functional effect. Also, some differentially expressed genes are a result of secondary affects due to the obese state. These genes are difficult to distinguish and other methodologies, such as animal studies and in vitro studies, are needed to help clarify this issue. This is beyond the scope of the present thesis.

4b.1: Changes in gene expression as a response to short-term aerobic exercise in obese men

Regular physical activity is known to contribute to the cure of obesity-associated metabolic diseases (Richterova *et al.*, 2004), and it appears to be one of the major factors determining the long-term success of weight loss (Pavlou *et al.*, 1989). One possible explanation for this observation may stem from the fact that regular physical activity protects against diseases characterized by chronic, low-grade systemic inflammation (Petersen and Pedersen, 2005), such as obesity (Das, 2001;Festa *et al.*, 2001;Weyer *et al.*, 2002;Bullo *et al.*, 2003). It has been shown that circulating levels of several markers of inflammation, including pro-inflammatory cytokines and acute-phase proteins such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), and

secretory phospholipase A2 (sPLS2), are elevated in obese persons. Pro-inflammatory cytokines refer to low molecular weight (8,000 to 40,000 dalton) proteins that promote inflammation, whereas cytokines that suppress the activity of proinflammatory cytokines are referred to as anti-inflammatory cytokines (Dinarello, 2000a). Obesity is also believed to be a consequence of inflammatory disease, and not vice-versa (Das, 2001). Moreover, the inflammatory response associated with obesity is thought to be triggered by, and reside mainly within, adipose tissue (Wellen and Hotamisligil, 2003).

In obese samples in our study, gene ontology analysis indicated a significant enrichment of up-regulated genes in sphingolipid metabolism and ceramide metabolism in response to aerobic exercise. Because TNF-α and interleukin-1 (IL-1) utilize the sphingomyelin pathway, it has been suggested that this signalling pathway is used in inflammation and immune responses (Kolesnick and Golde, 1994;Huwiler *et al.*, 1996), but its role has not been extensively studied with respect to obesity. The sphingomyelin pathway is an evolutionarily conserved, ubiquitous signalling system that is initiated by the hydrolysis of sphingomyelin, a plasma membrane phospholipid (Pena *et al.*, 1997). The phosphodiester bond of sphingomyelin is hydrolyzed by acid and neutral sphinogmyelinase isoforms generating ceramide and phosphocholine. Ceramide serves as a second messenger of this pathway by interacting with a number of direct targets including, ceramide-activated protein kinase (CAPK) (Mathias *et al.*, 1991), ceramide-activated protein phosphatase (CAPP) (Dobrowsky and Hannun, 1993), and protein kinase Cζ (PKCζ) (Muller *et al.*, 1995). As well, ceramide interacts with several signalling systems, such as mitogen-activated protein kinase (MAPK), Jun kinase (JNK),

caspase, and mitochondrial signalling systems, which affect numerous cellular functions, including proliferation, differentiation, growth arrest, and death (Mathias *et al.*, 1998). Furthermore, in response to diverse stresses, mammalian systems generate ceramide through rapid activation of neutral and acidic sphingomyelinases (Hannun *et al.*, 2001), and signalling through ceramide appears to play a role in the development of human diseases, including insulin resistance associated with obesity and diabetes (Mathias *et al.*, 1998). Our results indicated that within the subcutaneous adipose tissue of obese men there was nearly a 12-fold decrease in the expression of neutral sphinogmyelinase 2 (also known as sphingomyelin phosphodiesterase 3, *SMPD3*) in response to acute aerobic exercise. It has been reported that neutral sphinogmyelinase 2 is one of the key enzymes involved in ceramide generation, and that it is a TNF-responsive enzyme that plays an important role in the regulation of sphingomyelin metabolism (Marchesini *et al.*, 2003). It has also been reported that prolonged exercise of moderate intensity leads to reduced levels of ceramide in both fast-twitch red and slow-twitch red rat muscle, along with a corresponding decrease in neutral sphinogmyelinase activity (Dobrzyn and Gorski, 2002). To our knowledge, this is the first study to show a decrease in the expression of *SMPD3* within the subcutaneous adipose tissue of obese individuals in response to aerobic exercise. A down-regulation of *SMPD3* expression suggests a possible decrease in ceramide generation. Ceramide is known to affect many cell types and tissues with a wide range of effects, however, most research has focused on its role in apoptosis (Mathias *et al.*, 1998). For instance, ceramide has been observed to bind and activate protein kinase c-Raf in rat mesangial cells, which subsequently leads to the activation of the MAPK cascade, which signals inflammation (Huwiler *et al.*, 1996). In 3T3-L1

adipocytes, ceramide caused a similar effect to that of TNF-α in down-regulating the glucose transporter GLUT4 and impeding glucose uptake (Long and Pekala, 1996). This is a similar situation that often appears in the subcutaneous adipose tissue of obese individuals (Garvey *et al.*, 1991). Thus, it appears that the subcutaneous adipose tissue of obese individuals may respond to aerobic exercise by decreasing the amount of ceramide, which, in turn, may alleviate its harmful effects. Ceramide, however, can also be phosphorylated to ceramide-1-phosphate (C1P) by ceramide kinase (CERK) (Sugiura *et al.*, 2002). Obese individuals in our study experienced nearly a 2-fold increase in the expression of *CERK* in response to aerobic exercise. It has been shown that C1P is located mainly in the neutrophil plasma membrane and that it promotes phagolysosome formation, a critical event in phagocytosis (Hinkovska-Galcheva *et al.*, 1998). It has also been demonstrated that in fibroblasts C1P stimulates DNA synthesis, but does not alter the phosphorylation state of MAPK (Gomez-Munoz *et al.*, 1997). Furthermore, it is believed that the conversion of ceramide to C1P may serve as a mechanism to terminate the proapoptotic actions of ceramide (Sugiura *et al.*, 2002). Thus, an increase in *CERK* expression may increase the conversion of ceramide to C1P, which may, in turn, further decrease the levels of ceramide. We also found 2 other genes differentially regulated in obese individuals with roles in ceramide metabolism. The expression of *ASAH2* (N-acylsphingosine amidohydrolase 2) was decreased 2-fold in response to exercise, while the expression of *ST3GAL5* (ST3 β-galactoside α-2,3-sialyltransferase 5) was increased nearly 2-fold. The functions of both ASAH2 and ST3GAL5, however, have not been extensively studied with respect to ceramide metabolism. Taken together, it appears that

the abdominal subcutaneous adipose tissue of obese individuals respond to short-term aerobic exercise through the regulation of the ceramide/sphingomyelin pathway, possibly to reduce the harmful inflammatory effects of ceramide.

The regulation of sphingomylinases has also been linked to several cell surface receptors, including the interleukin-1 receptor (Mathias *et al.*, 1993). It is interleukin 1 receptor, type 1 (IL1R1), and not interleukin 1 receptor, type II (IL1RII), which is the signal-transducing receptor upon which IL-1 binds (Dinarello, 2000a). It is known that many cell types release IL-1 when exposed to an inflammatory environment (Huwiler *et al.*, 1996). IL-1 is a cytokine that stimulates the production of highly inflammatory substances, such as prostaglandins and nitric oxide, as well as promoting the synthesis of small proteins that aid in the entry of neutrophils, macrophages, and lymphocytes into tissues (Dinarello, 2000b). It is believed that the increased production of IL-1 that accompanies human inflammatory diseases contributes to the pathologic process following binding to its receptor. Thus, it has been suggested that administering agents that reduce the effects of IL-1 should improve inflammatory diseases. However, it is believed that the IL-1-mediated disease severity is regulated at the level of ligand production and activity, and not at the receptor level (Dinarello, 2005). It is known that type 1 IL-1 receptors are only increased 2- or 3-fold in the disease state, whereas IL-1 is increased over 100-fold. We found that in obese individuals there was nearly a 2-fold increase in expression of *IL1R1* in response to aerobic exercise, with no significant change in *IL-1* expression. Since IL-1-mediated disease severity is not regulated at the receptor level, it is difficult to evaluate the effect of a possible increase in IL1R1 in response to aerobic exercise. It has been previously reported, however, that physical

activity in obese individuals elicits a temporary inflammatory response with elevated plasma levels of IL-1, but not elevated *IL-1* gene expression levels in blood mononuclear cells (Moldoveanu *et al.*, 2000).

It is known that human adipose tissue expresses a local renin-angiotensin system that includes angiotensinogen, angiotensin converting enzyme (ACE), renin, and the type 1 angiotensin receptor (Schling *et al.*, 1999). Angiotensin II, the activation product of angiotensinogen, is a vasopressor hormone that regulates blood pressure and volume through its interaction with type 1 angiotensin II receptors (Murphy *et al.*, 1991). Angiotensin II, however, is also a proinflammatory peptide, and it has been suggested that the obstruction of its action by an angiotensin receptor blocker will likely exert an anti-inflammatory action (Phillips and Kagiyama, 2002). We found that the expression of *AGTR1* (angiotensin II, type 1) was up-regulated nearly 5-fold in obese individuals after exercise. An increase in *AGTR1* expression is likely to lead to an increase in angiotensin II receptor levels. An increase in receptor levels increases the possibility of angiotensin II exerting an inflammatory action, however, we did not find an increase in the expression of angiotensinogen or *ACE*. Thus, it is difficult to determine the net effect of an increase in the expression of *AGTR1*.

Physical activity is also known to promote the activation of the sympathetic nervous system (SNS) as well as increase the levels of circulating catecholamines (Galbo *et al.*, 1975;Christensen and Galbo, 1983). Catecholamines (epinephrine and norepinephrine) activate the lipolytic cascade through their interaction with β-adrenoceptors (β_1, β_2, and β_3), G-protein-coupled receptors, on the plasma membrane of

adipocytes (Horowitz, 2003). Catecholamines interaction with α_2-adrenoceptors, on the other hand, inhibits lipolytic activity. Upon the binding of catecholamines to the β-adrenoceptors, an activated complex is formed with a stimulatory G protein (Gs), and this complex is responsible for the activation of adenylate cyclase, which is responsible for the increase in the concentration of cyclic adenosine monophosphate (cAMP), and consequently the activation of cAMP-dependent protein kinase (PKA) (Lange, 2004) (**Figure 4.1**). The holoenzyme of PKA is a tetramer of 2 regulatory and 2 catalytic subunits (Orstavik *et al.*, 2005). PKA activation occurs upon the binding of 2 cAMP molecules to each regulatory subunit, which results in a conformational change that releases the active catalytic subunits. PKA is responsible for the phosphorylation of hormone sensitive lipase (HSL), generally though to be the rate-limiting enzyme, as well as perilipin, a lipid droplet surface protein (Tansey *et al.*, 2004). Our results indicate that in obese individuals there is over a 6-fold decrease in the catalytic alpha subunit of PKA (*PRKACA*) expression in response to acute aerobic exercise. The vital role of PKA, especially the catalytic alpha subunit, has previously been demonstrated. It was shown that a null mutation in the catalytic alpha subunit of PKA, which is the major catalytic subunit, lead to early postnatal lethality in the majority of the Cα knockout mice (Skalhegg *et al.*, 2002). Thus, due to the fact that the alpha catalytic subunit is the major catalytic subunit of PKA, it is likely that there is a decrease in the phosphorylation of HSL and perilipin in obese individuals. Since an increase in PKA phosphorylation of HSL and the perilipins is known to cause a dramatic increase in lipolysis (Holm, 2003), it is plausible that obese individuals have a decreased rate of lipolysis in response to acute

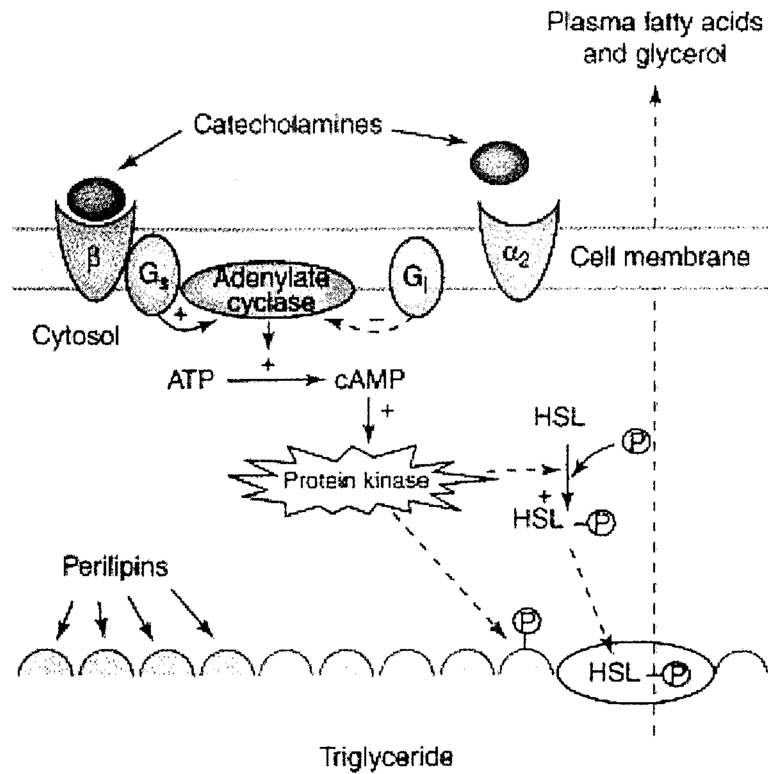

Figure 4.1 The activation of the lipolytic cascade through the interaction of catecholamines with the β-adrenoceptor (Horowitz, 2003).

aerobic exercise. To our knowledge, this is the first study to demonstrate that the expression of *PRKACA* is down-regulated in obese men in response to aerobic exercise. This result is also consistent with a previous study that demonstrated in males that the lipolytic response to exercise decreases with increasing adiposity (Mittendorfer *et al.*, 2004). They claim that the decrease in lipolytic rate limits the availability of plasma fatty acids as a fuel during exercise. We, however, also found that obese individuals have an up-regulation of a fatty acid transporter (*SLC27A6*) by nearly 2-fold. The up-regulation of *SLC27A6* might serve as a compensatory response to the reduced lipolytic rate or the increased demand on fatty acids during exercise. The actual role of HSL, however, in the lipolysis pathway is that, when phosphorylated, it moves from the cytosol of the adipocyte to the surface of the lipid droplet, where phosphorylated perilipin A permits access to intracellular TGs, so that efficient TG hydrolysis can occur (Sztalryd *et al.*, 2003) (**Figure 4.1**). It is, however, adipose triglyceride lipase (ATGL), rather than HSL, that predominantly hydrolyzes the first ester bond of TGs (Zimmermann *et al.*, 2004). HSL cleaves TGs as well as diglycerides (DGs), however, its specific activity for DGs is 10 fold that for TGs (Fredrikson *et al.*, 1981). Thus, it is both ATGL and HSL that catabolize stored TGs in adipose tissue of mammals (Zimmermann *et al.*, 2004). In our case, if the phosphorylation of HSL and perilipin were decreased, the rate of movement of phosphorylated HSL from the cytosol to the surface would be decreased, as would its access to TGs for hydrolysis.

4b.2: Changes in gene expression as a response to short-term aerobic exercise in lean men

In response to short-term aerobic exercise, lean individuals seem to regulate lipolysis at a number of levels. For instance, there appears to be regulation at the level of cAMP. We found that, in response to exercise, lean individuals had a down-regulation of adenylate cyclase 6 (*ADCY6*) expression by nearly 5-fold. Adenylate cyclase 6 is a membrane-associated enzyme that catalyzes the formation of cAMP. Thus, it appears that exercise may lead to a decreased level of cAMP, which could potentially lead to a decrease in the rate of lipolysis. However, we also report that lean individuals had an up-regulation of expression of adenylate cyclase activating polypeptide 1 (*ADCYAP1*), a gene that appears in the 2004 human obesity gene map (Perusse *et al.*, 2005), by approximately 2-fold. The encoded protein of *ADCYAP1* stimulates adenylate cyclase and subsequently increases the level of cAMP. Thus, an up-regulation of this gene will likely increase the level of cAMP. Taken together, both ADCY6 and ADCYAP1 may possibly work in unison to regulate the level of cAMP, and consequently possibly regulate the rate of lipolysis. Another level of regulation appears to be by protein phosphatase-1 (PP1) at the surface of the lipid droplet. PP1 is a serine/threonine-specific protein phosphatase involved in the dephosphorylation of numerous proteins, and work in opposition to the protein kinases to control the level of phosphorylation. PP1 has 3 catalytic subunits: alpha, beta (also known as delta), and gamma. It has been shown that the dephosphorylation of perilipin in adipocytes is mediated through PP1 (Clifford *et al.*, 1998). Dephosphorylated perilipin would deny access of HSL to intracellular TGs, and thus have an anti-lipolytic effect. We report that, in response to exercise, lean individuals

had nearly a 4-fold decrease in the mRNA for protein phosphatase 1, catalytic subunit, β isoform (*PPP1CB*). Consequently, it appears that the down-regulation of *PPP1CB* will likely have a lipolytic effect. The expression of *PPP1CB* may serve as another point of regulation for lipolysis. To our knowledge, this is the first finding that *PPP1CB* expression is down-regulation in lean individuals in response to aerobic exercise. This finding is also in agreement with the notion that the surface of the lipid droplet is a central site of regulation of lipolysis (Tansey *et al.*, 2004). There also appears to be regulation at the TG level via acyl-CoA:diacylglycerol acyltransferase 2 (*DGAT2*; also known as diacylglycerol O-acyltransferase homolog 2). DGAT2 has been recognized as a second mammalian DGAT with high expression in white adipose tissue, which is responsible for the final step in TG synthesis and believed to play a significant role in mammalian TG metabolism (Cases *et al.*, 2001). A recent gene knockout study in mice (Dgat2$^{-/-}$) demonstrated that DGAT2 is the DGAT responsible for the majority of triglyceride synthesis and that DGAT2 is required for survival (Stone *et al.*, 2004). DGAT2-deficient mice were reported to be lipopenic, with severely reduced triglyceride content in their tissues. Furthermore, since it has been reported that the tissue expression pattern of DGAT2 in mice is similar to that in humans (Cases *et al.*, 2001), it is suggested that DGAT2 plays a similar essential role in triglyceride metabolism in humans (Stone *et al.*, 2004). Lean individuals in our study, in response to exercise, experienced an up-regulation of *DGAT2* expression by approximately 3-fold. Thus, it is possible that the up-regulation of *DGAT2* will lead to a rise in TG synthesis and thus an increase in the availability of TGs for hydrolysis. Furthermore, it is possible that the up-regulation of

DGAT2 when lipolysis is up-regulated serves as a protective function against the accumulating free fatty acids inside the cells. Free fatty acids can behave like detergents. However, we also report a down-regulation of very low-density lipoprotein receptor (*VLDLR*) expression by approximately 4-fold. VLDLR is known for its involvement in the delivery of fatty acids to the adipose tissue and building of adipocyte TG stores (Tacken *et al.*, 2001). Thus, a decrease in the expression of *VLDLR* might decrease the availability of TGs for hydrolysis. The down-regulation of *VLDLR*, however, might be an indictor of a reduced availability of TGs in circulation. Nevertheless, it is possible that DGAT2 and VLDLR work in concert to regulate the level of TGs available for hydrolysis, and consequently potentially regulate the rate of lipolysis. In this case, the rate of lipolysis in lean individuals in response to exercise is likely increased. Many other studies have demonstrated that the increased energy demands of the body during physical activity are met, in part, by an increased rate of lipolysis in adipose tissue (Wolfe *et al.*, 1990;Klein *et al.*, 1994;Horowitz *et al.*, 1997;Stich and Berlan, 2004).

In lean individuals, in response to exercise, there seemed to be regulation of the insulin-signalling pathway through protein tyrosine phosphatases (PTPs). PTPs are involved in many cell-signalling systems, whereby they serve to dephosphorylate tyrosine residues of proteins (Stoker, 2005). Two main classes of PTPs are the classical receptor PTPs and the classical non-receptor PTPs. The PTPs that have been implicated in the dephosphorylation of the insulin receptor (IR) have received growing attention in the field of type 2 diabetes and obesity, since their inhibition would be expected to prolong insulin signalling and thereby facilitate glucose uptake, and in turn, lower blood glucose (Asante-Appiah and Kennedy, 2003). For instance, it was demonstrated that *PTP1B* knockout

mice were resistant to weight gain and remained insulin sensitive on a high-fat diet, while the wild-type mice rapidly gained weight and became insulin resistant (Elchebly *et al.*, 1999). It has been suggested that the loss of PTP1B specifically in the adipose tissue of the *PTP1B* knockout mice is a contributing factor for the observed obesity resistance (Asante-Appiah and Kennedy, 2003). Another PTP that has been implicated in the insulin-signalling pathway and the dephosphorylation of IR is SHP2 (also known as PTPN11), which falls into the non-receptor class. It has been shown that expression of *SHP2* in a transgenic mouse model lead to an insulin-resistant phenotype, thus indicating that it is a negative modulator of insulin signalling (Maegawa *et al.*, 1999). In our study, there was a 3-fold decrease in expression of *PTPN11* (*SHP2*) in lean individuals in response to exercise. Furthermore, there was a decrease in the expression of 2 other PTPs, one falling into the receptor class, *PTPRD* (10-fold decrease), and the other falling into the non-receptor class, *PTPN3* (2-fold decrease). Despite limited research on PTPRD and PTPN3, it is suggested that a wide range of PTPs could dephosphorylate IR and significantly influence the level of insulin signalling (Stoker, 2005). To our knowledge, this is the first study to reveal a decrease in the expression of PTPs in the abdominal subcutaneous adipose tissue of lean individuals in response to aerobic exercise. A possible decrease in the level of PTPs could potentially enhance insulin sensitivity and enable the facilitation of glucose uptake during exercise in lean individuals. This hypothesis is consistent with the fact that physical exercise, via mechanisms not fully clear, increases muscle glucose uptake, enhances post-exercise insulin sensitivity and leads to fatty acid oxidation in muscle (Fujii *et al.*, 2004).

Another interesting gene that we found up-regulated (4-fold) in the subcutaneous adipose tissue of lean individuals in response to aerobic exercise was peroxisome proliferative activated receptor, alpha (*PPARA*), a gene which appears in the 2004 human obesity gene map (Perusse *et al.*, 2005). PPARA, along with 2 other subtypes of PPAR, G (gamma) and D (delta), belong to the superfamily of nuclear hormone receptors (Berger and Moller, 2002). PPARs are lipid sensors that serve as transcriptional regulators of numerous genes involved in nutrient metabolism and energy homeostasis (Berger *et al.*, 2005). The crucial role of PPARA in energy homeostasis has been shown in PPARA null mice, which are characterized by hyperlipidemia, hypoglycermia, hypoketonimia, and hepatic steastosis upon fasting (Kersten *et al.*, 1999). A mutation (L162V) within PPARA has also been associated with reduced body fat percentage and BMI (Bosse *et al.*, 2003). Furthermore, since it has been shown that PPARA agonists decrease TG levels and increase HDL-C levels (Linton and Fazio, 2000), a possible increase in PPARA levels in lean individual in response to exercise may exert a similar effect to meet the increased demand of the body for fuel. Another gene in the PPAR gene family, which also appears in the 2004 human obesity gene map, is PPARG co-activator 1 (*PPARGC1A*) (Perusse *et al.*, 2005). Polymorphisms within this gene are associated with total body fat, BMI, and waist and hip circumferences (Esterbauer *et al.*, 2002). Furthermore, it has been shown that PGC1-alpha (also known as PPARGC1A) null mice were lean and resistant to diet-induced obesity, thus indicating an important role for PGC1-alpha in the control of energy metabolism (Lin *et al.*, 2004). We report the down-regulation (2-fold) of peroxisome proliferative activated receptor, gamma, coactivator-related 1 (*PPRC1*) in lean individuals in response to exercise. Although the biological

function of PPRC1 is still not clear, it is likely that it has a similar function to that of PPARG co-activator 1. To our knowledge, this is the first finding of the differential expression of *PPARA* and *PPRC1* in lean individuals in response to aerobic exercise. Taken together, in response to exercise, the differential regulation of PPARA and PPRC1 may function to favourably control energy metabolism in lean individuals.

Interestingly, in lean individuals in response to exercise there appeared to be a decrease in the generation of adenosine triphosphate (ATP) within the abdominal subcutaneous adipose tissue. We report a down-regulation of mitochondrial aconitase 2 (*ACO2*) expression by 2-fold. Mitochondrial aconitase catalyzes the conversion of citrate to isocitrate within the tricarboxylic acid (TCA) cycle. Inhibition of aconitase is known to divert metabolism from energy production to fat synthesis (Wlodek and Gonzales, 2003). The down-regulation of *ACO2* in lean individuals may possibly aim to restore the amount of TGs that were mobilized during exercise. Also, it is possible that, since TG synthesis requires more energy from ATP than lipolysis, an adaptation to a new steady state is occurring within the adipocytes where there is less TG synthesis compared with lipolysis. Less energy (ATP) is needed so fewer enzymes in ATP generating pathways are required and thus less mRNA for these enzymes are produced.

4b.3: A comparison of lean and obese individuals in their genome-wide expression response to short-term aerobic exercise

The first major difference that existed between lean and obese individuals in response to aerobic exercise was that the differential regulation of genes involved in

inflammation was unique to the subcutaneous adipose tissue of obese individuals. This finding is consistent with previous literature since obesity is associated with chronic, low-grade systemic inflammation. We found that within the abdominal subcutaneous adipose tissue of obese individuals, in response to acute aerobic exercise, there was nearly a 12-fold decrease in the expression of *SMPD3* as well as a 2-fold increase in the expression of *CERK*, which likely lead to a reduction in the levels of ceramide. We also found the differential regulation of other genes involved in ceramide metabolism (*ASAH2* and *ST3GAL5*), as well as other genes associated with inflammation (*IL1RII* and *AGTR1*). Since this is the first study, to our knowledge, to find the differential regulation of these 6 inflammatory related genes within the abdominal subcutaneous adipose tissue in response to aerobic exercise, it provides insight into the mechanisms of inflammation associated with the obese state.

The second difference that existed between lean obese individuals in response to aerobic exercise was the regulation of lipolysis. In obese individuals, our results indicated a likely decrease in the rate of lipolysis in response to exercise. Obese individuals experienced a 6-fold decrease in *PRKACA* expression, which likely lead to a decrease in the phosphorylation of HSL and the perilipins, as well as a compensatory 2-fold increase in the expression of *SLC27A6* to the reduced lipolytic rate or the increased demand on fatty acids. Lean individuals, on the other hand, experienced a tight regulation of lipolysis at several different levels with a possible increase in rate. There was regulation at the cAMP level with ADCY6 and ADCYAP1, regulation at the surface of the lipid droplet via dephosphorylation of perilipins by PPP1CB, and regulation at the triglyceride level by DGAT2 and VLDLR. The likely decrease in the rate of lipolysis in

obese individuals, despite aerobic exercise training, suggests a genetic basis of impairment in lipolysis regulation. However, it has been suggested that obese subjects who show impairments in the regulation of lipolysis could have their impairment corrected by dietary intervention (Stich and Berlan, 2004). To our knowledge, this is the first study to indicate that the differential regulation of numerous genes in the lipolytic pathway may be the molecular explanation for the differences in the regulation of lipolysis in response to aerobic exercise that exists between lean and obese individuals.

The third main difference that existed in our subjects in response to aerobic exercise was the down-regulation of protein tyrosine phosphatases within the abdominal subcutaneous adipose tissue of lean individuals. Since the significant down-regulation of PTPs were not observed in obese individuals, this indicates that the beneficial effect of exercise on insulin sensitivity in obese individuals may not go through the PTPs linked pathway. To our knowledge, this is the first study to provide a possible explanation of why insulin sensitivity is improved in lean individuals compared to obese individuals through the differential regulation of genes in the PTP pathway (*PTPN11*, *PTPRD*, and *PTPN3*). Furthermore, given that PTPs play a role in the regulation of the insulin-signalling pathway, they may represent potential pharmacological targets in insulin-resistant obesity.

The fourth major difference between lean and obese individuals was the differentially regulation of peroxisome proliferative activated receptors in response to aerobic exercise within the lean. Since it is believed that the modulation of PPAR activity might serve as an effective treatment for maladies associated with obesity (Berger *et al.*, 2005), and given the fact that obese individuals become fatigued faster than lean

individuals during physical exercise (Mattsson *et al.*, 1997), the differential regulation of *PPARA* and *PPRC1* may function to favourably control energy metabolism in lean individuals, while not in obese. To our knowledge, this is the first study to indicate that the differential regulation of *PPARA* and *PPRC1*, and, although only speculation, it may provide the molecular explanation of why obese individuals are more prone to fatigue during physical activity.

Chapter 5: Concluding remarks

Obesity rates among Canadian children and adults have increased dramatically during the past 25 years (Statistics Canada, 2005). For instance, obesity rates have tripled for adolescents aged 12 to 17 and doubled for adults aged 25 to 34. The obesity epidemic is of great concern for the health and well being of many Canadians, especially in Newfoundlanders and Labradoreans where the prevalence of obesity is significantly higher than the national average.

The current study has utilized two very different approaches to study the genetic factors that are involved in the common form of obesity. First, we have used the candidate gene association study approach to determine if the ghrelin precursor gene was associated with obesity phenotypes in the Newfoundland and Labrador population. The ghrelin precursor gene has been considered a genetic culprit involved in obesity, as it appears in the 2004 human obesity gene map (Perusse *et al.*, 2005). We have demonstrated that genetic variants within the ghrelin precursor gene do not appear to have a significant association with obesity phenotypes in the Newfoundland and Labrador population. Second, we have used microarray technology to simultaneously quantitate whole human genome mRNAs from abdominal subcutaneous adipose tissue of lean and obese young men to provide a comprehensive assessment of expression level in response to a 7-day aerobic exercise protocol. This particular study generated an enormous amount of data, which was deciphered using a number of methods. First, strict inclusion criteria were set to demonstrate significant expression changes in lean and obese samples in

response to exercise. Second, bioinformatic tools, such as gene ontology and systemic pathway analysis, were used to reveal the most relevant genes and pathways.

The current microarray expression study has numerous important discoveries in terms of the gene expression patterns and differences between obese and lean subjects. First, our data were consistent with previous reports that indicate inflammatory pathways are involved in obesity, and we demonstrated that aerobic exercise might reduce the effects of inflammation through the regulation of the ceramide/sphingomyelin pathway, with a possible reduction in the level of ceramide within the abdominal subcutaneous adipose tissue. To our knowledge, this is the first study to demonstrate the likely reduction of ceramide levels within the subcutaneous adipose tissue of obese men in response to aerobic exercise. Secondly, we also found that the decrease in the rate of lipolysis in response to acute aerobic exercise in obese individuals is probably through the dephosphorylation of HSL and perilipin by the down-regulation of *PRKACA*. Thirdly, our results indicated that short-term aerobic exercise in lean individuals might alter the insulin-signalling pathway through the regulation of protein tyrosine phosphatases, and that lean individuals might benefit from exercise by energy metabolism control through the differential regulation of the peroxisome proliferative activated receptors. Moreover, our findings provide insight into the role that physical exercise plays in contributing to controlling obesity by revealing possible mechanistic pathways that may be involved. As well, our study revealed numerous novel genes that warrant further research in the field of obesity and exercise physiology. In the future, these genes may serve as potential novel drugable targets, which may be invaluable to help against the global epidemic of obesity.

Chapter 6: Limitations of the present research

6.1: Candidate-gene association study

1) Although 1182 subjects is a reasonable number to perform association analyses, a second cohort would be ideal to replicate our findings.

6.2: Genome-wide expression study of adipose tissue in response to acute aerobic exercise using microarray technology

1) The sample size of this study was small (5 lean and 5 obese males) due to the high cost of the microarrays, as well as the invasiveness of the adipose tissue biopsy.
2) The current obesity candidate genes identified from this study were obtained from the subcutaneous adipose tissue. Genes with tissue specific expression, such as in the liver and the skeletal muscle, may offer a more complete picture of the mechanisms involved in obesity.
3) The current study investigated only males. The regulation of certain genes and pathways may be gender specific, thus an investigation into females is imperative.
4) The current study investigated only a very narrow age range (20 to 27 years). Since the regulation of certain genes and pathways may be age specific, it is necessary to study other age ranges.

5) Since transcript levels do not always correlated with protein abundance levels, and given that cellular functions are generally carried out by proteins, future studies need to address the involvement of different metabolic pathways using protein arrays or other proteomic methods.
6) The present study only investigated abdominal subcutaneous adipose tissue, and thus we cannot conclude that these data are representative of other adipose tissue depots in the body.

Chapter 7: Future plans

7.1: Candidate-gene association study

1) An independent family-based association study is in progress, and will eventually serve as a second cohort to replicate our findings.

7.2: Genome-wide expression study of adipose tissue in response to acute aerobic exercise using microarray technology

1) Subject recruitment is ongoing.
2) Expansion of the study to include the investigation of skeletal muscle is in progress.
3) Depending on future funding, the study will expand to include the investigation of females.
4) Depending on future funding, the study will expand to include the investigation of different age ranges.
5) Functional studies using protein microarrays are planned in the future with the collaboration of other researchers.

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Appendix 1

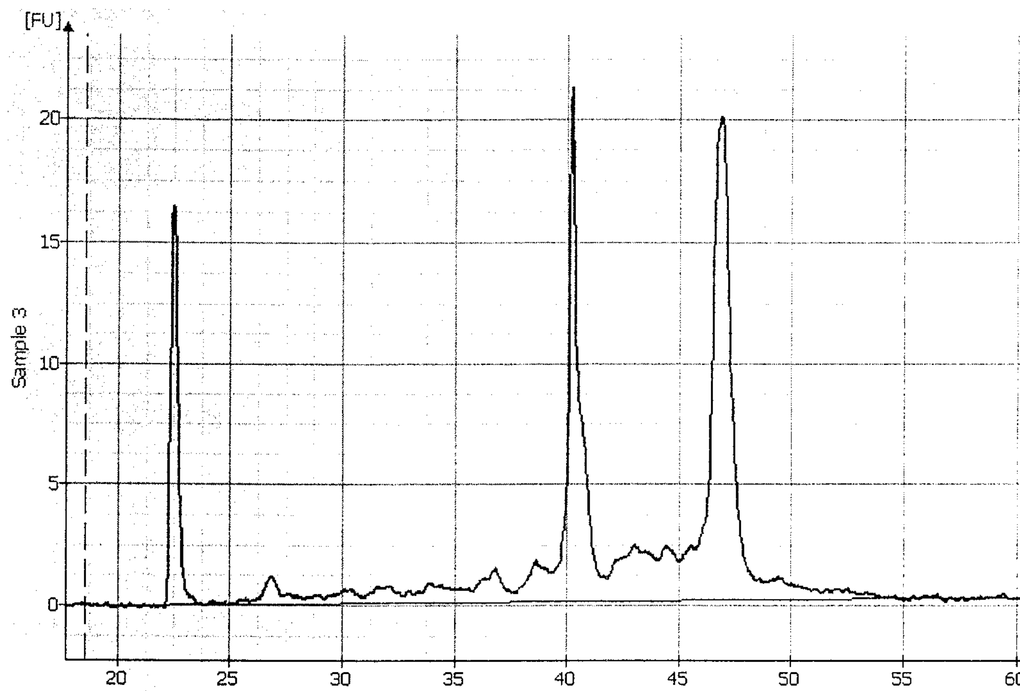

Figure A-1 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 1 (pre-exercise).

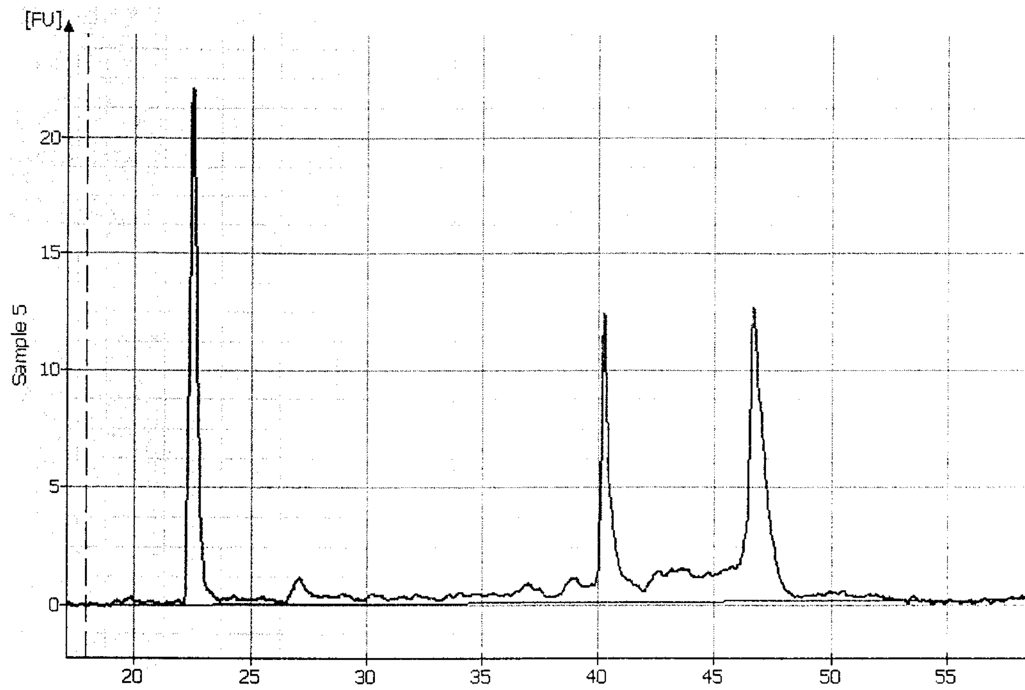

Figure A-2 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 1 (post exercise).

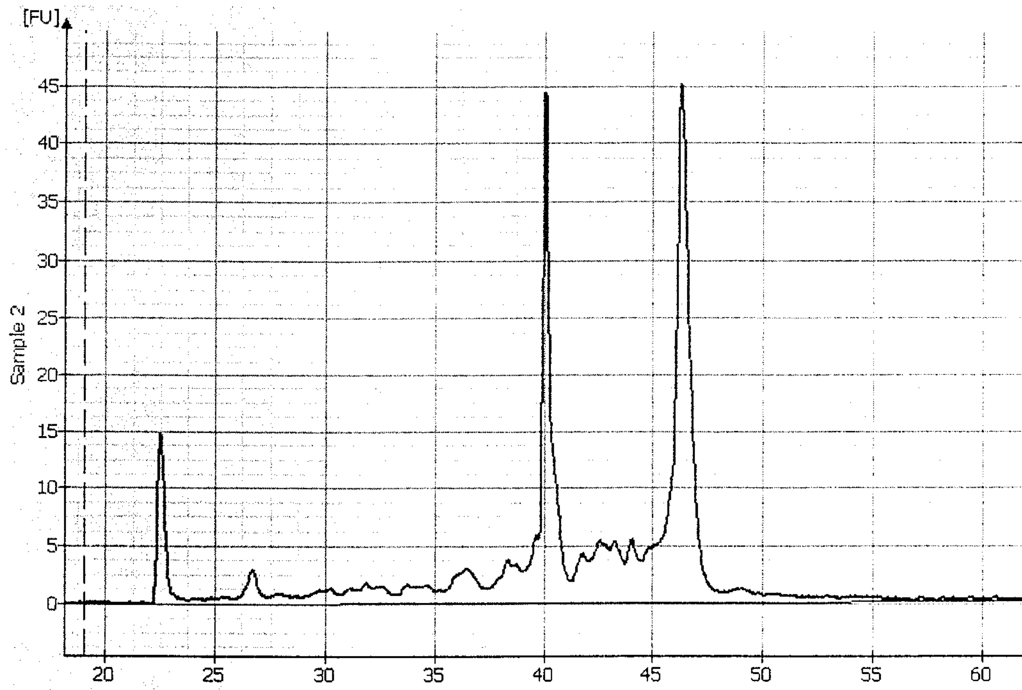

Figure A-3 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 2 (pre-exercise).

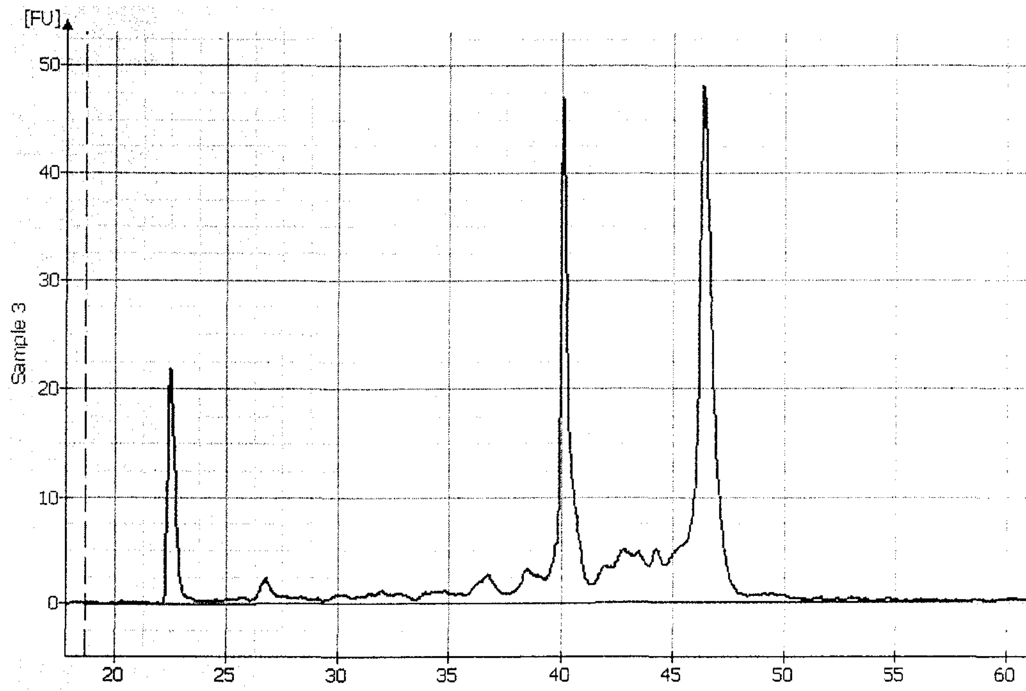

Figure A-4 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 2 (post exercise).

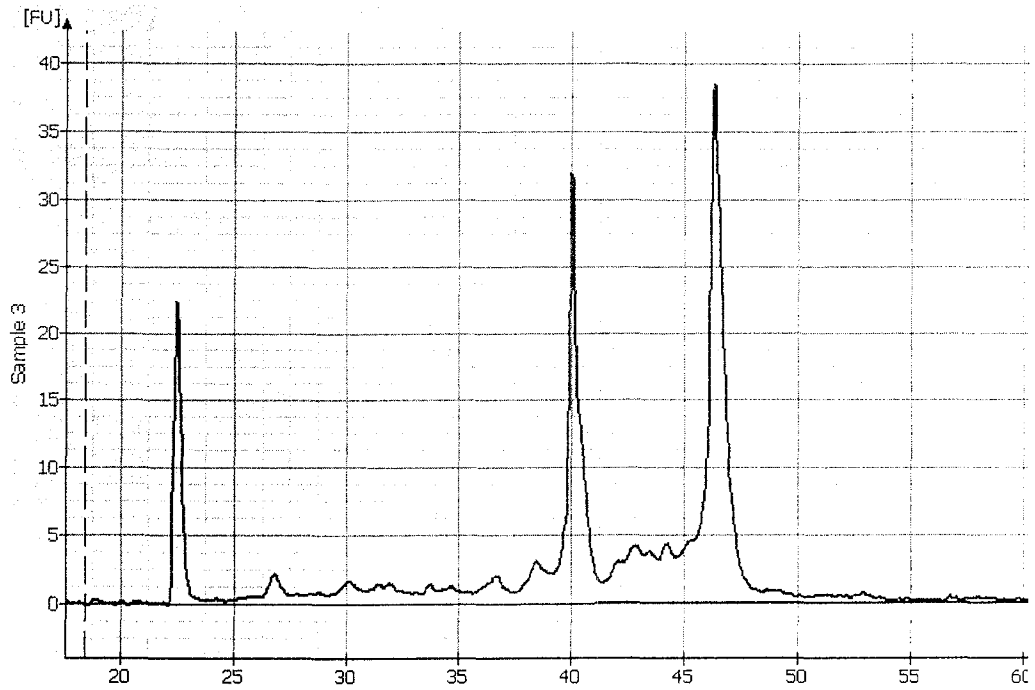

Figure A-5 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 3 (pre-exercise).

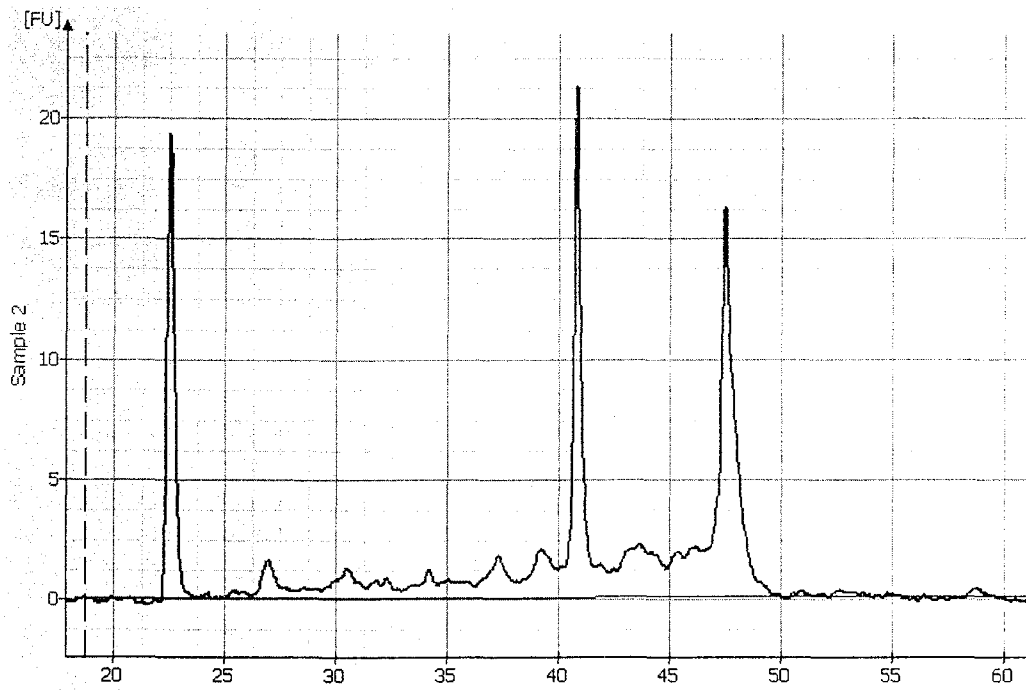

Figure A-6 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 3 (post exercise).

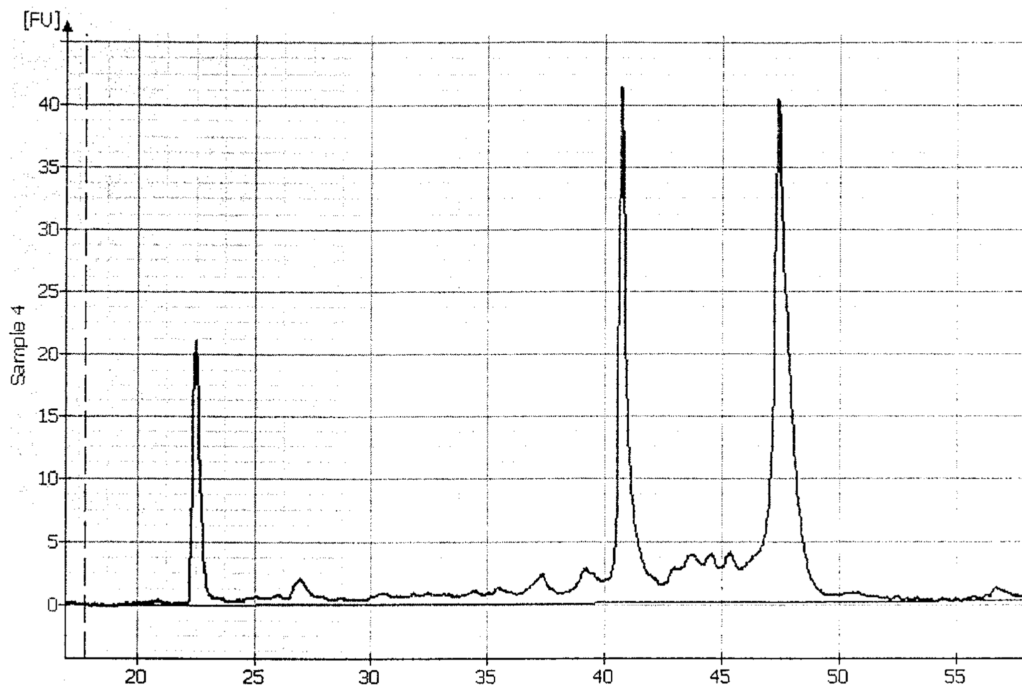

Figure A-7 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 4 (pre-exercise).

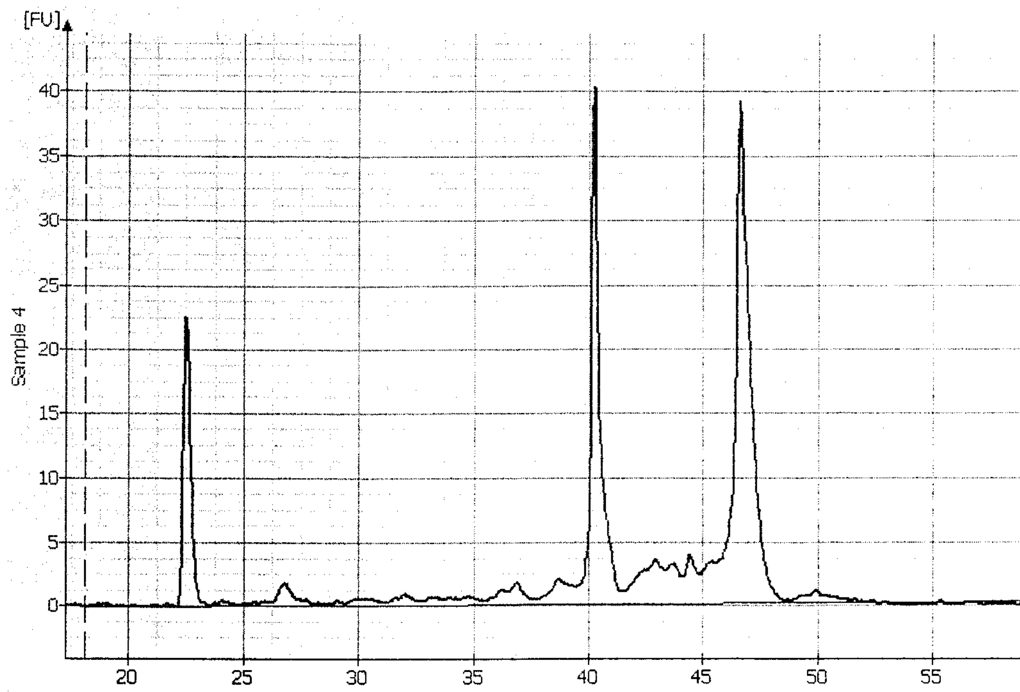

Figure A-8 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 4 (post exercise).

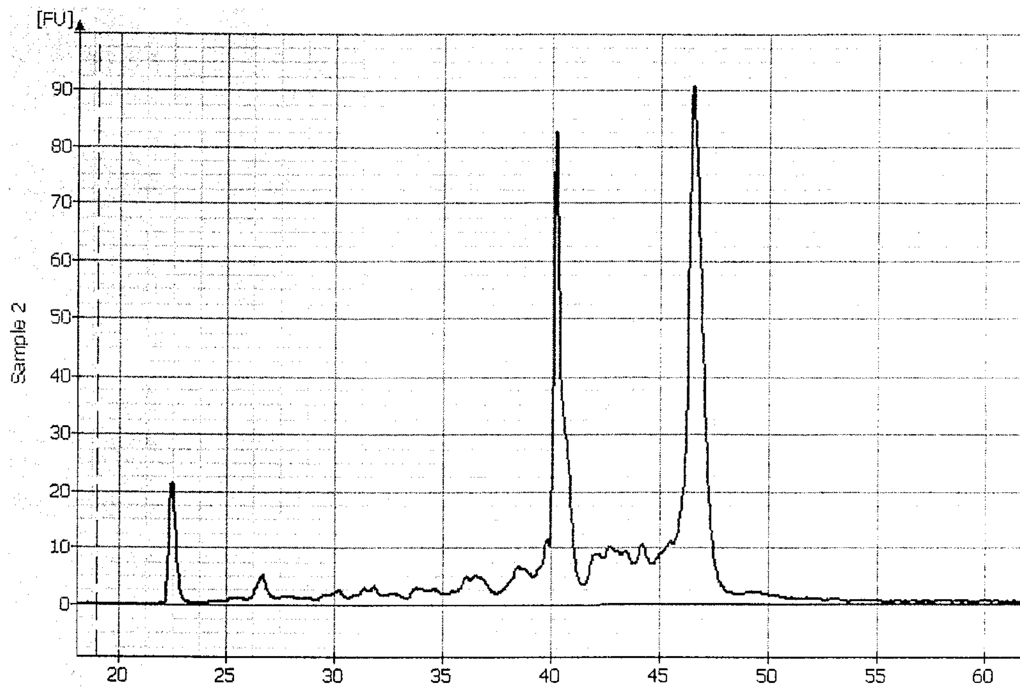

Figure A-9 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 5 (pre-exercise).

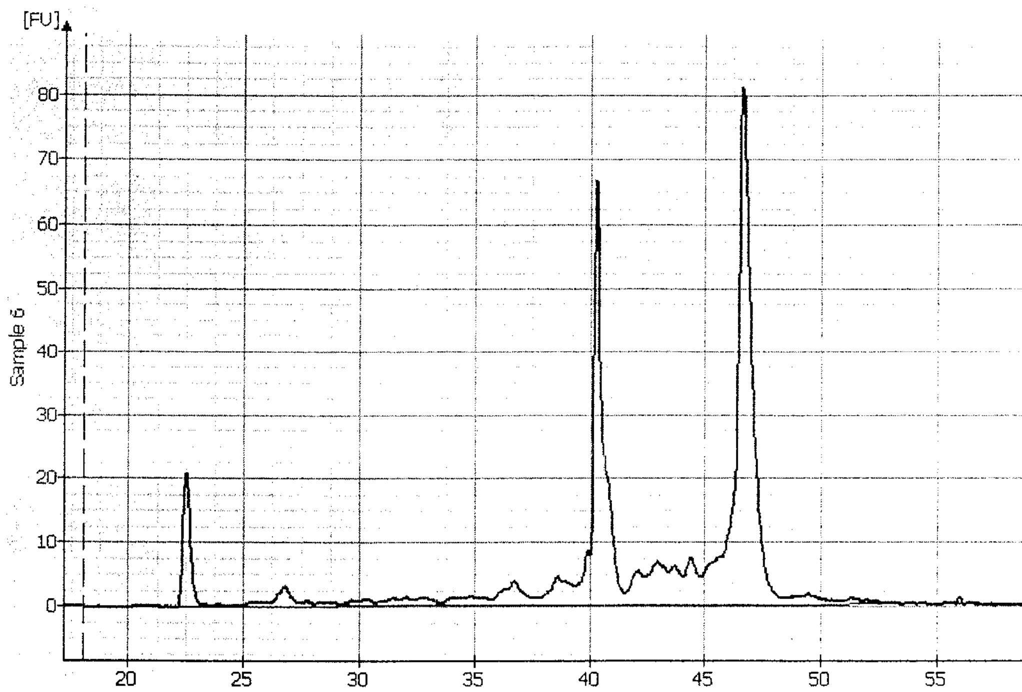

Figure A-10 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from lean subject 5 (post exercise).

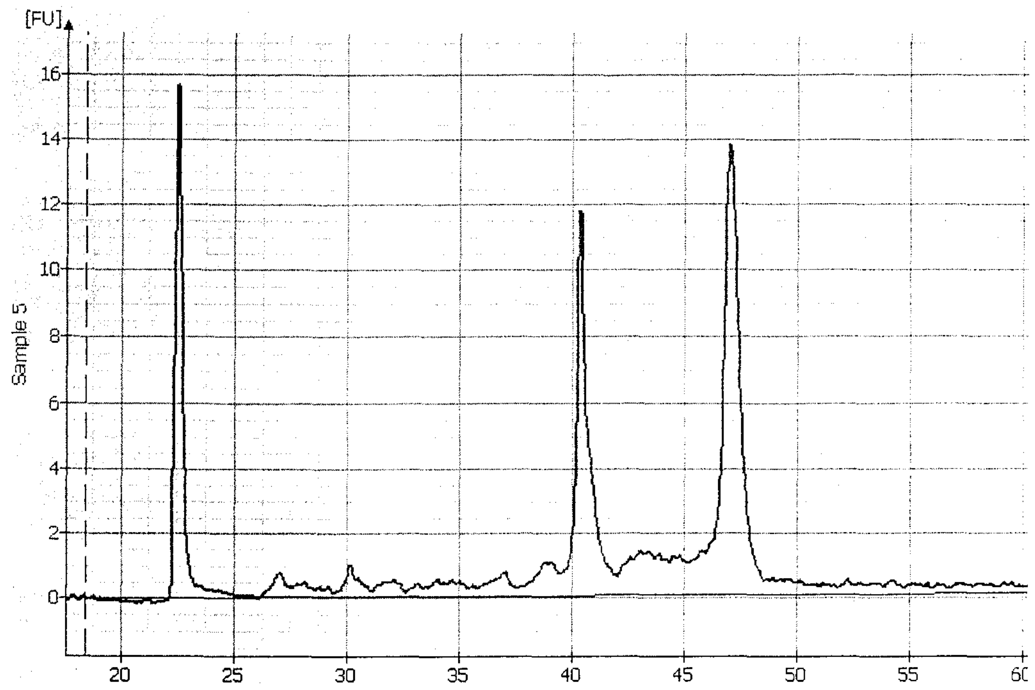

Figure A-11 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 1 (pre-exercise).

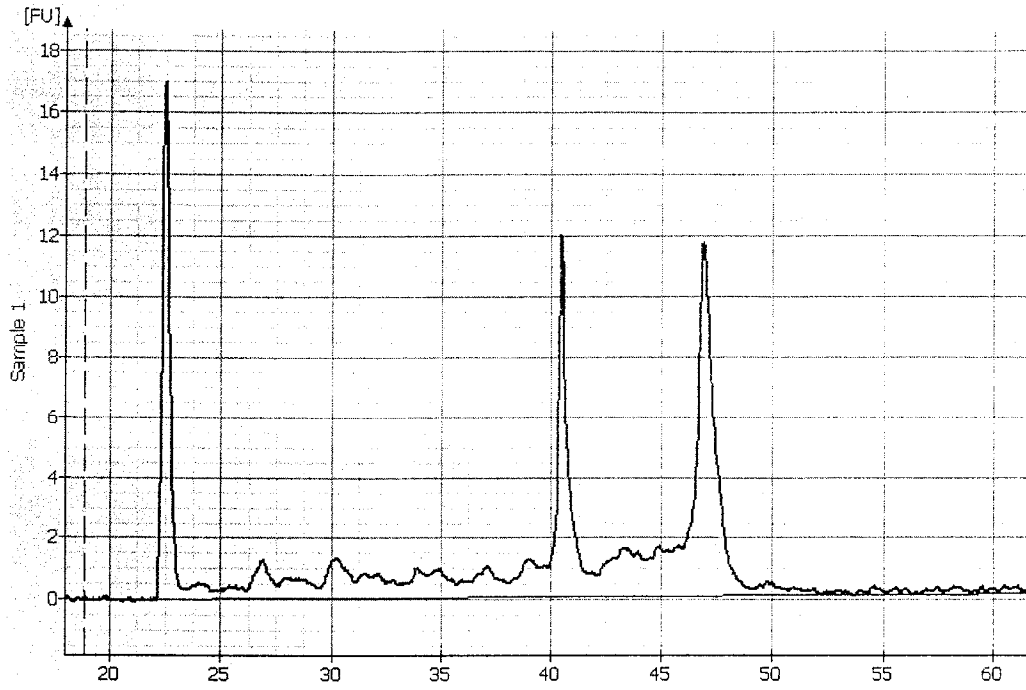

Figure A-12 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 1 (post exercise).

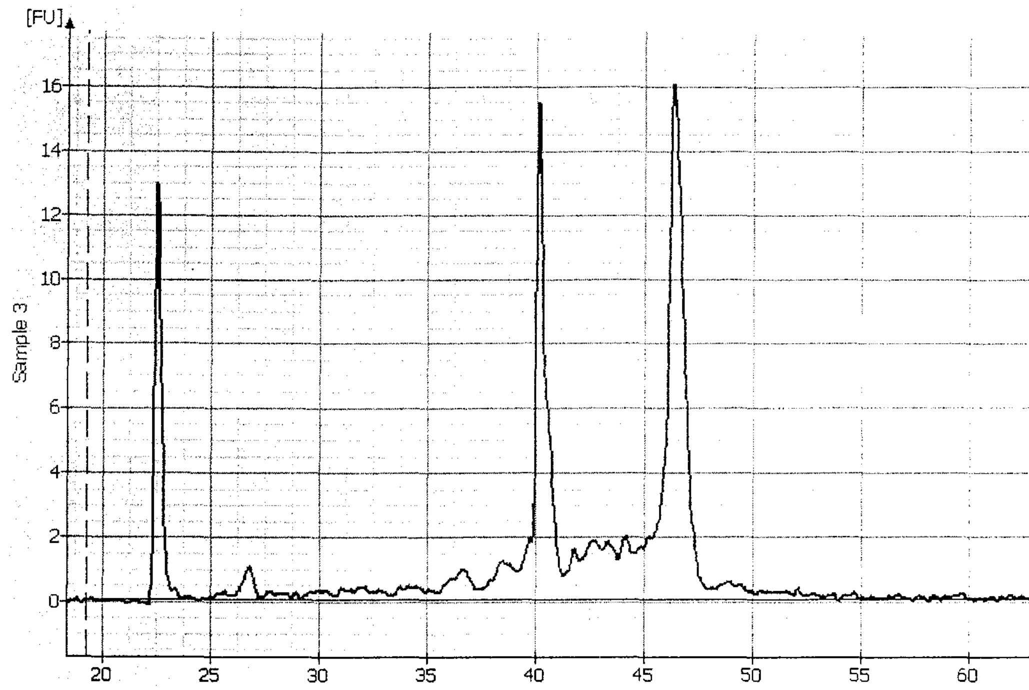

Figure A-13 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 2 (pre-exercise).

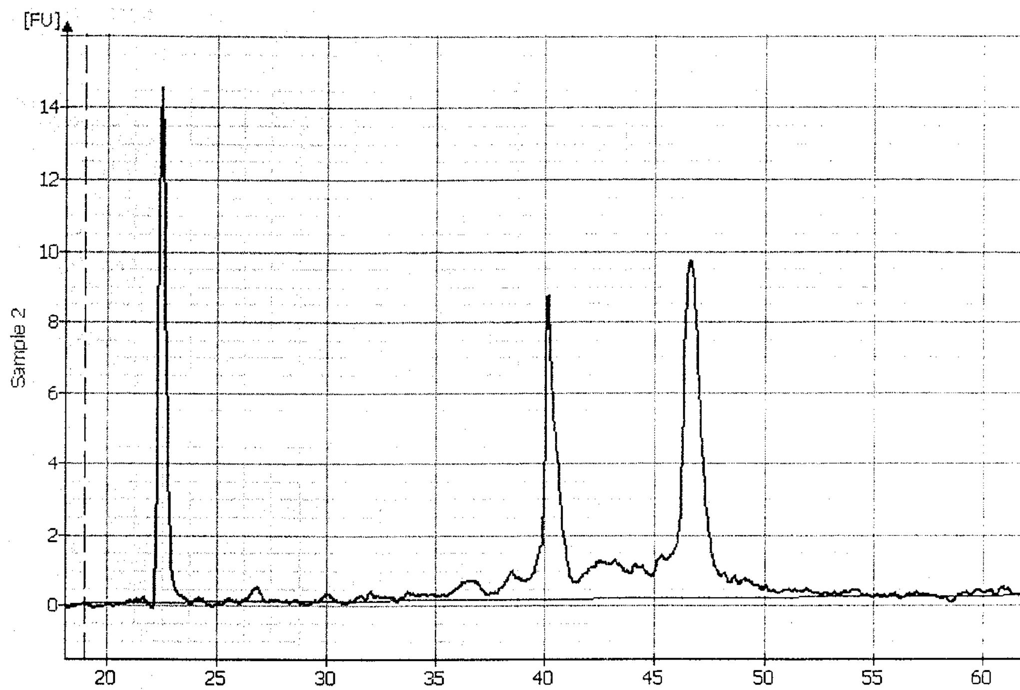

Figure A-14 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 2 (post exercise).

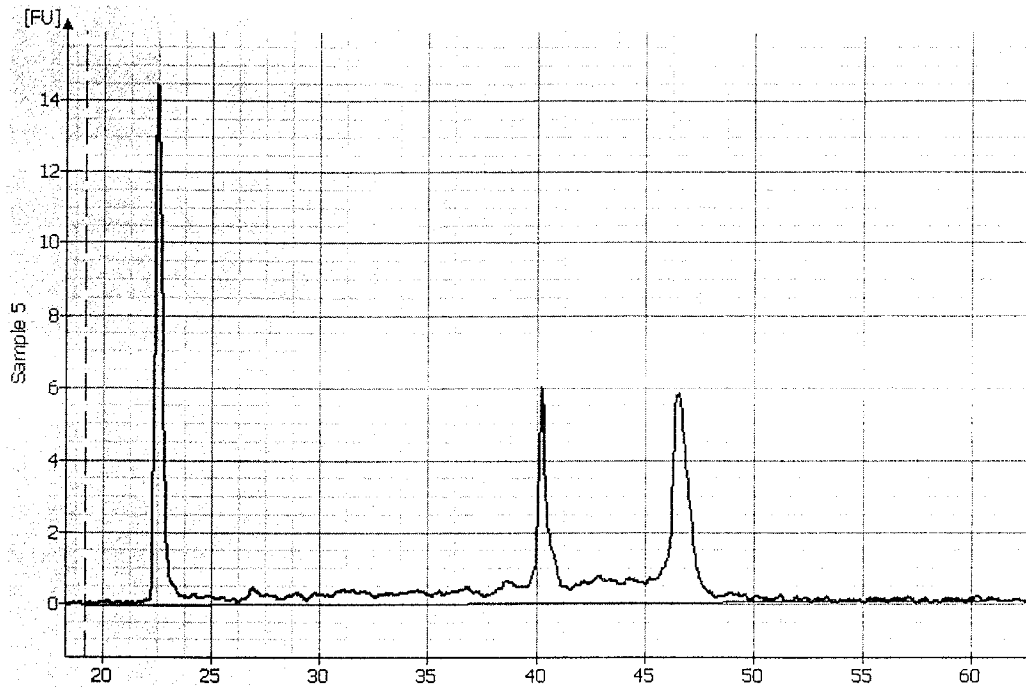

Figure A-15 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 3 (pre-exercise).

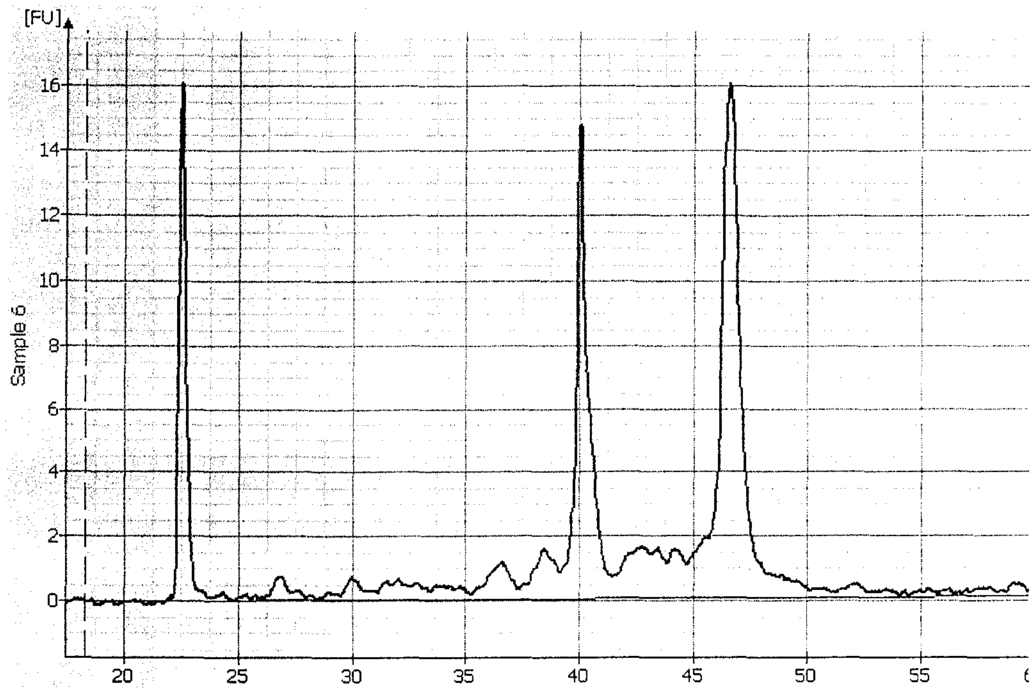

Figure A-16 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 3 (post exercise).

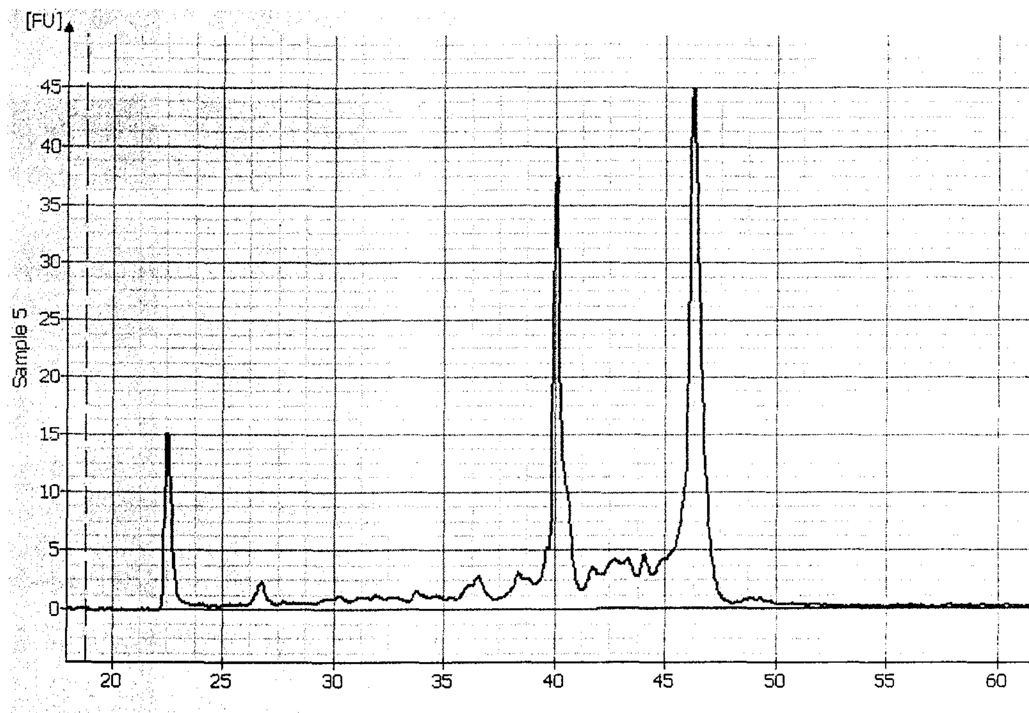

Figure A-17 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 4 (pre-exercise).

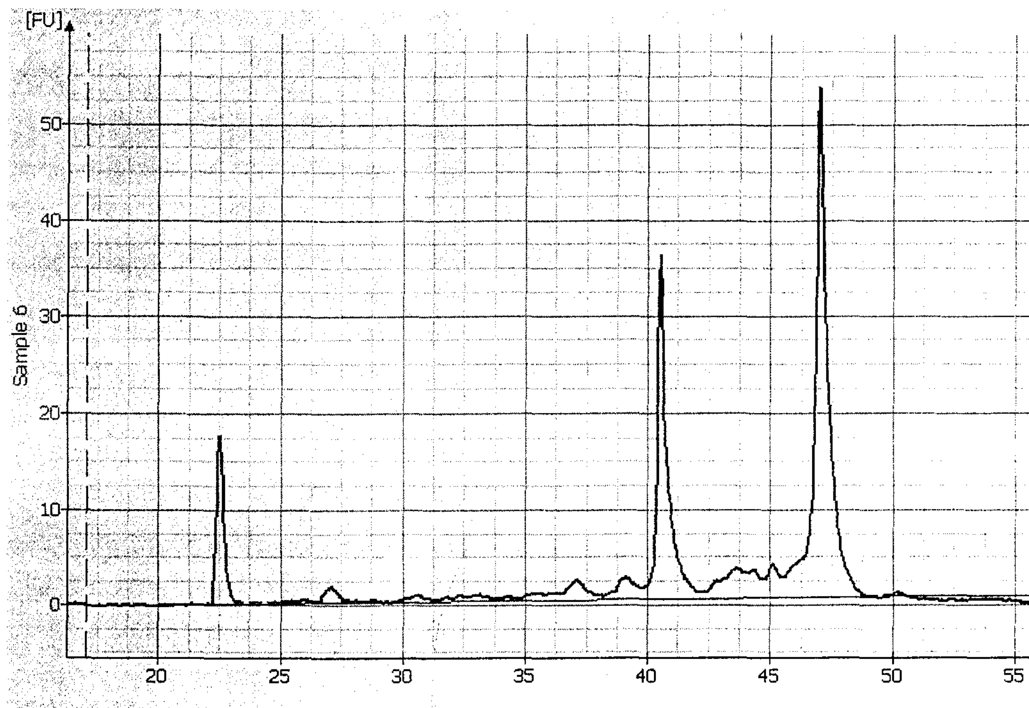

Figure A-18 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 4 (post exercise).

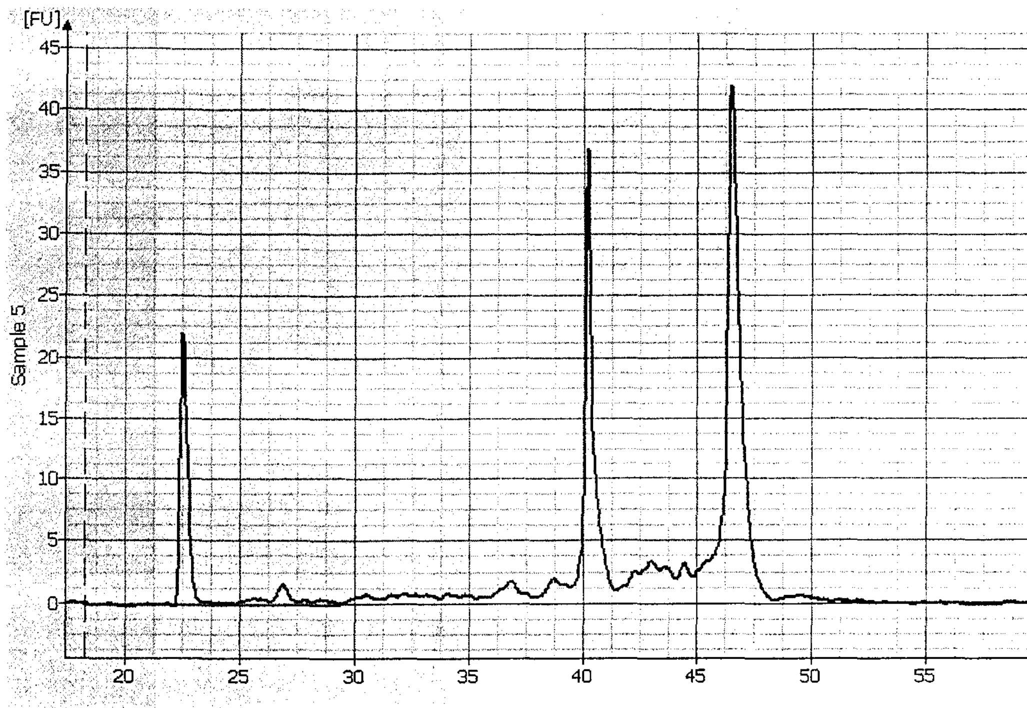

Figure A-19 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 5 (pre-exercise).

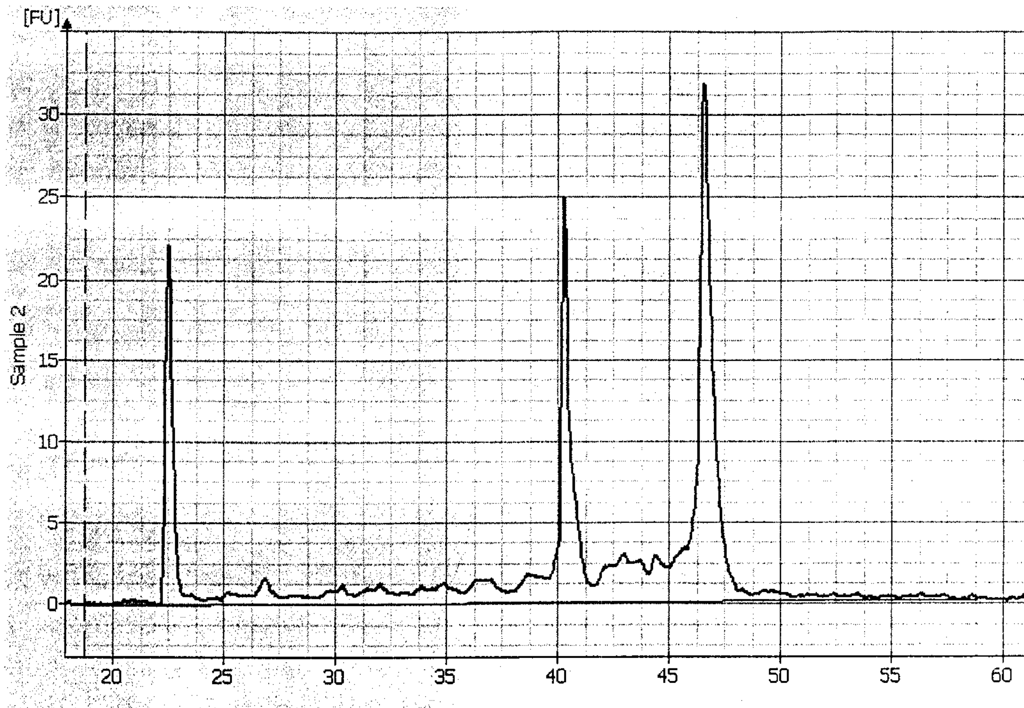

Figure A-20 The electropherogram of total RNA of abdominal subcutaneous adipose tissue from obese subject 5 (post exercise).

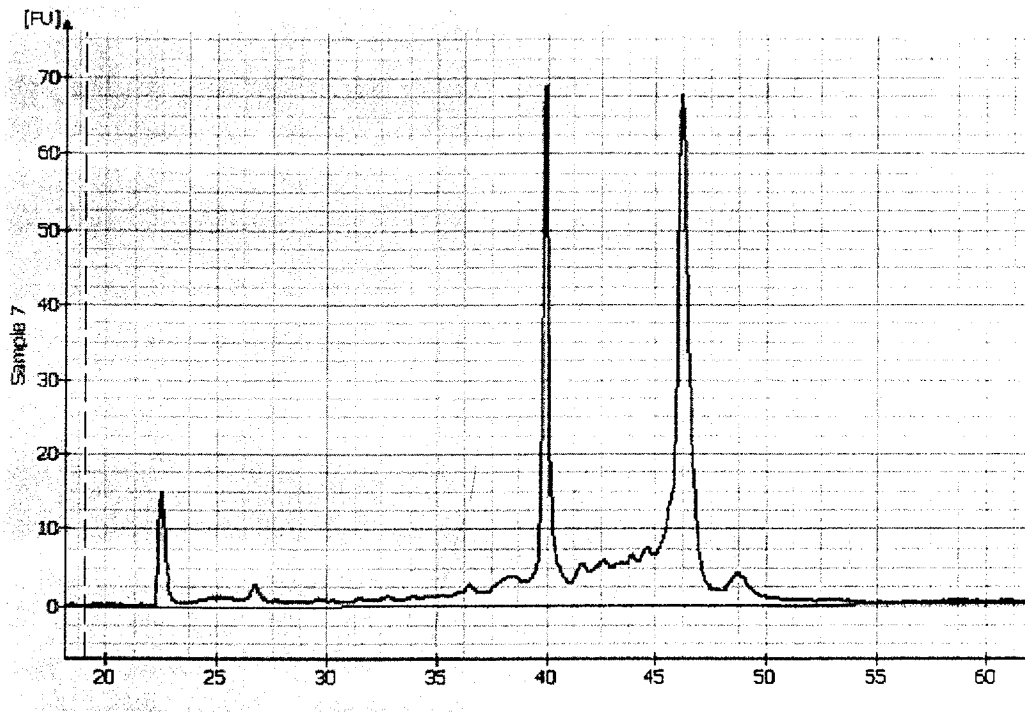

Figure A-21 The electropherogram of Universal Human Reference RNA.