

ABSTRACT

Title of Document: THE INFLUENCE OF VISFATIN AND VISFATIN GENE POLYMORPHISMS ON GLUCOSE AND OBESITY-RELATED VARIABLES AND THEIR RESPONSES TO AEROBIC EXERCISE TRAINING

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Adipokines, soluble factors produced by adipocytes, may help to connect diabetes and obesity; one such adipokine is visfatin. Previous research has linked visfatin and visfatin gene (PBEF1) polymorphisms with glucose and obesity-related conditions; however, less is known regarding visfatin's response to an aerobic exercise training intervention, and no one, to our knowledge, has examined whether polymorphic variation in the PBEF1 gene affects aerobic exercise training-induced changes in glucose and obesity-related variables.

Thus, this retrospective study investigated whether 6 months of aerobic exercise training reduced plasma visfatin levels in individuals with impaired glucose tolerance (IGT) or normal glucose tolerance (NGT). In addition, we examined the influence of common PBEF1 gene polymorphisms (-4689 G>T, -1543 C>T, -1001 T>G, -948 G>T,

and SER301SER) and haplotypes on glucose and obesity-related variables and their responses to aerobic exercise training.

Following the completion of 6 weeks of dietary stabilization, 116 healthy, sedentary, middle-aged, Caucasian men and women underwent 6 months of aerobic exercise training. Glucose total area under the curve (AUC), insulin AUC, and insulin sensitivity were measured via oral glucose tolerance tests. Plasma visfatin was measured using an enzyme immunoassay in 67 of the participants (22 with IGT, 45 with NGT), and standard techniques were used to assess lipoprotein-lipid and body composition variables. Restriction fragment length polymorphism techniques and TaqMan assays were used to determine PBEF1 genotypes.

We found that plasma visfatin levels were comparable in IGT and NGT individuals at baseline and increased similarly in both groups in response to aerobic exercise training. We also found associations at baseline between glucose and obesity-related variables and PBEF1 gene variants, with -4689, -1001, -948, and SER301SER variant allele groups and PBEF1 variant allele-containing haplotypes having higher insulin sensitivity. Last, PBEF1 genetic variation influenced the aerobic exercise training-induced change in glucose and obesity-related variables. Moreover, the -948 polymorphism, TCGTT haplotype, and TCGGT haplotype were associated with lipoprotein-lipid changes with training, and the SER301SER polymorphism influenced changes in BMI and body fat. Future studies need to address the functional significance of PBEF1 polymorphisms and haplotypes and clarify mechanisms connecting visfatin to glucose and obesity-related phenotypes.

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ON GLUCOSE AND OBESITY-RELATED PHENOTYPES AND THEIR
RESPONSES TO AEROBIC EXERCISE TRAINING

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Dedication

To my family, especially Olin and Judy McKenzie, Tim Smith, Jr., and Cory Smith,
for all of your support, encouragement, understanding, love, and sunshine...

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I consider myself very fortunate to have had the opportunity to work with so many great people. First, I would like to thank the participants of the Gene Exercise Research Study (GERS), who helped to make this dissertation project possible. Much appreciation must also be directed to all of the GERS staff members, many of whom I will always consider great colleagues and friends. My advisor, Dr. Hagberg, deserves many, many thanks – you have been a great mentor and truly are an inspiration. I would also like to thank my dissertation committee members, Drs. Roth, Spangenburg, Kleinman, and Damcott – for being so giving of your time, insight, guidance, and/or laboratory space. Finally, I would like to thank my fellow “Super Lab” colleagues, especially Sarah Witkowski and Andy Ludlow, who have been so very helpful, patient, and thought provoking. Thank you all so much...

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List of Abbreviations

ACE – Angiotensin converting enzyme

ANCOVA – Analysis of covariance

ANOVA – Analysis of variance

Apo – Apolipoprotein

APO-E – Apolipoprotein E

AUC – Area under the curve

BMI – Body mass index

CKD – Chronic kidney disease

DNA – Deoxyribonucleic acid

dL – Deciliter

EDTA – Ethylenediaminetetraacetic acid

et al. – *et alii*

etc. – *et cetera* (and other things, and so on)

FFA – Free fatty acid

g – Gram

GDM – Gestational diabetes mellitus

GERS – Gene Exercise Research Study

HDL – High density lipoprotein

HERITAGE – HEalth, RIsk factors, exercise Training, And Genetics study

HIF1 – Hypoxia inducible factor-1

HOMA-IR – Homeostasis model assessment insulin resistance

i.e. – *id est* (that is)

IFG – Impaired fasting glucose

IGT – Impaired glucose tolerance

IL – Interleukin

IR – Insulin resistance

IRS – Insulin receptor substrate

ISI – Insulin sensitivity index

IU – International units

kcal – Kilocalorie

kg – Kilogram

L – Liter

LDL – Low density lipoprotein

m – Meter

mg – Milligram

min – Minute

mL – Milliliter

mmol – Millimole

mRNA – Messenger RNA

N – Normal (concentration)

NAD – Nicotinamide adenine dinucleotide

Nampt – Nicotinamide phosphoribosyltransferase

NFAT – Nuclear factor of activated-T cells

ng – Nanogram

NGT – Normal glucose tolerance

nm – Nanometer

NMN – Nicotinamide mononucleotide

Nmnat – NMN adenylyltransferase

OGTT – Oral glucose tolerance test

PBEF – Pre-B cell colony-enhancing factor

PCOS – Polycystic ovary syndrome

pM/pm – Picomole

PPAR – Peroxisome proliferator activated receptor

RNA – Ribonucleic acid

SER – Serine

SIRT – Sirtuin

SNP – Single nucleotide polymorphism

TNF- α – Tumor necrosis factor- α

TZD – Thiazolidinedione

T1D – Insulin dependent diabetes mellitus, type 1 diabetes

T2D – Non-insulin dependent diabetes mellitus, type 2 diabetes

VLDL – Very low density lipoprotein

VO₂max – Maximal oxygen consumption

vs – Versus

wks – Weeks

yr – Year

μ – Micro

% – Percent

Chapter 1: Introduction

The prevalence of diabetes is reaching epidemic proportions in the United States and throughout the World with estimates predicting that over 360 million people will be afflicted by the year 2030 (124). Associated with type 2 diabetes (T2D) are insulin resistance and obesity, risk factors that often precede the development of the disease. Consequently, the prevalence of obesity is also reaching epidemic status in the United States, with over 65 % of American adults reportedly overweight or obese (101). Furthermore, adipose tissue is a metabolically active organ and adipokines, soluble factors produced by adipocytes, may further link obesity and insulin resistance; one such adipokine is visfatin.

Visfatin History

In 2004, Fukuhara and colleagues reported the isolation of an adipokine prevalent in the visceral fat of humans and mice, which they termed visfatin (35). Previously isolated in lymphocytes and also found to be expressed in skeletal muscle, liver, and bone marrow, visfatin was first identified in 1994 as a cytokine-like molecule, enhancing the effects of interleukin-7 and stem cell factor on pre-B-lymphocyte colony formation, known as pre-B cell colony-enhancing factor (PBEF) (102). Additionally, Jia and colleagues reported that PBEF was an inflammatory cytokine since it was upregulated in neutrophils and acted to inhibit/delay neutrophil apoptosis in experimental inflammation and sepsis (54). PBEF also was reported to function as a nicotinamide phosphoribosyltransferase (100) known as Nampt, active

in nicotinamide adenine dinucleotide (NAD) salvage pathways and suggested to be a regulator of mammalian health and longevity (127).

Visfatin & Metabolism

Fukuhara and colleagues, via their series of experiments involving cell culture, rodent models, and humans, extended the realm of PBEF/Nampt/visfatin to include glucose metabolism and obesity, asserting its insulin-like effects (35). Until its retraction in 2007 (36), Fukuhara and colleagues' paper was considered seminal in this area, and although Xie and colleagues (126) reported similar insulin-mimetic actions for visfatin in osteoblasts, other researchers have not been able to replicate the earlier findings of Fukuhara's group (97). Furthermore, Revollo and colleagues have proposed that visfatin's connection to glucose and obesity-related variables is via its role as Nampt. Moreover, they proposed a mechanism linking Nampt to NAD biosynthesis and metabolism through the maintenance of nicotinamide mononucleotide (NMN) and NAD levels and subsequent activation of NAD-dependent factors such as the sirtuin family (95; 97).

Briefly, the pathway proposed by Revollo and colleagues begins with nicotinamide absorption from the diet and distribution to the body's tissues. If taken up by cells, nicotinamide's conversion to NMN would be mediated by intracellular Nampt. If not removed from the circulation, extracellular Nampt could convert nicotinamide to NMN, which would then be transported to the tissues for uptake. Once inside of cells, NMN adenylyltransferase (Nmnat) would complete the reaction to produce NAD. Then, via its involvement in transcriptional regulation, NAD would regulate metabolism. (95; 97)

One such target of NAD is the sirtuin family, as NAD cleavage is required for their deacetylase and ADP-ribosylation reactions. There are 7 mammalian sirtuins (SIRT1-7), and SIRT1 has been shown to regulate genes involved in pancreatic beta cell insulin secretion (14; 80), beta cell survival (60), adipogenesis (91), lipolysis (38; 91; 99), gluconeogenesis (98; 99), and cholesterol degradation (99). SIRT3 may also be involved, as it reportedly activates acetyl-coenzyme A synthetase (47), an enzyme important in the formation of acetyl coenzyme A, which is involved in the citric acid cycle, cholesterol synthesis, and fatty acid synthesis. Finally, SIRT4 has been linked to amino acid-stimulated insulin secretion (46). Thus, visfatin may be mechanistically linked to metabolic processes via its function as Nampt.

Regardless of the mechanism, numerous investigators have examined visfatin's potential role in glucose and obesity-related conditions. Although the results have not been entirely consistent (Tables 1-1 and 1-2), most studies have found increased circulating levels of visfatin in type 1 diabetes [T1D; (43; 74)], T2D (22; 29; 31; 74; 94; 104), gestational diabetes [GDM;(68; 72)], obesity (7; 25; 37; 41; 45; 56; 76; 131), polycystic ovary syndrome [PCOS; (19; 66; 113)], advanced chronic kidney disease [CKD; (9; 130)], and metabolic syndrome (7; 32; 133) patients as compared to healthy or less-diseased individuals. As shown in Table 1-3, several studies have also found significant correlations between circulating visfatin and glucose and obesity-related variables including fasting insulin (69; 72; 74; 113; 131), insulin sensitivity [assessed via homeostasis model assessment-insulin resistance; HOMA-IR; (69; 113; 120)], fasting glucose (32; 131), visceral fat (7; 120), body mass index [BMI; (13; 19; 22; 73; 88)], and triglyceride levels (32; 69; 120). There

have also been correlations reported between the surgical weight loss-related change in visfatin levels and the change in fasting insulin (45; 69) and insulin sensitivity (45; 69). However, these relationships have not always been consistent (Table 1-3).

The disparity in previous visfatin studies may be partly due to study designs that have failed to account for the effects of diet and/or exercise. Furthermore, only 10 studies have mentioned some sort of subject dietary and/or physical activity criteria. Of these, 5 studies included dietary information/exclusion criteria (17; 40; 56; 69; 88), 4 included physical activity information (25; 43; 77; 89), and 1 included both dietary and physical activity information (110). In addition, very few of the studies involving female participants reported menopausal status or hormone replacement therapy usage, either of which could also have influenced many of the outcome variables. Thus, studies assessing visfatin levels that also account for the effects of diet, physical activity, and/or female menopausal status may help to clarify some of the discrepant findings from previous reports.

Visfatin & Genetics

The visfatin gene (PBEF1) is located on chromosome 7q22. This chromosomal region has previously been reported to have linkage with the insulin response to aerobic exercise training in Caucasians (70), insulin resistance syndrome phenotypes in Mexican-Americans (8), and BMI in the National Heart, Lung, and Blood Institute's Family Blood Pressure Program (125). Thus, based on its chromosomal location, the visfatin gene may be a candidate for glucose and obesity-related phenotypes. Furthermore, over 52 single nucleotide polymorphisms have been reported in PBEF1, and PBEF1 promoter polymorphisms have been linked to

diseases, including glucose and obesity-related conditions. Two such polymorphisms are the -1001 T>G and -948 G>T. Moreover, the -1001 polymorphism has been associated with fasting glucose (11), fasting insulin (11), and waist-to-hip ratio (64), whereas the -948 variant has been associated with T2D (132), fasting insulin (11; 16), 2-hour oral glucose tolerance test (OGTT) glucose (16), lipoprotein-lipids (11), and diastolic blood pressure (64). Both of these SNPs occur at a relatively common frequency in the population, with reports ranging from 0.25-0.26 (9; 11; 64) and 0.12-0.19 (11; 16; 64; 132), respectively.

An additional promoter polymorphism, the -1543 C>T (also known as -1535), has been found to have a functional effect, reducing transcription in human lung microvascular endothelial cells (129), but a more recent study reported no alteration in transcription in mouse adipocytes (115). The -1543 C>T polymorphism has not been studied in connection with glucose and obesity-related traits in Caucasians, although in Japanese individuals it was associated with differences in HDL-cholesterol and triglyceride levels, but not with T2D, glucose, insulin, or insulin sensitivity (115). Its frequency has been reported as ~ 0.31 (129).

Along with these 3 SNPs, the -4689 G>T polymorphism covers much of the variation in the promoter region of the PBEF1 gene. Moreover, Bailey and colleagues reported a weak association for this polymorphism and fat mass and apolipoprotein B levels in a French Canadian population, and the frequency of the T allele was 0.41 (11).

Last, based on the linkage disequilibrium structure of the gene, one additional polymorphism helps to account for variation in the remainder of the PBEF1 gene,

rs2302559 (SER301SER). This exon 7 polymorphism results in a synonymous amino acid change at position 301 (serine) and has a minor allele frequency of ~ 0.41. However, no one has examined this polymorphism in connection with glucose or obesity-related traits.

Thus, variation within the visfatin gene can be marked by 5 polymorphisms, some of which have been previously associated with glucose and obesity-related conditions. However, the majority of genotype association studies have failed to account for the effects of diet, physical activity, and/or female menopausal status. Thus, a study that accounts for these factors may help to clarify some of the discrepant findings from previous reports.

Visfatin & Exercise

Many beneficial physiological changes occur with aerobic exercise training, including improvements in glucose tolerance and insulin sensitivity. Furthermore, adipokines such as adiponectin are responsive to exercise training. However, the response of visfatin to acute exercise and to aerobic exercise training has only been investigated minimally.

Acute Exercise

Only one study, thus far, has examined visfatin's response to acute exercise in humans. Frydelund-Larsen and colleagues studied visfatin mRNA expression in 15 healthy men, approximately 25 yrs of age, with a BMI of ~ 25 kg/m². The 15 men were divided into an exercise group (n = 8) and a control group (n = 7). The exercise group cycled on an ergometer for 3 hours at 60 % of their maximal oxygen

consumption (VO_2max) and then underwent 6 hours of recovery. In contrast, the control group rested for 9 hours. The researchers found that as compared to the control participants and as compared to baseline, abdominal adipose tissue visfatin mRNA expression increased 3-fold in response to exercise, with significant increases at the 3, 4.5, and 6 hours time points. However, skeletal muscle visfatin mRNA expression and plasma visfatin levels did not significantly change with acute exercise. (34)

Exercise Training

Three studies, thus far, have examined visfatin's response to aerobic exercise training in humans. Haider and colleagues examined the effects of aerobic exercise training on plasma visfatin levels in 18 (11 women and 7 men, 42 ± 10 yrs of age) T1D patients. The participants were compared to healthy, non-exercising controls (7 women and 7 men, 29 ± 5 yrs of age). The exercise training program consisted of 4 months of stationary cycling for 1 hour, at an intensity of 60-70 % of heart rate reserve. At baseline, the T1D patients had greater fasting plasma glucose and visfatin levels than the controls. Moreover, there was no correlation between visfatin and BMI or age at baseline. After both 2 and 4 months of exercise training, visfatin levels decreased significantly in the T1D patients (from 64.1 ± 12 ng/mL to 27.8 ± 2.6 ng/mL and 17.5 ± 3.4 ng/mL, respectively) and remained lower 8 months after the cessation of exercise training. However, BMI, fasting glucose, glycosylated hemoglobin, and lipoprotein-lipid levels were unchanged with the exercise training program. In addition, the researchers did not measure VO_2max or a similar training effect variable, and to be included in the study, participants only had to attend > 60 %

of the exercise sessions. Thus, it is difficult to ascertain whether an actual aerobic exercise training effect occurred. Lastly, the researchers did not assess diet, and differences in diet could have influenced the outcome measures. (43)

Choi and colleagues evaluated the effect of a combined aerobic and resistance exercise training program on plasma visfatin levels in non-diabetic Korean women. Healthy, overweight or obese women (n = 48) aged 30-55 yrs were recruited for the study. The participants had to be sedentary (< 20 minutes of exercise twice per wk) and could not be taking laboratory test result-altering medications. The 12 wk exercise training intervention consisted of ~ 45 minutes of aerobic exercise and ~ 20 minutes of resistance exercise, 5 times per week. At baseline, the obese women had higher plasma visfatin levels than the overweight women. Furthermore, plasma visfatin levels were associated with body weight, BMI, and waist circumference in simple regression analyses and BMI in multiple regression analyses. After 12 wks of exercise training, the participants lost 4-5 kg of weight, decreased BMI, waist circumference, blood pressure, percent body fat, fasting glucose, HOMA-IR, and visfatin levels. Thus, a combined aerobic and resistance exercise training program with weight loss significantly decreased plasma visfatin levels and improved glucose and obesity-related variables in healthy, overweight or obese Korean women. However, diet was not assessed in the study and part of the weight loss was likely due to dietary changes since the caloric cost of the exercise training was estimated by the researchers as ~ 400 kcal per session. Thus, it is difficult to assess whether the exercise training or weight loss caused the reported differences in visfatin. (25)

Finally, Brema and colleagues compared plasma visfatin levels in 15-30 yr old Caucasian age- and BMI-matched obese and T2D patients before and after the completion of an aerobic exercise training intervention. The training program lasted 12 weeks and consisted of 4 supervised sessions per week at an intensity of 75 % VO₂max and a duration of 1 hour. Dietary intake reportedly remained unchanged during the program, as did nearly every physiological variable measured, with the exception of waist circumference in the T2D group and VO₂max in the obese group. Furthermore, visfatin levels were comparable in the obese and T2D patients before the intervention and decreased in both groups with training, with a greater decrease occurring in the T2D patients. Also, in the T2D patients, visfatin was correlated with percent body fat ($r = -0.933$) and HDL-cholesterol ($r = -0.893$) at baseline. As VO₂max did not change in the T2D patients and most outcomes did not change with the program, it is difficult to assess if a training effect occurred. Furthermore, there was no indication made as to whether the participants were sedentary before the program started. In addition, gender was not accounted for and differences between the groups could have affected many of the outcome variables. (17)

Summary: Visfatin & Exercise

In sum, acute exercise seems to increase visfatin mRNA expression but not visfatin plasma levels in healthy, young men. In contrast, plasma visfatin levels seem to decrease in response to exercise training. However, no research has been reported regarding the independent effects of 6 months of aerobic exercise training on plasma visfatin levels in normal or impaired glucose tolerant individuals. In addition, previous studies in T2D patients, T1D patients, and non-diabetic Korean women have

not provided evidence that an actual training effect occurred and was responsible for the reported visfatin changes. The study involving T1D patients and Korean women also failed to control for the effects of diet. Thus, studies taking these factors into account would help to further elucidate the effects of aerobic exercise training on plasma visfatin levels.

Aerobic Exercise Training Responses & Their Genetic Link

Coincidentally, the response of many variables to aerobic exercise training tends to be highly variable. In fact, part of the variation in the response of glucose and obesity-related phenotypes to aerobic exercise training has been attributed to common genetic polymorphisms (93). With its links to glucose and obesity-related phenotypes, its polymorphisms associated with glucose and obesity-related phenotypes, and its responsiveness to exercise training, visfatin is a candidate gene for aerobic exercise training-induced changes in glucose and obesity-related phenotypes. However, this avenue of research has not currently been investigated.

Specific Aims & Hypotheses

Thus, the purpose of this study was to investigate the response of visfatin to 6 months of aerobic exercise training in generally healthy, normal glucose tolerant (NGT) and impaired glucose tolerant (IGT) individuals. In addition, common polymorphisms in the visfatin gene, and their corresponding haplotypes, were examined for their potential involvement in the response of glucose and obesity-related phenotypes to aerobic exercise training. The hypotheses examined were:

Hypothesis 1: As compared to baseline values, plasma visfatin concentration will decrease with aerobic exercise training in overall healthy, normal glucose tolerant individuals and impaired glucose tolerant individuals. Moreover, there will be no differences in the magnitude of aerobic exercise training-induced change between the normal and impaired glucose tolerance groups.

Hypothesis 2: The visfatin -948 G>T, -1001 T>G, -1543 C>T, -4689 G>T, and SER301SER gene polymorphisms will be associated with plasma visfatin levels and glucose and obesity-related phenotypes at baseline. Specifically, -948 T allele carriers will have greater plasma visfatin levels, greater OGTT glucose total area under the curve (AUC), greater OGTT insulin total AUC, and lower insulin sensitivity than CC homozygotes. Carriers of the -1001 G allele will have lower plasma visfatin levels, lower OGTT glucose total AUC, lower OGTT insulin total AUC, and higher insulin sensitivity than TT homozygotes. Lastly, for the PBEF1 -1543 C>T, -4689 G>T, and SER301SER polymorphisms, analyses will be carried out to determine the direction of association between genotypes and plasma visfatin levels, OGTT glucose total AUC, OGTT insulin total AUC, and insulin sensitivity.

Hypothesis 3: The visfatin -948 G>T, -1001 T>G, -1543 C>T, -4689 G>T, and SER301SER gene polymorphisms will influence aerobic exercise training-induced changes in glucose and obesity-related phenotypes, including dyslipidemia. As no current data exist on this topic, a specific directional hypothesis would be conjecture.

Table 1-1. Circulating visfatin in diabetes-related conditions.

	Sample	Comparison	Result	Source
T1D	plasma	T1D vs healthy	↑	(43)
	serum	T1D vs non-diabetic	↑	(74)
	serum	T1D vs T2D	↑	(74)
T2D	plasma	T2D vs healthy	ND	(112)
	plasma	T2D vs healthy	↑	(29)
	plasma	T2D vs healthy	↑	(22)
	serum	T2D vs non-diabetic	↑	(74)
	serum	T2D vs non-diabetic	↑	(94)
	serum	T2D vs non-diabetic	↑ or ND	(104)
	serum	T2D newly diagnosed vs NGT	↑	(31)
	plasma	T2D vs NGT	↓	(73)
	serum	T2D vs NGT	ND	(55)
	serum	T2D vs IGT	ND	(55)
	plasma	T2D vs IGT	ND	(29)
	serum	T2D newly diagnosed vs IFG/IGT	↑	(31)
GDM	serum	GDM vs healthy	↑	(72)
	plasma	GDM vs healthy pregnant	↓	(40)
	?	GDM vs healthy pregnant	↑	(68)
	plasma	GDM vs healthy pregnant	↓	(20)
IGT	plasma	IGT vs healthy	ND	(29)
	serum	IGT vs NGT	ND	(55)
	plasma	IGT vs NGT	ND	(73)

Results are statistically significant unless indicated otherwise. T1D, type 1 diabetes; T2D, type 2 diabetes; GDM, gestational diabetes mellitus; IGT, impaired glucose tolerance; ?, not clearly stated; ↑, higher; ND, no difference; ↓, lower; vs, versus; NGT, normal glucose tolerance, IFG, impaired fasting glucose.

Table 1-2. Circulating visfatin in obesity and other related conditions.

	Sample	Comparison	Result	Source
Obesity and overweight	serum	Obese vs normal weight	↓	(55)
	serum	Obese vs normal weight	↑	(131)
	plasma	Obese vs normal weight	↓	(88)
	plasma	Obese vs normal weight	↑	(76)
	serum	Obese vs normal weight	↑	(56)
	plasma	Obese vs healthy	↑	(41)
	plasma	Obese vs healthy	↑	(45)
	plasma	Obese vs non-obese	↑	(7)
	serum	Obese vs overweight	↓	(55)
	plasma	Obese vs overweight	↑	(25)
	plasma	Severely obese vs healthy	↑	(37)
	plasma	Severely obese w/NGT vs healthy	ND	(37)
	plasma	Severely obese w/IFG or T2D vs healthy	↑	(37)
	serum	Subcutaneous obese, visceral obese, lean	ND	(65)
	serum	Overweight vs normal weight	ND	(55)
plasma	Overweight T2D, lean T2D, overweight healthy, lean healthy	ND	(116)	
plasma	Obese vs obese w/T2D	ND	(17)	
PCOS	plasma	PCOS vs healthy	↑	(19)
	serum	PCOS vs healthy	↑	(66)
	serum	Lean PCOS vs healthy	↑	(66)
	plasma	PCOS vs healthy w/infertility	↑	(113)
CKD	serum	CKD stage 5 vs CKD stage 3-4	↑	(9)
	serum	CKD stage 5 vs healthy	↑	(9)
	serum	CKD stage 3, 4, or 5 vs healthy	↑	(130)
	serum	CKD stage 1 or 2 vs healthy	ND	(130)
	serum	CKD diabetics vs CKD non-diabetics	ND	(9)
Other	plasma	Metab. syndrome vs w/o metab. syndrome	↑	(32)
	serum	Metab. syndrome vs w/o metab. syndrome	↑	(133)
	plasma	Obese w/metab. syndrome vs obese	↑	(7)
	plasma	Obese w/metab. syndrome vs non-obese	↑	(7)
	plasma	FCHL family members vs FCHL spouses	ND	(120)
	plasma	Hypertensive vs healthy	ND	(30)

Results are statistically significant unless indicated otherwise. PCOS, polycystic ovary syndrome; CKD, chronic kidney disease; vs, versus; w/, with; NGT, normal glucose tolerance; IFG, impaired fasting glucose; T2D, type 2 diabetes; metab., metabolic; w/o, without; FCHL, familial combined hyperlipidemia; ↓, lower; ↑, higher; ND, no difference.

Table 1-3. Correlations between circulating visfatin and glucose and obesity-related variables.

	Positive Correlation	Negative Correlation
Fasting insulin	(72; 113; 131)	(69; 74)
Acute insulin response to glucose		(74)
30-minute OGTT insulin		(74)
2-hour OGTT insulin	(72)	
Insulin sensitivity	(74)	(66)
Insulin resistance (HOMA-IR)	(113)	(69; 120)
Fasting glucose	(32; 131)	
2-hour OGTT glucose		(73)
Glycosylated hemoglobin	(74)	(73)
Visceral fat	(7)	(120)
Percent body fat	(13)	(17)
Body mass index	(13; 19; 73)	(22; 88)
Waist to hip ratio	(73)	
Waist circumference	(32)	
Triglyceride levels	(32)	(69; 120)
Subcutaneous fat visfatin mRNA	(88; 113)	(13)
Omental fat visfatin mRNA	(113)	
Visceral fat visfatin mRNA	(13)	
Visfatin protein levels	(113)	
Change in fasting insulin		(45; 69)
Change in HOMA-IR		(45; 69)
Change in body mass index	(45)	
Change in weight	(45)	
Change in waist circumference		(45)
Waist reduction percentage		(37)

Studies included in the table reported statistically significant correlations.
OGTT, oral glucose tolerance test.

Chapter 2: Methods

This study was a retrospective analysis of data collected from participants in the Gene Exercise Research Study (GERS) at the University of Maryland, College Park. The main purpose of the GERS was to investigate the effects of genetic polymorphisms on aerobic exercise training-induced changes in plasma lipoprotein lipids and/or blood pressure. In contrast, this study examined relationships between visfatin, visfatin gene polymorphisms, glucose and obesity-related phenotypes, and the response of these variables to aerobic exercise training. Visfatin levels and visfatin gene polymorphisms were assessed from banked plasma and DNA samples. All other variables were previously assayed by the GERS staff. Last, before this retrospective study was conducted, written approval was obtained from the University of Maryland Institutional Review Board via an addendum to the original approved GERS Institutional Review Board application.

Participants & Screening

Participants in the GERS were sedentary volunteers, aged 50-75 yrs, living in the vicinity of the College Park campus (Washington, D.C. metropolitan area) and were recruited via media advertisements. All were screened initially via telephone and then through a series of laboratory screening visits to verify eligibility. During the first laboratory visit, potential participants met with study personnel, were informed of the study requirements, provided written informed consent, and completed health history and physical activity questionnaires. During subsequent screening visits, participants underwent a fasted blood draw for standard blood

chemistry tests, a 2-hour OGTT to assess diabetes status, blood pressure, height, weight, and BMI measurements, a general physical examination, and a maximal graded treadmill exercise test using the Bruce protocol, to screen for signs and symptoms of cardiovascular disease (6). A study physician conducted the general physical examination and oversaw the maximal graded treadmill exercise test, which included blood pressure, electrocardiogram, and heart rate recordings.

Briefly, participants had to be ≥ 50 yrs of age but ≤ 75 yrs of age, sedentary (< 20 minutes of physical activity \leq twice per week), non-diabetic (fasting glucose concentration < 126 mg/dL and 2-hour glucose concentration < 200 mg/dL), non-smoking, have no history or characteristics of cardiovascular disease, have no history or characteristics of lung, liver, or kidney disease, have a BMI < 37 kg/m², have a hematocrit value > 35 , and have no physical or orthopedic conditions that would preclude exercise. In addition, female participants had to be postmenopausal for at least 2 years and willing to maintain their hormone therapy status, either receiving or not receiving hormone replacement therapy (HRT), throughout the duration of the study. Furthermore, subjects had to qualify for either the GERS apolipoprotein-E gene (APO-E) and/or angiotensin converting enzyme gene (ACE) projects. Thus, for APO-E, subjects had to additionally have at least 1 National Cholesterol Education Program (NCEP) lipid abnormality (total cholesterol > 200 mg/dL, LDL-cholesterol > 130 mg/dL, HDL-cholesterol < 35 mg/dL, or triglyceride levels > 200 mg/dL but < 400 mg/dL) (2) and be normotensive or have blood pressure controlled with non-lipid and non-glucose altering medication. For the ACE project, participants had to have resting blood pressure measurements that remained $> 120/80$ mmHg but $< 160/100$

mmHg even after dietary stabilization classes and physician approved hypertension medication tapering (as described below).

Dietary Stabilization and ACE Study Medication Tapering

After screening into the study, participants were required to attend dietary stabilization classes twice a week for 6 weeks. A registered dietician provided instructional sessions on the principles of the American Heart Association Step 1 diet, similar to the American Heart Association Dietary Guidelines for the General Population (1), which emphasized the consumption of 55-60 % of dietary calories from carbohydrates, < 30 % of dietary calories from fat, < 6 grams of salt per day, and alcohol in moderation. Participants maintained the diet throughout the study's duration, with periodic dietary recalls and food frequency checks to ensure adherence. In addition, before the start of aerobic exercise training and during week 24 of the exercise training intervention, participants recorded 7 day dietary logs which were analyzed by the dietician using Computrition dietary software (Computrition, Inc., Chatsworth, CA).

Furthermore, if a participant enrolled in the ACE study had been taking blood pressure medication, he/she was gradually tapered off of the medication during the dietary stabilization period, provided that his/her physician granted approval. If physician approval was not received for medication tapering, the participant did not undergo the medication taper and did not continue past the dietary stabilization portion of the study.

Blood pressure measurements were taken and recorded during each dietary class to ensure the safety of all participants. In addition, automated blood pressure

monitors were available for home blood pressure measurement by the participants. Moreover, resting blood pressure measurements had to remain $> 120/80$ mmHg but $< 160/100$ mmHg for the participant to continue to take part in the ACE study. If a participant's blood pressure dropped below this range for 3 consecutive weeks, he/she was excluded from the ACE study. In addition, if a participant's blood pressure exceeded this range for 3 consecutive weeks, he/she was excluded from further participation in the ACE study and referred to his/her physician.

Testing Procedures

Following the completion of the dietary classes, participants underwent baseline testing before beginning the aerobic exercise training intervention. The baseline assessments determined main outcome variables in the GERS and included fasting blood draws, a 2-hour OGTT, body composition scans, and a maximal oxygen consumption ($VO_2\text{max}$) test. Final testing commenced following the completion of 24 weeks of aerobic exercise training. Final testing procedures were similar to baseline testing procedures except all final testing took place 24-36 hours after an exercise training bout. This time frame ensured measurement of training rather than acute exercise effects.

Lipoprotein-Lipid Levels

Following a 12-hour overnight fast on at least 2 separate occasions, venous blood samples were drawn from the antecubital region into 10 and/or 15 mL tubes containing 15 % potassium EDTA. Blood samples were placed on ice until centrifuged at 4 degrees Celsius, for 20 minutes, at 3000 revolutions per minute.

Plasma was separated from the centrifuged whole blood and analyzed for conventional plasma lipoprotein-lipid levels at the Baltimore Veteran's Affairs Medical Center Clinical Chemistry Laboratory. Plasma total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol were measured using standard procedures (5; 33; 103; 122). The lipoprotein-lipid values from the separate blood draws were then averaged according to the GERS protocol.

OGTT

Following a 12-hour overnight fast, a 2-hour OGTT began between the hours of 6:30 and 9:00 AM. A 20- or 22-gauge indwelling catheter was placed into a vein in the antecubital region, and blood sampling occurred before and every 30 minutes after the ingestion of a 75 gram D-glucose solution, for 2 hours. The blood samples were immediately transferred into 10 and 15 mL tubes containing 15 % potassium EDTA. Blood samples were placed on ice until centrifuged at 4 degrees Celsius, for 20 minutes, at 3000 revolutions per minute. Plasma was separated from the centrifuged whole blood and stored at -80 degrees Celsius until assayed for glucose and insulin levels. In addition, extra fasting plasma samples (from the OGTT and from the lipoprotein-lipid fasting blood draws) were stored for assessment of other variables, including plasma visfatin levels.

Glucose & Insulin

The glucose oxidase method was used to determine plasma glucose concentration via a glucose analyzer (YSI 2300 Stat Plus, YSI, Inc., Yellow Springs, OH). Each sample was measured in duplicate and averaged, provided that the values

did not differ by > 2 mg/dL. If measurements differed by > 2 mg/dL, the sample was reanalyzed.

A competitive radioimmunoassay (kit HI-14K, Linco Research, St. Charles, MO) was used to determine plasma insulin concentration. Each sample was measured in duplicate and averaged, provided that the coefficient of variation for the sample duplicates was $\leq 10\%$. However, if the coefficient of variation for the sample duplicates was $> 10\%$, the sample was reanalyzed in duplicate.

Glucose & Insulin-Related Variables

Glucose total AUC was calculated via the trapezoidal method using fasting, 30, 60, 90, and 120 minute glucose concentrations from the OGTT. The formula was as follows: glucose total AUC = (fasting glucose * 15) + (30 minute glucose * 30) + (60 minute glucose * 30) + (90 minute glucose * 30) + (120 minute glucose * 15). Insulin total AUC was calculated in a similar manner, except using OGTT insulin concentrations. An insulin sensitivity index (ISI) was calculated using the method of Matsuda and DeFronzo where $ISI = 10,000 / \text{square root of } [(fasting\ glucose * fasting\ insulin) * (\text{mean of fasting, 30, 60, 90, and 120 minute OGTT glucose} * \text{mean of fasting, 30, 60, 90, and 120 minute OGTT insulin})]$ (78).

Participants were classified as having impaired glucose tolerance (IGT) or normal glucose tolerance (NGT) based on their 120 minute OGTT glucose concentration. Glucose concentrations ≥ 140 mg/dL but < 200 mg/dL were classified as IGT, and glucose concentrations < 140 mg/dL were classified as NGT (3). Individuals with a 120 minute glucose concentration ≥ 200 mg/dL were excluded from analysis.

Plasma Visfatin

A commercially available competitive enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA, catalog number: EK-003-80, lot number 600508) was used to determine plasma visfatin concentration. Briefly, 50 μ L of standard, plasma sample, or control was added to an assigned microplate well, followed by 25 μ L of primary antiserum and 25 μ L of biotinylated peptide. Then, the microplate was sealed and incubated at room temperature for 2 hours with orbital shaking throughout the incubation period. Following the completion of the first incubation, the microplate was washed 4 times with assay buffer. Next, 100 μ L of streptavidin-horseradish peroxidase was added to each well. The microplate was sealed again and incubated at room temperature for 1 hour with orbital shaking throughout the incubation. Following the completion of the second incubation, 100 μ L of substrate solution was added to each well, but only after the microplate had been washed 4 times with assay buffer. The microplate was again sealed, incubated at room temperature for 1 hour, and orbitally shaken. During this step, the microplate was also protected from light. After the third incubation was completed, 100 μ L of 2N hydrochloric acid was added to each well to terminate the reaction. Then, the absorbance of the wells was read at 450 nm using a microplate reader, and curve-fitting software was used to calculate the standard curve and sample concentrations.

The only deviation from the manufacturer's recommendations was that manual washing was used in lieu of a plate washer, and this contributed to a higher intra-assay coefficient of variation (12.5 %) than the manufacturer's reported < 5 %. However, the plasma samples analyzed in this project were samples that had been

stored for 2-8 years at -80 degrees Celsius, and this may have affected their measurement. Moreover, only one study has examined the influence of storage on visfatin levels, and the researchers found that storage at -25 degrees Celsius for 1 month resulted in measurement of 92.7 ± 31.1 % of the initial value (83). Despite this potential limitation, we found no relationship between plasma sample age and the baseline or final visfatin concentration (data not shown, both $p > 0.05$).

Each sample was measured in duplicate and averaged, with reanalysis of sample duplicates having a coefficient of variation > 20 %. The inter-assay coefficient of variation was approximately 18 % versus the manufacturer's reported < 14 %.

Plasma C-Reactive Protein

As previously reported (84), an enzyme-linked immunosorbent assay (Alpha Diagnostic International, San Antonio, TX) was used to measure plasma C-reactive protein.

Body Composition

Body composition was measured at the Baltimore Veteran's Affairs Medical Center. Total percent body fat was measured via dual-energy x-ray absorptiometry (DPX-L or DPX-IQ, Lunar Corporation, Madison, WI), whereas visceral and subcutaneous adipose tissue intra-abdominal fat were measured using single slice computed tomography (GE Hi-Light CT scanner). Standard procedures were used as previously described (81).

Maximal Graded Treadmill Exercise Test

VO₂max and heart rate were measured during a maximal graded treadmill exercise test, as previously described (28). A physician presided over the test, and electrocardiogram and blood pressure measurements were recorded throughout the test. Standard criteria were used to ensure that VO₂max was achieved (6), and the resting and maximum heart rates measured from the electrocardiogram recording during the test were used in the aerobic exercise training prescription.

Genotyping

A 10 mL blood sample was collected during the screening process and DNA extracted and stored for later genotyping projects. The 5 PBEF1 polymorphisms of interest in this project [-948 G>T, -1001 T>G, -1543 C>T, -4689 G>T, and SER301SER] were genotyped in the Functional Genomics Laboratory in the Department of Kinesiology at the University of Maryland School of Public Health, College Park, MD, using restriction fragment length polymorphism techniques (-1543, -4689, SER301SER) or a TaqMan allelic discrimination assay (-948, -1001).

For the PBEF1 -1543 polymorphism, the primer sequences were: forward primer, 5'-AGCGAGACTCCGTCTCAAAA-3'; reverse primer, 5'-GGCAAAGATCATGGAAGTGG-3'. Microplate wells containing a total reaction volume of 25 µL, consisting of 2.5 µL of 10X Taq PCR Buffer with ammonium sulfate ((NH₄)₂SO₄), 4.0 µL of 1.25 mM 2'-deoxynucleoside 5'-triphosphate (dNTP) mix, 1.0 µL of 25 mM magnesium chloride (MgCl₂), 0.4 µL of 20 mM forward primer, 0.4 µL of 20 mM reverse primer, 0.2 µL of *Taq* polymerase, 1.0 µL of dimethyl sulfoxide (DMSO), 13.5 µL of distilled deionized water, and 2.0 µL of

genomic DNA, underwent the polymerase chain reaction (PCR). The thermal cycling conditions for the PCR involved: 1) initial denaturation at 95 degrees Celsius for 5 minutes, 2) 30 cycles of denaturation (95 degrees Celsius for 30 seconds), annealing (54 degrees Celsius for 30 seconds), and elongation (72 degrees Celsius for 30 seconds), and 3) final extension at 72 degrees Celsius for 5 minutes. Ten μL of each PCR product was then amplified using gel electrophoresis. Following the amplification check, the remaining 15 μL of PCR product was digested overnight at a temperature of 60 degrees Celsius with *Bst*NI (New England Biolabs, Inc., Beverly, MA). The digestion mixture included 15 μL of PCR product, 0.5 μL of enzyme, 2.3 μL of distilled deionized water, 2.0 μL of manufacturer provided buffer 2, and 0.2 μL of manufacturer provided bovine serum albumin (BSA). Following the digestion, 5 μL of Orange G running buffer was added to each sample, and 10 μL of the total volume was transferred into the wells of a 3 % agarose gel (9 g agarose in 300 mL Tris-acetate-EDTA buffer). The agarose gel contained 5 μL of ethidium bromide for every 100 mL of Tris-acetate-EDTA buffer. The gel then underwent electrophoresis for approximately 75-90 minutes and was visualized under ultraviolet light. The typical gel patterns for genotyping the -1543 polymorphism were: homozygous TT with 1 band at 206 bp; homozygous CC with 2 bands, 1 at 174 bp and 1 at 32 bp; and heterozygous CT with 3 bands, 1 at 206 bp, 174 bp, and 34 bp. For quality control and genotype assignment purposes, genotype-known controls (obtained via direct sequencing) were included in each run.

The procedures for genotyping the -4689 polymorphism were similar to those used for the -1543 polymorphism except that the primer sequences used were:

forward primer, 5'-TGCTGTTTTACATCCTCCA-3'; reverse primer, 5'-GTTTCCTCAGACCTGCTTGC-3'. Furthermore, the restriction endonuclease used was *Alu I*, the digestion mixture included 15 µL of PCR product, 0.6 µL of enzyme, 2.4 µL of distilled deionized water, and 2.0 µL of manufacturer provided buffer 2, and the overnight digestion required a temperature of 37 degrees Celsius. The -4689 polymorphism banding patterns were: homozygous GG with 1 band at 188 bp; homozygous TT with 2 bands, 1 at 143 bp and 1 at 45 bp; and heterozygous GT with 3 bands, 1 at 188 bp, 143 bp, and 45 bp.

Slightly different PCR and digestion conditions were used for the SER301SER polymorphism. First, the primer sequences for the SER301SER polymorphism were: forward primer, 5'-TTGGGTGAAGTCATCAGACG-3'; reverse primer, 5'-GTATTGCCGGAAGGAAAAG-3'. Next, the PCR reaction mixture contained 2.5 µL of 10X Taq PCR Buffer, 4.0 µL of 1.25 mM dNTP mix, 1.0 µL of 25 mM MgCl₂, 0.4 µL of 20 mM forward primer, 0.4 µL of 20 mM reverse primer, 0.2 µL of *Taq* polymerase, 14.5 µL of distilled deionized water, and 2.0 µL of genomic DNA, equaling a 25 µL total volume. The annealing step of the PCR also involved a different temperature, 56 degrees Celsius. Overnight digestion of the PCR product occurred using the *Taq I* enzyme and a temperature of 65 degrees Celsius. The digestion mixture included 15 µL of PCR product, 0.6 µL of enzyme, 2.2 µL of distilled deionized water, 2.0 µL of manufacturer provided buffer 3, and 0.2 µL of BSA. Gel electrophoresis remained as previously described. The bands visualized for genotyping the Ser301Ser polymorphism were: homozygous TT with 1 band at

361 bp; homozygous CC with 2 bands, 1 at 204 bp and 1 at 157 bp; and heterozygous CT with 3 bands, 1 at 361 bp, 204 bp, and 157 bp.

Custom TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA) were developed for genotyping the PBEF1 -948 and -1001 polymorphisms based on a previously published report (64). The primers and reporters for the -948 polymorphism were: forward primer, 5'-GCCCGTTGCCTTTTCCTT-3'; reverse primer, 5'-GGTGGAAATTCAGTCCTCACAGATAA-3'; VIC reporter, CCTAATTGAACCGAGTATT; FAM reporter, CCTAATTGAACAGAGTATT. The primers and reporters for the -1001 polymorphism were: forward primer, 5'-CCAACTCGTTTCCCAGGATTTAAAG-3'; reverse primer, 5'-ACGGGCCAAGCCTTTGA-3'; VIC reporter, CAGTGTCGCACCCTG; FAM reporter, CAGTTTCGCACCCTG. All reactions were carried out using an Applied Biosystems 7300 Real Time PCR System and optical reaction plates. Plate wells contained a total reaction volume of 12.5 μ L consisting of 6.25 μ L of 2X TaqMan Universal PCR Master Mix (Perkin-Elmer, Applied Biosystems Division), 0.625 μ L of 20X diluted SNP mix, 1.5 μ L of genomic DNA, and 4.125 μ L of nuclease free water. The PCR cycling protocol consisted of 2 minutes at 50 degrees Celsius; 10 minutes at 95 degrees Celsius (for AmpliTaq Gold DNA polymerase activation); and 50 cycles of 92 degrees Celsius for 15 seconds (for melting/denaturation) and 60 degrees Celsius for 1 minute (for annealing and extending). Fluorescence (via allele specific VIC and FAM dyes) was measured and genotypes were determined using the Applied Biosystems 7300 System Sequence Detection Software. For quality control

purposes, each plate contained 4 no template controls and 4 positive control samples of known genotype (determined previously via direct sequencing).

Haplotype Estimation

The computational inference program PHASE, version 2.1 (108; 109) was used to construct haplotypes from the 5 PBEF1 polymorphisms investigated in this study. A dataset containing the genotypes for the study participants was input into the PHASE program as described in the program's documentation. Resulting haplotypes with a frequency $\geq 5\%$ were used in exploratory analyses involving glucose and obesity-related variables.

Aerobic Exercise Training

Following the completion of baseline testing, subjects initiated 24 weeks of supervised aerobic exercise training. Briefly, a participant's resting and maximum heart rate data, obtained during the baseline maximal graded treadmill test, were used in the Karvonen (heart rate reserve) formula to determine his/her exercise training heart rates. A heart rate range was determined for each participant corresponding to the percent intensity desired $\pm 5\%$. During the exercise training sessions, Polar heart rate monitors were used to assess heart rate. Training equipment available to the participants included stair climbers, stationary bicycles, recombiant bicycles, rowing ergometers, treadmills, elliptical machines, and cross country ski machines. To gradually acclimatize the sedentary participants to exercise, the first week of training involved 20 minutes of activity at 50% intensity for 3 days per week. Exercise duration then progressed by 5 minutes weekly, until 40 minutes was obtained. Then,

exercise intensity increased gradually, 5 % per week, until 70 % was attained. Thus, from week 9 through week 24, participants exercised for 40 minutes at an intensity of 70 %. In week 10, participants were also asked to add in an unsupervised home workout. Participants recorded resting and exercise heart rates and blood pressure measurements in training logs provided by the GERS.

Statistical Analysis

All statistical analyses were performed using SAS (version 9.1) and SPSS (version 14.0) software. Before any statistical analyses were conducted, the assumptions for each procedure were examined, and as a result, transformation using the common logarithm was required for visfatin, triglycerides, intra-abdominal fat, insulin AUC, ISI, and C-reactive protein in order to meet the assumption(s) of normality and/or homogeneity of variance. Chi-square tests were used to assess Hardy-Weinberg equilibrium, pairwise linkage disequilibrium, and to compare differences in categorical variables between groups. However, a Fisher's Exact Probability Test was used when the frequency of categorical data in a cell was ≤ 5 . Pearson correlation coefficients were used to examine relationships between potential covariates and between plasma visfatin levels and glucose and obesity-related variables. Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) techniques were used to compare baseline and after aerobic exercise training concentrations of plasma visfatin between normal glucose tolerant (NGT) and impaired glucose tolerant (IGT) individuals. BMI was used as a covariate in the baseline visfatin analysis, and BMI and the baseline plasma visfatin level were covariates in the after training analysis. Gender was included in the visfatin analyses

as a potential moderating factor. Paired t-tests were used to determine significant changes within the IGT and NGT groups with aerobic exercise training. ANCOVA was also used to compare differences in glucose and obesity-related variables between PBEF1 genotype groups and haplotype groups at baseline and in response to aerobic exercise training. When appropriate, covariates for the baseline analyses included age and BMI, and covariates for the after aerobic exercise training analyses included age, BMI, and the baseline value of the outcome variable. Gender was also included as a potential moderating factor in all of the analyses. When significant genotype, haplotype, or interaction effects were detected, Bonferroni adjusted post-hoc multiple comparisons were undertaken. Paired t-tests were used to determine significant changes within groups with aerobic exercise training. Statistical significance was set at $p \leq 0.05$.

Chapter 3: Results

Hypothesis 1: Plasma Visfatin Concentration

Baseline

Sixty seven participants with an average age of 59 ± 1 yr had viable plasma samples available for analysis of fasting visfatin concentration. Furthermore, 22 of the participants (14 men, 8 women) had impaired glucose tolerance (IGT) at baseline, whereas 45 participants (21 men, 24 women) were classified with normal glucose tolerance (NGT). Hormone replacement therapy (HRT) use among women in the study was 63 % in the IGT group and 54 % in the NGT group and was not significantly different between the groups ($p > 0.999$). Table 3-1 displays baseline characteristics for the IGT and NGT groups. Besides having lower insulin sensitivity (via ISI) and elevated fasting glucose, glucose total area under the curve (AUC), and insulin AUC, the IGT group also had higher body mass index (BMI; $p = 0.032$) and intra-abdominal fat measurements ($p = 0.020$) than the NGT group. As the BMI and intra-abdominal fat measurements were correlated ($r = 0.599$, $p < 0.001$), and BMI data were available on all 67 participants as compared to 65 participants with intra-abdominal fat measurements, BMI was used as a covariate in the analyses involving visfatin.

Correlations involving plasma visfatin concentration and glucose and obesity-related variables are displayed in Table 3-2. Although the variables examined were not significantly correlated with the baseline visfatin concentration, there was a

tendency towards a negative relationship between insulin AUC and baseline visfatin ($r = -0.242$, $p = 0.070$). However, insulin AUC was not included as a covariate because of its relatedness to the 120 minute OGTT glucose concentration (used to determine IGT/NGT status; $r = 0.487$, $p < 0.001$). There were also no significant correlations involving plasma visfatin concentration and glucose and obesity-related variables when the IGT and NGT groups were analyzed separately (data not shown). One-way ANOVA techniques were used to examine the influence of plasma sample age, study qualification (APO-E, ACE, both), cardiovascular disease risk factor quantity, and HRT usage on baseline levels of visfatin. However, these factors were not statistically related to the baseline visfatin concentration ($p = 0.156$, $p = 0.522$, $p = 0.744$, and $p = 0.217$, respectively).

After including BMI as a covariate and gender as a potentially moderating factor in the analysis, baseline plasma visfatin levels did not differ between the IGT and NGT groups [Figure 3-1; 18 (14-23) ng/mL versus 20 (17-23) ng/mL, $p = 0.527$].

Table 3-1. Characteristics of visfatin study participants at baseline.

	IGT (n = 17-22)	NGT (n = 40-45)	P-value
Men/Women	14/8	21/24	0.192
HRT use (Yes/No)	5/3	13/11	1.000
Age (yrs)	59 ± 2	59 ± 1	0.851
VO ₂ max (mL/kg/min)	25.8 ± 1.1	26.2 ± 0.7	0.750
Weight (kg)	84.2 ± 3.2	79.1 ± 2.4	0.216
BMI (kg/m ²)	29.0 ± 0.9	26.6 ± 0.6	0.032*
Intra-abdominal fat (cm ²)	144 (119-175)	110 (97-125)	0.020*
Subcutaneous fat (cm ²)	310 ± 21	294 ± 19	0.624
Total fat (%)	35.8 ± 2.1	34.7 ± 1.4	0.654
Total cholesterol (mg/dL)	192 ± 7	196 ± 4	0.615
Triglycerides (mg/dL)	133 (117-151)	126 (108-148)	0.660
HDL-cholesterol (mg/dL)	43 ± 3	47 ± 2	0.242
LDL-cholesterol (mg/dL)	122 ± 6	119 ± 4	0.665
Fasting glucose (mg/dL)	95 ± 2	90 ± 1	0.039*
Fasting insulin (pmol/L)	84 ± 7	72 ± 4	0.116
ISI	2.5 (2.0-3.0)	4.2 (3.6-4.8)	< 0.001*
Glucose AUC (mg/dL x 120 min)	19913 ± 561	14632 ± 431	< 0.001*
Insulin AUC (pmol/L x 120 min)	65826 (53015- 81733)	40365 (34882- 46709)	< 0.001*
C-reactive protein (mg/L)	22 (12-40)	14(11-19)	0.150

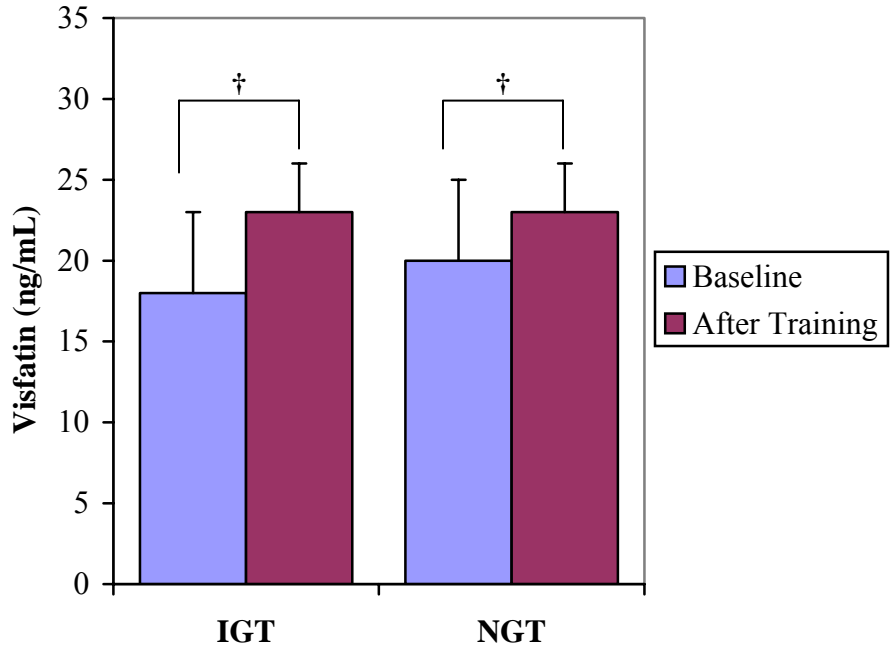
Data are expressed as mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and C-reactive protein, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; VO₂max, maximal oxygen consumption; BMI, body mass index; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. P-values are from χ^2 or Fisher Exact tests (categorical variables) and ANOVA (continuous variables). * indicates significant difference between groups.

Table 3-2. Correlations between plasma visfatin concentration and glucose and obesity-related variables at baseline.

	N	Pearson's correlation	P-value
Weight (kg)	67	-0.056	0.654
BMI (kg/m ²)	67	-0.100	0.421
Intra-abdominal fat (cm ²)	65	-0.160	0.202
Subcutaneous fat (cm ²)	65	-0.125	0.323
Total fat (%)	66	-0.109	0.383
Total cholesterol (mg/dL)	67	-0.170	0.170
Triglycerides (mg/dL)	67	-0.031	0.802
HDL-cholesterol (mg/dL)	67	-0.052	0.676
LDL-cholesterol (mg/dL)	67	-0.149	0.228
Fasting glucose (mg/dL)	67	-0.034	0.788
Fasting insulin (pmol/L)	58	-0.076	0.573
ISI	57	0.182	0.175
Glucose AUC (mg/dL x 120 min)	66	-0.056	0.657
Insulin AUC (pmol/L x 120 min)	57	-0.242	0.070
C-reactive protein (mg/L)	57	-0.003	0.985

Intra-abdominal fat, triglycerides, ISI, insulin AUC, C-reactive protein, and visfatin were transformed using the common logarithm prior to analysis. BMI, body mass index; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants.

Figure 3-1. Plasma visfatin concentration at baseline and after aerobic exercise training, grouped by glucose tolerance status. IGT, impaired glucose tolerance; NGT, normal glucose tolerance. Values are geometric mean + 95 % confidence interval. * indicates statistically significant difference between groups. † indicates significant change within group with aerobic exercise training (via paired t-test).



After Aerobic Exercise Training

Table 3-3 displays participant characteristics following the completion of 24 weeks of aerobic exercise training, and fasting insulin ($p = 0.012$), glucose AUC ($p < 0.001$), insulin AUC ($p = 0.014$), and ISI ($p = 0.004$) still differed between the IGT and NGT groups. In general, aerobic exercise training adherence for the IGT and NGT groups did not differ ($p = 0.716$), averaging 89.5 ± 1.6 and 90.3 ± 1.3 %, respectively. Furthermore, the IGT and NGT groups similarly and significantly improved VO_2 max (both $p < 0.001$), HDL-cholesterol ($p = 0.001$, $p < 0.001$), and insulin AUC ($p = 0.014$, $p = 0.003$) with training. In contrast, changes in weight, BMI, total body fat, and fasting insulin were not consistent between the groups.

Correlations involving the aerobic exercise training-induced change in plasma visfatin concentration and the aerobic exercise training-induced change in glucose and obesity-related variables are displayed in Table 3-4. However, none of the variable changes were significantly correlated with the aerobic exercise training-induced change in plasma visfatin concentration. Similar results were also found for correlations involving the NGT group (data not shown). However, in the IGT group, there was a weak correlation between the aerobic exercise training-induced increase in plasma visfatin and the non-significant decrease in C-reactive protein levels ($r = 0.547$, $p = 0.043$). But after accounting for gender, this relationship was no longer significant ($r = 0.531$, $p = 0.062$).

Although the IGT and NGT groups both significantly increased plasma visfatin levels with training (Figure 3-1, $p = 0.028$, $p = 0.027$), the changes were similar, resulting in no difference between the groups after training [Figure 3-1; 23 (19-28) ng/mL versus 23 (20-26) ng/mL, $p = 0.949$].

Table 3-3. Characteristics of visfatin study participants after 24 weeks of aerobic exercise training.

	IGT (n = 17-22)	NGT (n = 40-45)	P-value ¹	P-value ²
VO ₂ max (mL/kg/min)	30.7 ± 1.2†	29.7 ± 1.0†	0.524	0.132
Weight (kg)	82.2 ± 3.1†	78.6 ± 2.4	0.376	0.022*
Body mass index (kg/m ²)	28.3 ± 0.8†	26.5 ± 0.6	0.100	0.023*
Intra-abdominal fat (cm ²)	128 (101-161)†	104 (91-119)	0.115	0.878
Subcutaneous fat (cm ²)	303 ± 26	277 ± 17	0.410	0.690
Total fat (%)	33.8 ± 1.7†	33.4 ± 1.4†	0.880	0.004*
Total cholesterol (mg/dL)	187 ± 8	198 ± 4	0.215	0.278
Triglycerides (mg/dL)	120 (104-140)	117 (102-135)†	0.819	0.807
HDL-cholesterol (mg/dL)	48 ± 4†	51 ± 2†	0.418	0.971
LDL-cholesterol (mg/dL)	114 ± 7	119 ± 4	0.485	0.278
Fasting glucose (mg/dL)	95 ± 2	93 ± 1†	0.510	0.459
Fasting insulin (pmol/L)	84 ± 8	65 ± 3†	0.012*	0.037*
Insulin sensitivity index	3.0 (2.3-3.8)	4.4 (3.8-4.9)	0.004*	0.856
Glucose AUC (mg/dL x 120 min)	18251 ± 708†	15150 ± 453	<0.001*	0.611
Insulin AUC (pmol/L x 120 min)	51892 (39619-67967)†	36796 (31915-42433)†	0.014*	0.741
C-reactive protein (mg/L)	19 (11-34)	13 (9-20)	0.297	0.737

Data are expressed as mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and C-reactive protein, which are presented as geometric mean (95 % confidence interval). IGT, impaired glucose tolerance; NGT, normal glucose tolerance; VO₂max, maximal oxygen consumption; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. P-value¹ from ANOVA; P-value² from ANCOVA using baseline variable value as a covariate and gender as a moderating factor. * indicates significant difference between groups. † indicates significant change within group with aerobic exercise training (via paired t-test).

Table 3-4. Correlations between aerobic exercise training-induced change in plasma visfatin concentration and glucose and obesity-related variables.

	N	Pearson's correlation	P-value
Weight (kg)	67	-0.089	0.475
BMI (kg/m ²)	67	-0.073	0.555
Intra-abdominal fat (cm ²)	59	-0.146	0.271
Subcutaneous fat (cm ²)	58	-0.093	0.487
Total fat (%)	64	-0.137	0.280
Total cholesterol (mg/dL)	64	-0.022	0.864
Triglycerides (mg/dL)	63	0.025	0.844
HDL-cholesterol (mg/dL)	63	0.126	0.325
LDL-cholesterol (mg/dL)	63	-0.114	0.373
Fasting glucose (mg/dL)	66	0.070	0.579
Fasting insulin (pmol/L)	56	-0.149	0.272
ISI	53	0.133	0.341
Glucose AUC (mg/dL x 120 min)	63	-0.089	0.488
Insulin AUC (pmol/L x 120 min)	53	-0.046	0.744
C-reactive protein (mg/L)	51	0.150	0.294

Intra-abdominal fat, triglycerides, ISI, insulin AUC, C-reactive protein, and visfatin were transformed using the common logarithm prior to analysis. BMI, body mass index; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants.

Hypothesis 2: Baseline

Data from 116 participants (55 men, 61 women) were included in the PBEF1 genotype analyses. General baseline characteristics of this sample are presented in Table 3-5. On average, the participants were overweight, had borderline-high risk levels of total cholesterol and LDL-cholesterol (2), and had below average VO₂max values (6). In addition, approximately 47.5 % of women used HRT while participating in the GERS. Among the 116 participants, there were 80 who qualified only for the GERS APO-E study, 20 who qualified only for the GERS ACE study, and 16 who qualified for both studies. Results from ANOVA analyses indicated significant differences in total cholesterol ($p < 0.001$), LDL-cholesterol ($p < 0.001$), and triglyceride ($p = 0.001$) levels between the groups. Post-hoc multiple comparisons with Bonferroni correction identified that in all 3 instances, those individuals qualifying only for the ACE study had significantly different values than those qualifying for both studies ($p < 0.001$, $p = 0.002$, $p = 0.032$) or for only the APO-E study ($p < 0.001$, $p < 0.001$, $p = 0.001$). Thus, only data from APO-E qualifiers were used in the analyses involving lipoprotein-lipid levels. As blood pressure was not investigated in this project and other GERS entrance criteria were uniform between the ACE and APO-E studies, the groups were combined for the analyses involving glucose and obesity-related variables. As suggested by correlation matrices, age and BMI were included as covariates, when appropriate, and gender was included as a potential moderating variable in the analyses.

Table 3-5. Characteristics of study participants at baseline.

Gender (men/women)	(n = 116)	55/61
Age (yrs)	(n = 116)	59 ± 1
VO ₂ max (mL/kg/min)	(n = 116)	25.7 ± 0.4
Weight (kg)	(n = 116)	81.7 ± 1.4
BMI (kg/m ²)	(n = 116)	27.9 ± 0.4
Intra-abdominal fat (cm ²)	(n = 113)	124 (116-133)
Subcutaneous fat (cm ²)	(n = 112)	307 ± 11
Total fat (%)	(n = 114)	36.2 ± 0.8
Total cholesterol (mg/dL)	(n = 96)	210 ± 3
Triglycerides (mg/dL)	(n = 96)	147 (134-161)
HDL-cholesterol (mg/dL)	(n = 95)	47 ± 1
LDL-cholesterol (mg/dL)	(n = 95)	131 ± 3
Fasting glucose (mg/dL)	(n = 85)	92 ± 1
Fasting insulin (pmol/L)	(n = 72)	80 ± 3
ISI	(n = 71)	3.3 (3.0-3.7)
Glucose AUC (mg/dL x 120 min)	(n = 82)	16495 ± 397
Insulin AUC (pmol/L x 120 min)	(n = 71)	49774 (44627-55514)
Fasting visfatin (ng/mL)	(n = 63)	20 (18-23)

Data are expressed as mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. VO₂max, maximal oxygen consumption; BMI, body mass index; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants.

PBEF1 -4689 G>T (rs2110385)

Genotypes for the PBEF1 -4689 polymorphism were available on 115 of the 116 possible participants, and the genotype frequencies were 37 % GG, 47 % GT, and 16 % TT. The resultant T allele frequency was 39 %, which was consistent with a previous report (11). In addition, the genotype frequencies did not vary from Hardy-Weinberg equilibrium ($\chi^2 = 0.023$, $p = 0.878$), and gender ($\chi^2 = 0.511$, $p = 0.774$) and HRT use ($p = 0.592$) did not differ by genotype group.

There were no significant genotype group differences at baseline for visfatin, lipoprotein-lipid, or body composition variables (Table 3-6). However, there was a significant genotype effect for insulin AUC ($p = 0.002$), with the GG group having a significantly higher insulin AUC value than the TT group ($p = 0.004$) and the GT group ($p = 0.021$). In addition, there was a significant gender by genotype interaction effect for ISI (Figure 3-2; $p = 0.028$), as women with the TT genotype had a significantly higher ISI value than women with the GG genotype ($p = 0.001$), men with the GG genotype ($p = 0.025$), or men with the GT genotype ($p = 0.011$). The main effects for gender ($p = 0.02$) and genotype ($p = 0.012$) were also significant, with women having a higher ISI than men and the GG genotype group having a lower ISI than the TT genotype group ($p = 0.010$).

As displayed in Figure 3-2, there were significant genotype by gender interaction effects for fasting glucose ($p = 0.017$) and fasting insulin ($p = 0.041$). Pairwise comparisons indicated that women with the TT genotype had a significantly lower fasting glucose concentration than men with the GG genotype ($p = 0.001$), GT genotype ($p = 0.041$), or TT genotype ($p = 0.002$) and a significantly lower fasting insulin concentration than men with the GT genotype ($p = 0.032$). However, these findings should be viewed with caution as there were only 6 women in our study with the TT genotype. For fasting glucose, the main effect of gender was also significant (men > women; $p < 0.001$).

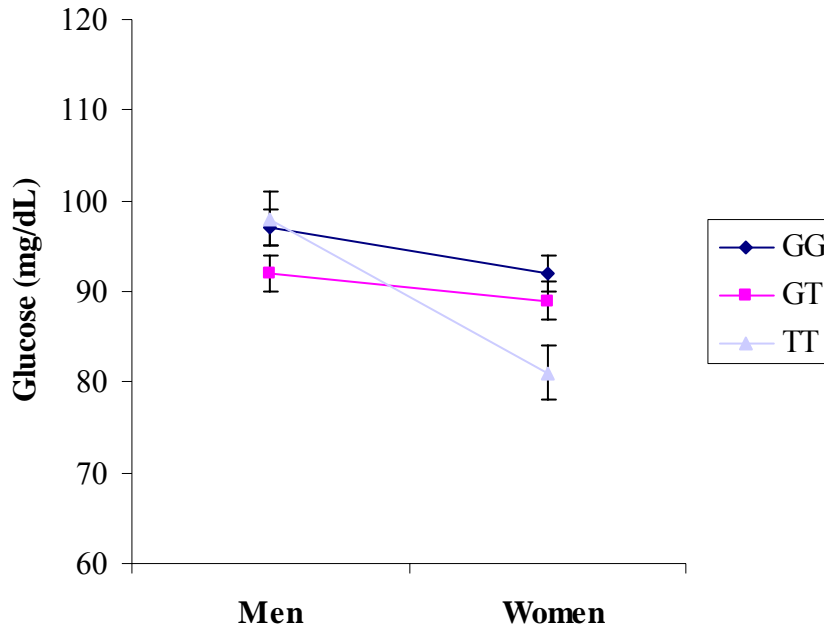
Table 3-6. Baseline characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 -4689 polymorphism.

	GG (n = 24-43)	GT (n = 35-54)	TT (n = 12-18)	P-value
Men/women	20/23	25/29	10/8	---
HRT use (Y/N)	11/12	12/17	5/3	---
Age (yrs)	58 ± 1	58 ± 1	60 ± 1	0.629
VO ₂ max	26.3 ± 0.5	25.9 ± 0.5	24.2 ± 0.8	0.103
Weight (kg)	80.8 ± 2.1	82.6 ± 1.8	81.1 ± 3.2	0.796
BMI (kg/m ²)	28.0 ± 0.6	27.8 ± 0.6	27.9 ± 1.0	0.981
IA fat (cm ²)	129 (116-143)	128 (117-141)	108 (92-126)	0.140
SC fat (cm ²)	294 ± 16	308 ± 15	321 ± 25	0.644
Total fat (%)	35.6 ± 1.0	35.9 ± 0.9	37.7 ± 1.5	0.458
TC (mg/dL)	210 ± 5	208 ± 5	217 ± 9	0.684
TG (mg/dL)	162 (140-188)	138 (122-157)	138 (107-177)	0.239
HDL (mg/dL)	45 ± 2	46 ± 2	51 ± 4	0.348
LDL (mg/dL)	129 ± 5	131 ± 4	131 ± 8	0.953
Glucose (mg/dL)	94 ± 1	91 ± 1	90 ± 2	0.085
Insulin (pmol/L)	84 ± 5	81 ± 4	66 ± 6	0.074
ISI	2.9 (2.5-3.3)	3.4 (3.0-3.9)	4.2 (3.4-5.2)	0.012*
Glucose AUC	17027 ± 619	16562 ± 544	15064 ± 941	0.221
Insulin AUC	61944 (52966- 72444)	46559 (40926- 52966)	39084 (31333- 48753)	0.002*
Visfatin (ng/mL)	20 (16-25)	19 (15-23)	23 (17-31)	0.621

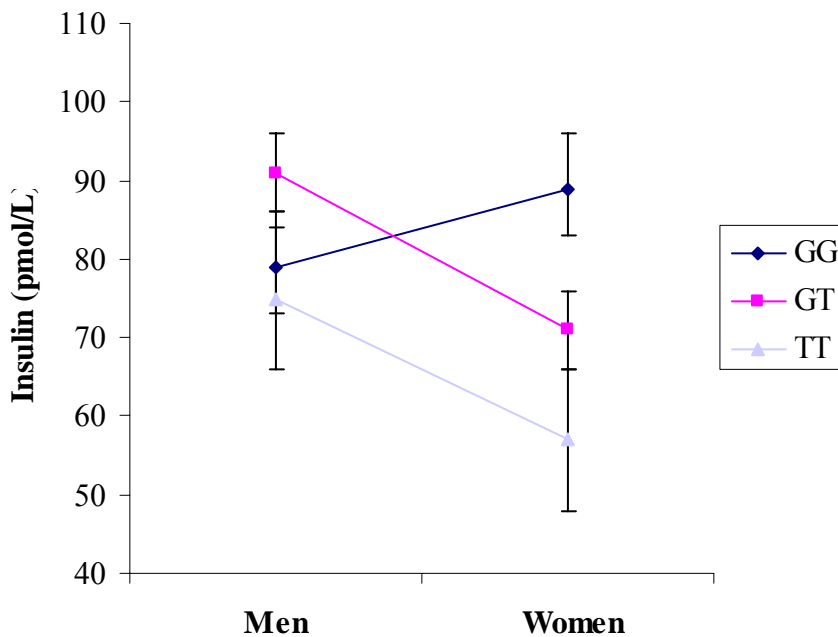
Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data which are presented as count. HRT, hormone replacement therapy; Y, yes; N, no; VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: VO₂max, mL/kg/min; glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of genotype. * indicates significant difference between genotype groups.

Figure 3-2. Interaction between gender and the PBEF1 -4689 polymorphism for A) fasting glucose ($p = 0.017$), B) fasting insulin ($p = 0.041$), and C) ISI ($p = 0.028$). Data are presented as adjusted mean \pm SE except for ISI, which is presented as geometric mean \pm 95 % confidence interval.

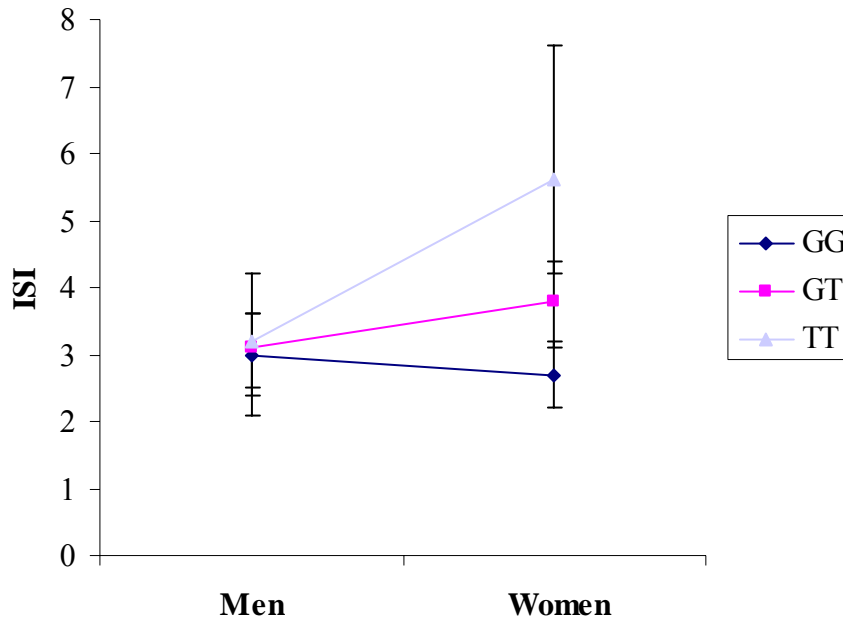
A.



B.



C.



PBEF1 -1543 C>T

Genotypes for the -1543 polymorphism were available on 115 of the 116 possible participants. Genotype frequencies for the PBEF1 -1543 polymorphism were 54 % CC, 39 % CT, and 7 % TT. The T allele frequency was approximately 27 %, consistent with previous reports (9; 129). In addition, the genotype frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 0.002$, $p = 0.966$), and both gender ($p > 0.999$) and HRT use ($p = 0.845$) did not differ by genotype group. Due to the low frequency of individuals with the TT genotype and non-statistically different CT and TT group means for the major outcome variables (all $p > 0.398$, data not shown), the CT and TT genotype groups were combined for analysis.

There were no significant genotype group differences at baseline for visfatin, lipoprotein-lipid, body composition, or OGTT variables (Table 3-7). However, as shown in Figure 3-3, there was a significant gender by genotype interaction effect for

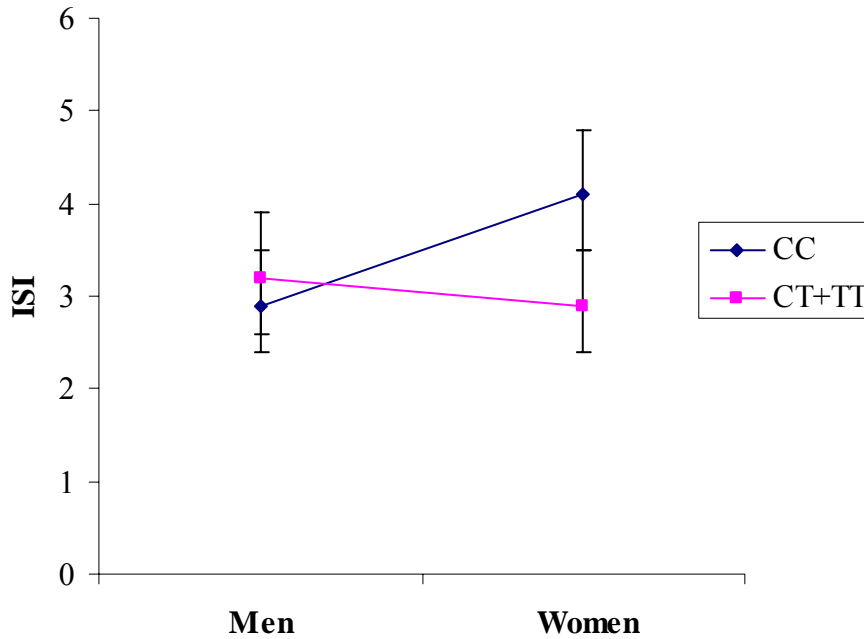
ISI ($p = 0.024$), but after Bonferroni adjustment, none of the pairwise comparisons were significantly different.

Table 3-7. Baseline characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 -1543 polymorphism.

	CC (n = 38-62)	CT+TT (n = 33-53)	P-value
Men/Women	30/32	25/28	---
HRT use (Y/N)	16/16	12/16	---
Age (yrs)	59 ± 1	58 ± 1	0.563
VO ₂ max (mL/kg/min)	25.4 ± 0.4	26.0 ± 0.5	0.366
Weight (kg)	81.1 ± 1.7	82.8 ± 1.8	0.506
BMI (kg/m ²)	27.8 ± 0.5	28.2 ± 0.6	0.575
IA fat (cm ²)	120 (110-131)	131 (119-144)	0.208
SC fat (cm ²)	308 ± 13	309 ± 15	0.965
Total fat (%)	36.6 ± 0.8	35.7 ± 0.9	0.435
TC (mg/dL)	214 ± 4	208 ± 4	0.376
TG (mg/dL)	147 (129-167)	148 (130-168)	0.957
HDL (mg/dL)	46 ± 2	47 ± 2	0.765
LDL (mg/dL)	133 ± 4	129 ± 4	0.432
Glucose (mg/dL)	91 ± 1	93 ± 1	0.162
Insulin (pmol/L)	75 ± 4	86 ± 4	0.067
ISI	3.5 (3.1-4.0)	3.1 (2.7-3.5)	0.139
Glucose AUC	16511 ± 527	16549 ± 561	0.960
Insulin AUC	46989 (41115-53703)	54325 (46989-62951)	0.145
Visfatin (ng/mL)	21 (18-25)	18 (15-22)	0.271

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; Y, yes; N, no; VO₂max, maximal oxygen consumption; BMI, body mass index; IA, intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of genotype. * indicates significant difference between genotype groups.

Figure 3-3. Interaction between gender and the PBEF1 -1543 polymorphism for ISI (p = 0.024). Data are presented as geometric mean \pm 95 % confidence interval.



PBEF1 -1001 T>G (rs9770242)

Genotypes for the PBEF1 -1001 polymorphism were available for all 116 participants, and the genotype frequencies were approximately 8 % GG, 33 % GT, and 59 % TT, which were consistent with Hardy-Weinberg equilibrium ($\chi^2 = 1.29$, $p = 0.256$). The G allele frequency of 24 % was congruent with previous reports (9; 64), and both gender ($p = 0.879$) and HRT use ($p = 0.845$) did not differ between the PBEF1 -1001 genotype groups. However, as there were few GG homozygotes in the study and baseline variables did not differ significantly between the GT and GG groups (all $p > 0.120$, data not shown), the GG genotype group was combined with the GT genotype group for analyses involving outcome variables.

Baseline characteristics of the participants grouped by -1001 genotype appear in Table 3-8. The TT genotype group had significantly higher VO_2max ($p = 0.038$), BMI ($p = 0.017$), and fasting insulin ($p = 0.027$) values and a significantly lower ISI value ($p = 0.040$) than the GG+GT group. As depicted in Figure 3-4, there were also significant gender by genotype interaction effects detected for total cholesterol ($p = 0.024$) and LDL-cholesterol ($p = 0.025$). Furthermore, pairwise comparisons indicated that women with the G allele had significantly higher total cholesterol values than men with the G allele ($p = 0.046$), but for LDL-cholesterol, none of the pairwise comparisons were significant after Bonferroni adjustment.

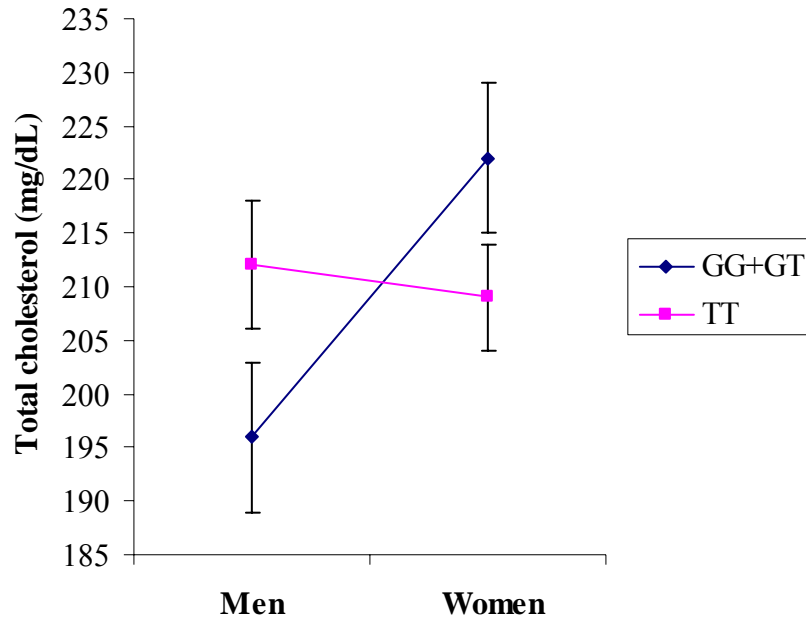
Table 3-8. Baseline characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 -1001 polymorphism.

	GG+GT (n = 32-47)	TT (n = 38-69)	P-value
Men/Women	23/24	32/37	---
HRT use (Y/N)	13/11	16/21	---
Age (yrs)	58 ± 1	59 ± 1	0.560
VO ₂ max (mL/kg/min)	24.9 ± 0.5	26.3 ± 0.4	0.038*
Weight (kg)	83.8 ± 1.9	80.3 ± 1.6	0.169
BMI (kg/m ²)	27.2 ± 0.5	28.8 ± 0.4	0.017*
IA fat (cm ²)	124 (112-137)	124 (115-135)	0.962
SC fat (cm ²)	329 ± 15	292 ± 13	0.061
Total fat (%)	37.3 ± 0.9	35.4 ± 0.8	0.124
TC (mg/dL)	209 ± 5	211 ± 4	0.782
TG (mg/dL)	137 (119-158)	154 (137-174)	0.205
HDL (mg/dL)	48 ± 2	46 ± 2	0.433
LDL (mg/dL)	130 ± 4	132 ± 4	0.799
Glucose (mg/dL)	91 ± 1	93 ± 1	0.175
Insulin (pmol/L)	72 ± 5	86 ± 4	0.027*
ISI	3.8 (3.2-4.4)	3.0 (2.6-3.5)	0.040*
Glucose AUC	15370 ± 651	16996 ± 575	0.069
Insulin AUC	45186 (38548-53088)	53827 (46666-62087)	0.113
Visfatin (ng/mL)	22 (18-27)	19 (16-22)	0.296

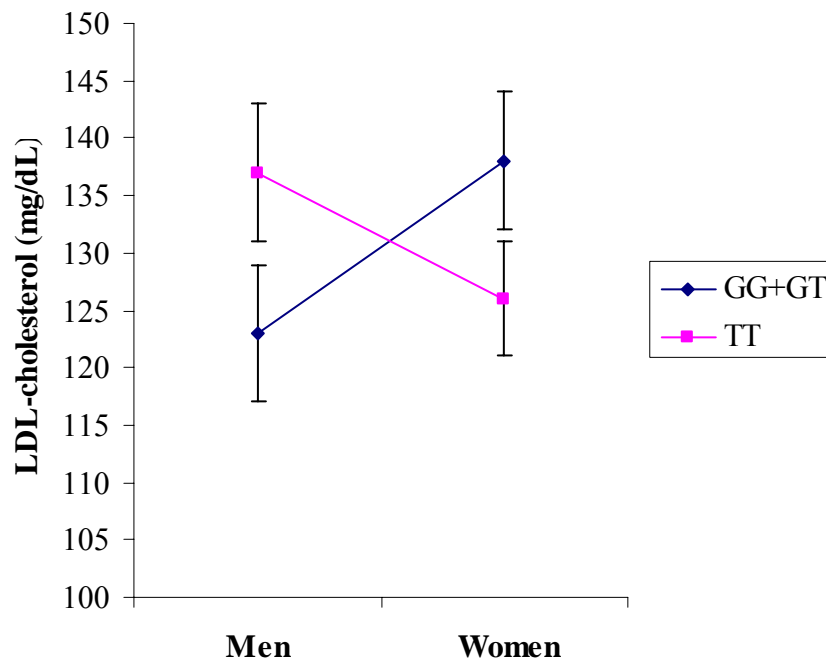
Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; Y, yes; N, No; VO₂max, maximal oxygen consumption; BMI, body mass index; IA, intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of genotype. * indicates significant difference between genotype groups.

Figure 3-4. Interaction between gender and the PBEF1 -1001 polymorphism for A) total cholesterol ($p = 0.024$) and B) LDL-cholesterol ($p = 0.025$). Data are presented as adjusted mean \pm SE.

A.



B.



PBEF1 -948 G>T

PBEF1 -948 G>T genotypes were available for all 116 participants. The genotype frequencies were 71 % GG, 26 % GT, and 3 % TT, resulting in a T allele frequency of 16 %, similar to previous reports (11; 64; 132). In addition, the genotype frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 0.36$, $p = 0.547$), and gender ($p = 0.524$) and HRT use ($p = 0.883$) were not different between the groups. Due to the low frequency of the TT genotype and no statistical differences between the GT and TT groups at baseline (all $p > 0.301$, data not shown), the TT group was combined with the GT group for analysis.

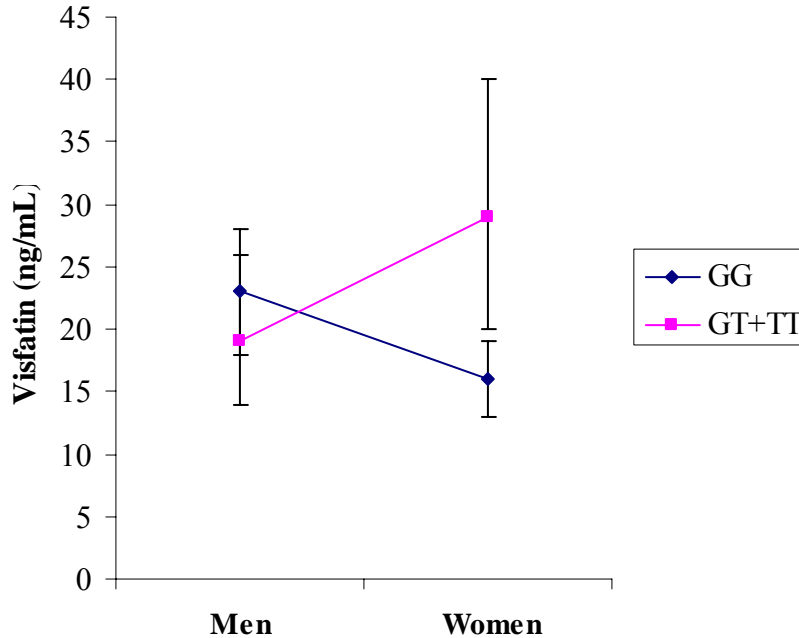
As displayed in Table 3-9, the GG genotype group had significantly higher glucose AUC ($p = 0.030$) and insulin AUC ($p = 0.018$) values and a significantly lower ISI value ($p = 0.017$) than the combined GT and TT group. The GG group also tended to have a higher fasting insulin concentration than the T allele carriers ($p = 0.051$). Last, as shown in Figure 3-5, there was a significant gender by genotype interaction detected for the baseline visfatin concentration ($p = 0.006$), as women with the T allele had significantly higher baseline visfatin levels than women with the GG genotype ($p = 0.028$).

Table 3-9. Baseline characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 -948 polymorphism.

	GG (n = 49-82)	GT+TT (n = 19-34)	P-value
Men/Women	39/43	16/18	---
HRT use (Y/N)	20/23	9/9	---
Age (yrs)	59 ± 1	58 ± 1	0.323
VO ₂ max (mL/kg/min)	25.9 ± 0.4	25.3 ± 0.6	0.394
Weight (kg)	81.4 ± 1.5	82.5 ± 2.3	0.695
BMI (kg/m ²)	28.4 ± 0.4	27.4 ± 0.6	0.150
IA fat (cm ²)	128 (118-138)	117 (104-131)	0.198
SC fat (cm ²)	310 ± 12	301 ± 18	0.678
Total fat (%)	36.3 ± 0.7	36.0 ± 1.1	0.819
TC (mg/dL)	213 ± 4	203 ± 6	0.156
TG (mg/dL)	154 (138-171)	130 (109-154)	0.102
HDL (mg/dL)	46 ± 2	49 ± 2	0.232
LDL (mg/dL)	133 ± 3	125 ± 5	0.226
Glucose (mg/dL)	92 ± 1	91 ± 2	0.586
Insulin (pmol/L)	84 ± 4	70 ± 6	0.051
ISI	3.1 (2.7-3.5)	4.0 (3.3-4.9)	0.017*
Glucose AUC	16942 ± 515	14882 ± 759	0.030*
Insulin AUC	54325 (47863-61518)	41020 (33884-49659)	0.018*
Visfatin (ng/mL)	19 (16-22)	23 (18-30)	0.139

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; Y, yes; N, no; VO₂max, maximal oxygen consumption; BMI, body mass index; IA, intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of genotype. * indicates significant difference between genotype groups.

Figure 3-5. Interaction between gender and the PBEF1 -948 polymorphism for fasting plasma visfatin concentration ($p = 0.006$). Data are presented as geometric mean \pm 95 % confidence interval.



PBEF1 SER301SER C>T (rs2302559)

Genotypes for the PBEF1 SER301SER polymorphism were available on 113 of the 116 participants. Genotype frequencies for the PBEF1 SER301SER polymorphism were 42 % CC, 45 % CT, and 12 % TT. The T allele frequency of 35 % was slightly lower than previously reported (132). However, the genotype frequencies met Hardy-Weinberg expectancies ($\chi^2 = 0.006$, $p = 0.937$), and both gender ($\chi^2 = 0.697$, $p = 0.706$) and HRT use ($p = 0.931$) did not differ by genotype group.

As presented in Table 3-10, intra-abdominal fat ($p = 0.026$), HDL-cholesterol ($p = 0.039$), fasting insulin ($p = 0.018$), insulin AUC ($p = 0.002$), and insulin sensitivity ($p = 0.004$) differed by SER301SER genotype group. Moreover, the CC

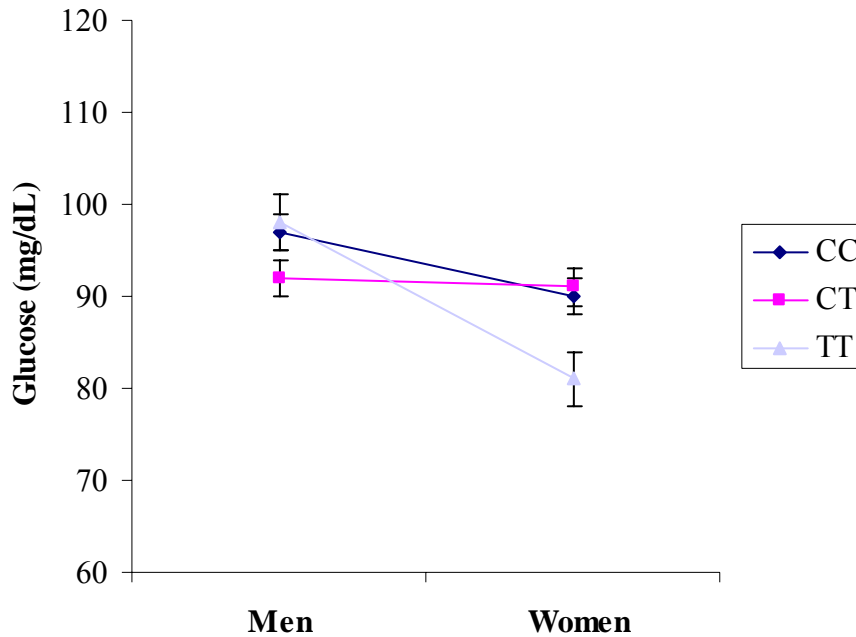
and CT groups had significantly higher intra-abdominal fat ($p = 0.037$ and 0.029) and significantly lower insulin sensitivity ($p = 0.003$ and 0.037) than the TT group. The CC group also had significantly higher fasting insulin ($p = 0.014$) and higher insulin AUC ($p = 0.002$) values than the TT group. Pairwise comparisons for HDL-cholesterol indicated no significant differences after Bonferroni adjustment. Finally, there was a significant gender by genotype interaction effect for fasting glucose (Figure 3-6; $p = 0.007$) with a significant gender, but not a genotype, main effect (men > women, $p < 0.001$). Pairwise comparisons indicated that women with the TT genotype had significantly lower fasting glucose levels than men with the CC ($p = 0.001$) or TT ($p = 0.004$) genotype. However, these results should be viewed with caution as there were only 6 women in the TT genotype group.

Table 3-10. Baseline characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 SER301SER polymorphism.

	CC (n =26-48)	CT (n = 31-51)	TT (n = 9-14)	P-value
Men/Women	24/24	23/28	8/6	---
HRT use (Y/N)	10/14	13/15	3/3	---
Age (yrs)	58 ± 1	59 ± 1	59 ± 1	0.757
VO ₂ max	26.4 ± 0.5	25.6 ± 0.5	24.5 ± 0.9	0.167
Weight (kg)	82.1 ± 2.0	82.4 ± 1.9	80.0 ± 3.6	0.835
BMI (kg/m ²)	28.2 ± 0.6	27.8 ± 0.6	27.5 ± 1.1	0.760
IA fat (cm ²)	129 (117-143)	130 (119-143)	100 (84-119)	0.026*
SC fat (cm ²)	289 ± 15	316 ± 15	318 ± 28	0.393
Total fat (%)	35.2 ± 0.9	36.4 ± 0.9	37.1 ± 1.7	0.515
TC (mg/dL)	209 ± 5	212 ± 5	210 ± 10	0.841
TG (mg/dL)	161 (140-185)	144 (126-164)	118 (88-159)	0.152
HDL (mg/dL)	43 ± 2	48 ± 2	53 ± 4	0.039*
LDL (mg/dL)	131 ± 4	131 ± 4	128 ± 9	0.949
Glucose (mg/dL)	94 ± 1	91 ± 1	90 ± 2	0.265
Insulin (pmol/L)	86 ± 4	79 ± 4	64 ± 7	0.018*
ISI	2.9 (2.5-3.4)	3.3 (2.9-3.8)	4.6 (3.7-5.8)	0.004*
Glucose AUC	16737 ± 589	16957 ± 564	14674 ± 975	0.123
Insulin AUC	59156 (50933- 68549)	49091 (42855- 56364)	35810 (28379- 45186)	0.002*
Visfatin (ng/mL)	19 (15-23)	20 (16-25)	22 (16-31)	0.633

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; Y, yes; N, no; VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: VO₂max, mL/kg/min; glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of genotype. * indicates significant difference between genotype groups.

Figure 3-6. Interaction between gender and the PBEF1 SER301SER polymorphism for fasting glucose ($p = 0.007$). Data are presented as adjusted mean \pm SE.



PBEF1 Haplotypes

Pairwise linkage disequilibrium values for the PBEF1 -4689, -1543, -1001, -948, and SER301SER polymorphisms appear in Table 3-11. Moreover, a strong association was found for the PBEF1 -4689 and SER301SER polymorphisms ($r^2 = 0.65$, $p < 0.001$).

PBEF1 haplotypes were inferred from the PBEF1 -4689, -1543, -1001, -948, and SER301SER genotype data using the program PHASE, version 2.1. As shown in Table 3-12, 12 haplotypes were identified in our participant population, and 5 of these haplotypes occurred at a frequency $> 5\%$. Furthermore, there were 14 individuals homozygous for the GCTGC haplotype, 7 homozygous for the GTTGC haplotype, 4 homozygous for the TCGTT haplotype, and 1 homozygous for the TCTGT haplotype. Due in part to these low frequencies, analyses involving glucose and

obesity-related variables were conducted by comparing haplotype carriers to non-carriers for the 5 haplotypes present at a frequency > 5 % (GCTGC, GTTGC, TCGTT, TCTGT, and TCGGT).

Table 3-11. PBEF1 polymorphism pairwise linkage disequilibrium values.

	-4689	-1543	-1001	-948
-1543	0.04 (p < 0.001)			
-1001	0.25 (p < 0.001)	0.06 (p = 0.005)		
-948	0.25 (p < 0.001)	0.01 (p = 0.143)	0.16 (p < 0.001)	
SER301SER	0.65 (p < 0.001)	0.04 (p < 0.001)	0.25 (p < 0.001)	0.32 (p < 0.001)

Values are r^2 . P-values are from χ^2 or Fisher Exact tests.

Table 3-12. PBEF1 haplotypes and their frequencies.

Haplotype	Count	Frequency
GCTGC	80	34.5 %
GTTGC	57	24.6 %
TCGTT	35	15.1 %
TCTGT	20	8.6 %
TCGGT	19	8.2 %
TCTGC	11	4.7 %
GTTGT	3	1.3 %
TTTGC	2	0.9 %
TCTTT	2	0.9 %
GCGGC	1	0.4 %
TCGTC	1	0.4 %
GCTGT	1	0.4 %

Haplotype GCTGC

The GCTGC haplotype, comprised of common alleles for the 5 PBEF1 polymorphisms, was present in 66 participants (14 homozygous). Neither gender ($\chi^2 = 0.741$, $p = 0.389$) nor HRT use ($\chi^2 = 0.096$, $p = 0.757$) differed between the

GCTGC haplotype carriers and non-carriers. As shown in Table 3-13, baseline glucose AUC ($p = 0.049$) and insulin AUC ($p = 0.048$) differed between the GCTGC haplotype groups, with carriers having greater values than non-carriers.

Table 3-13. Baseline characteristics of Gene Exercise Research Study participants grouped by PBEF1 GCTGC haplotype status.

	GCTGC carriers (n = 40-66)	GCTGC non-carriers (n = 31-50)	P-value
Men/Women	29/37	26/24	---
HRT use (Y/N)	17/20	12/12	---
Age (yrs)	58 ± 1	59 ± 1	0.553
VO ₂ max (mL/kg/min)	25.9 ± 0.4	25.5 ± 0.5	0.592
Weight (kg)	81.1 ± 1.7	82.5 ± 1.9	0.570
BMI (kg/m ²)	27.8 ± 0.4	28.6 ± 0.5	0.244
IA fat (cm ²)	127 (117-138)	121 (109-133)	0.423
SC fat (cm ²)	299 ± 13	318 ± 15	0.356
Total fat (%)	36.4 ± 0.8	36.0 ± 0.9	0.733
TC (mg/dL)	211 ± 4	209 ± 5	0.766
TG (mg/dL)	155 (137-175)	137 (119-157)	0.187
HDL (mg/dL)	46 ± 2	48 ± 2	0.508
LDL (mg/dL)	130 ± 4	132 ± 4	0.814
Glucose (mg/dL)	93 ± 1	91 ± 1	0.343
Insulin (pmol/L)	82 ± 4	77 ± 5	0.392
ISI	3.0 (2.6-3.5)	3.7 (3.2-4.4)	0.055
Glucose AUC	17015 ± 559	15299 ± 646	0.049*
Insulin AUC	54828 (47643-63096)	44259 (37757-51761)	0.048*
Visfatin (ng/mL)	20 (16-23)	21 (17-25)	0.718

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; Y, yes; N, no; VO₂max, maximal oxygen consumption; BMI, body mass index; IA, intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups.

Haplotype GTTGC

The common alleles for the -4689, -1001, -948, and SER301SER polymorphisms and the variant allele for the -1543 polymorphism create haplotype GTTGC. There were 50 carriers (7 homozygous) and 66 non-carriers of the GTTGC haplotype, and gender ($\chi^2 = 0.070$, $p = 0.791$) and HRT use ($\chi^2 = 0.186$, $p = 0.666$) did not differ between the groups. In fact, the only variable that varied between the GTTGC carriers and non-carriers was BMI ($p = 0.012$), which was greater in the GTTGC carriers (Table 3-14).

Haplotype TCGTT

The TCGTT haplotype contains the common allele for the -1543 polymorphism and the variant alleles for the other 4 polymorphisms. There were 31 carriers (4 homozygous) and 85 non-carriers of the TCGTT haplotype, and gender ($\chi^2 = 0.016$, $p = 0.899$) and HRT use ($\chi^2 = 0.053$, $p = 0.819$) did not differ between the groups. Table 3-15 displays haplotype associations with glucose and obesity-related variables. In the TCGTT haplotype carrier group, fasting insulin ($p = 0.026$), glucose AUC ($p = 0.040$), and insulin AUC ($p = 0.040$) values were all significantly lower than and the ISI ($p = 0.020$) value was significantly greater than the TCGTT haplotype non-carriers. As shown in Figure 3-7, there were significant gender by haplotype interaction effects found for visfatin ($p = 0.024$) and total cholesterol ($p = 0.030$), and although neither the gender nor the haplotype main effects were significant for visfatin, the gender main effect was significant for total cholesterol (women > men, $p = 0.020$). Pairwise comparisons indicated that men with the TCGTT haplotype had a lower total cholesterol value than women TCGTT haplotype

non-carriers ($p = 0.019$), but there were no differences between the groups for visfatin after Bonferroni correction.

Table 3-14. Baseline characteristics of Gene Exercise Research Study participants grouped by PBEF1 GTTGC haplotype status.

	GTTGC carriers (n = 24-50)	GTTGC non-carriers (n = 39-66)	P-value
Men/Women	23/27	32/34	---
HRT use (Y/N)	12/15	17/17	---
Age (yrs)	58 ± 1	59 ± 1	0.301
VO ₂ max (mL/kg/min)	26.2 ± 0.5	25.4 ± 0.4	0.215
Weight (kg)	82.6 ± 1.9	81.0 ± 1.7	0.533
BMI (kg/m ²)	29.1 ± 0.5	27.4 ± 0.4	0.012*
IA fat (cm ²)	129 (117-142)	121 (111-132)	0.360
SC fat (cm ²)	307 ± 15	308 ± 13	0.947
Total fat (%)	35.3 ± 0.9	36.9 ± 0.8	0.191
TC (mg/dL)	207 ± 5	214 ± 4	0.288
TG (mg/dL)	148 (129-169)	146 (129-166)	0.901
HDL (mg/dL)	45 ± 2	48 ± 2	0.358
LDL (mg/dL)	130 ± 4	131 ± 4	0.856
Glucose (mg/dL)	92 ± 1	92 ± 1	0.668
Insulin (pmol/L)	86 ± 5	75 ± 4	0.083
ISI	3.1 (2.6-3.7)	3.5 (3.1-4.0)	0.250
Glucose AUC	16463 ± 672	16142 ± 579	0.723
Insulin AUC	53827 (45604-63680)	47098 (40926-54200)	0.232
Visfatin (ng/mL)	19 (16-24)	20 (17-24)	0.701

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; Y, yes; N, no; VO₂max, maximal oxygen consumption; BMI, body mass index; IA, intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups.

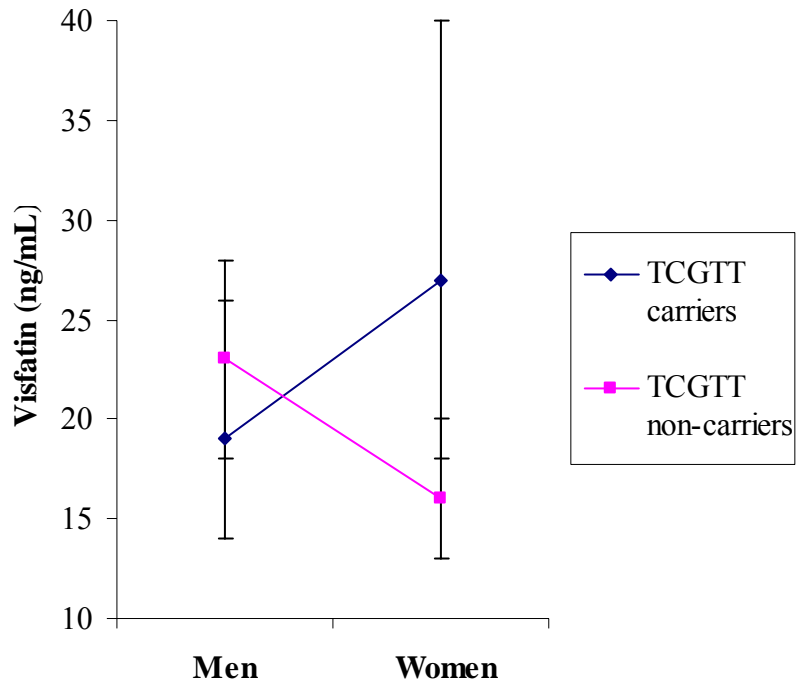
Table 3-15. Baseline characteristics of Gene Exercise Research Study participants grouped by PBEF1 TCGTT haplotype status.

	TCGTT carriers (n = 19-31)	TCGTT non-carriers (n = 49-85)	P-value
Men/Women	15/16	40/45	---
HRT use (Y/N)	8/8	21/24	---
Age (yrs)	58 ± 1	59 ± 1	0.411
VO ₂ max (mL/kg/min)	25.3 ± 0.6	25.9 ± 0.4	0.387
Weight (kg)	82.5 ± 2.4	81.4 ± 1.5	0.681
BMI (kg/m ²)	27.2 ± 0.6	28.5 ± 0.4	0.093
IA fat (cm ²)	116 (103-132)	127 (118-137)	0.214
SC fat (cm ²)	300 ± 19	310 ± 12	0.659
Total fat (%)	36.1 ± 1.1	36.2 ± 0.7	0.908
TC (mg/dL)	201 ± 6	213 ± 4	0.105
TG (mg/dL)	129 (107-154)	153 (138-170)	0.097
HDL (mg/dL)	49 ± 3	46 ± 2	0.398
LDL (mg/dL)	127 ± 6	132 ± 3	0.382
Glucose (mg/dL)	91 ± 2	92 ± 1	0.575
Insulin (pmol/L)	69 ± 6	84 ± 4	0.026*
ISI	4.1 (3.3-4.9)	3.1 (2.7-3.5)	0.020*
Glucose AUC	14926 ± 773	16886 ± 512	0.040*
Insulin AUC	41783 (34356-50933)	53580 (47315-60674)	0.040*
Visfatin (ng/mL)	22 (17-29)	19 (17-22)	0.312

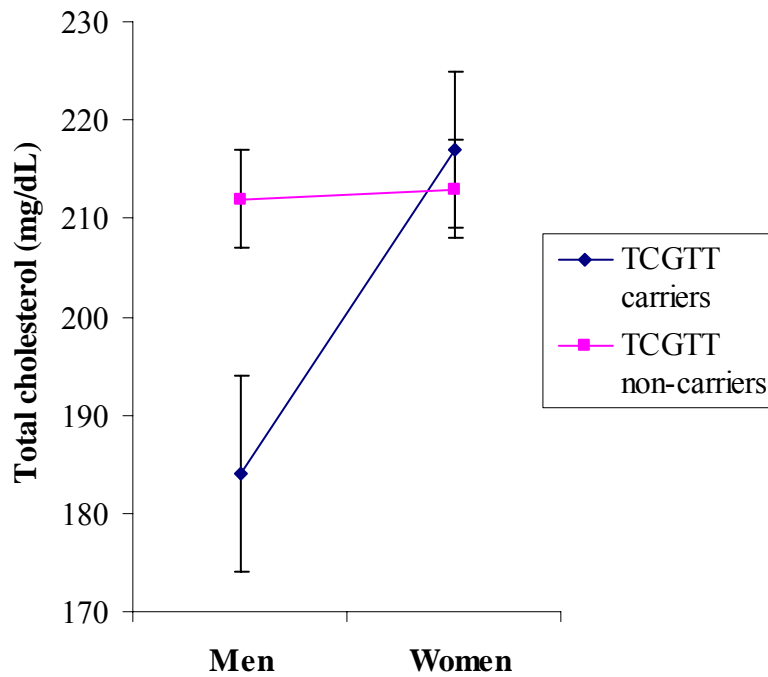
Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; Y, yes; N, no; VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups.

Figure 3-7. Interaction between gender and the PBEF1 TCGTT haplotype for A) visfatin ($p = 0.024$) and B) total cholesterol ($p = 0.030$). Data are geometric mean \pm 95 % confidence interval (visfatin) and adjusted mean \pm SE (total cholesterol).

A)



B)



Haplotype TCTGT

The TCTGT haplotype, composed of the -4689 and SER301SER variant alleles and -1543, -1001, and -948 common alleles, was present in 19 participants (1 homozygous). Gender ($\chi^2 = 1.001$, $p = 0.317$) and HRT use ($p = 0.260$) did not differ between the groups. Although age differed between the groups ($p = 0.041$), there were no other statistically significant TCTGT haplotype effects detected for any of the outcome variables (Table 3-16).

Haplotype TCGGT

Haplotype TCGGT contains the variant alleles for the -4689, -1001, and SER301SER polymorphisms and the common alleles for the -1543 and -948 polymorphisms. In the study population, there were 19 carriers and 97 non-carriers of the TCGGT haplotype. Although gender ($\chi^2 = 0.257$, $p = 0.612$) and HRT use ($\chi^2 = 0.264$, $p = 0.607$) did not differ between the groups, the TCGGT haplotype carriers had significantly greater subcutaneous fat ($p = 0.020$) and significantly lower fasting glucose ($p = 0.010$) than TCGGT haplotype non-carriers (Table 3-17).

Table 3-16. Baseline characteristics of Gene Exercise Research Study participants grouped by PBEF1 TCTGT haplotype status.

	TCTGT carriers (n = 11-19)	TCTGT non-carriers (n = 51-97)	P-value
Men/Women	11/8	44/53	---
HRT use (Y/N)	2/6	27/26	---
Age (yrs)	61 ± 1	58 ± 1	0.041*
VO ₂ max (mL/kg/min)	25.3 ± 0.8	25.8 ± 0.4	0.612
Weight (kg)	77.1 ± 3.1	82.6 ± 1.4	0.110
BMI (kg/m ²)	29.2 ± 0.8	27.9 ± 0.4	0.146
IA fat (cm ²)	116 (99-136)	126 (117-135)	0.349
SC fat (cm ²)	293 ± 25	310 ± 11	0.543
Total fat (%)	36.0 ± 1.5	36.2 ± 0.6	0.892
TC (mg/dL)	219 ± 9	209 ± 3	0.307
TG (mg/dL)	148 (115-191)	147 (132-162)	0.938
HDL (mg/dL)	50 ± 4	46 ± 1	0.381
LDL (mg/dL)	135 ± 8	130 ± 3	0.580
Glucose (mg/dL)	92 ± 2	92 ± 1	0.942
Insulin (pmol/L)	80 ± 8	79 ± 3	0.913
ISI	3.3 (2.5-4.4)	3.3 (3.0-3.8)	0.952
Glucose AUC	17454 ± 1161	16084 ± 465	0.280
Insulin AUC	45290 (34041-60256)	50699 (45082-57016)	0.472
Visfatin (ng/mL)	18 (14-25)	20 (18-24)	0.538

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; Y, yes; N, no; VO₂max, maximal oxygen consumption; BMI, body mass index; IA, intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups.

Table 3-17. Baseline characteristics of Gene Exercise Research Study participants grouped by PBEF1 TCGGT haplotype status.

	TCGGT carriers (n = 11-19)	TCGGT non-carriers (n = 52-97)	P-value
Men/Women	8/11	47/50	---
HRT use (Y/N)	6/5	23/27	---
Age (yrs)	59 ± 1	58 ± 1	0.490
VO ₂ max (mL/kg/min)	25.3 ± 0.8	25.8 ± 0.4	0.572
Weight (kg)	82.9 ± 3.1	81.5 ± 1.4	0.668
BMI (kg/m ²)	27.1 ± 0.8	28.3 ± 0.4	0.186
IA fat (cm ²)	126 (108-147)	124 (116-133)	0.843
SC fat (cm ²)	358 ± 24	297 ± 11	0.020*
Total fat (%)	38.3 ± 1.4	35.8 ± 0.6	0.108
TC (mg/dL)	219 ± 7	209 ± 3	0.222
TG (mg/dL)	135 (109-167)	150 (135-166)	0.399
HDL (mg/dL)	47 ± 3	47 ± 1	0.968
LDL (mg/dL)	138 ± 6	129 ± 3	0.215
Glucose (mg/dL)	87 ± 2	93 ± 1	0.010*
Insulin (pmol/L)	66 (55-81)	78 (71-85)	0.132
ISI	4.1 (3.2-5.2)	3.2 (2.8-3.6)	0.073
Glucose AUC	15156 ± 1037	16524 ± 475	0.237
Insulin AUC	42560 (33266-54450)	51761 (45920-58345)	0.159
Visfatin (ng/mL)	21 (15-29)	20 (17-23)	0.800

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, insulin, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. HRT, hormone replacement therapy; Y, yes; N, no; VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups.

Hypothesis 3: After Aerobic Exercise Training

Average attendance for the 24 week aerobic exercise training intervention was 90.8 ± 0.7 %. Table 3-18 displays participant characteristics following the completion of the intervention. Furthermore, some of the advantageous changes occurring with aerobic exercise training included improvements in insulin sensitivity [decreased fasting insulin ($p = 0.001$), decreased insulin AUC ($p < 0.001$), and increased ISI ($p < 0.001$)], lipoprotein-lipid levels [increased HDL-cholesterol ($p < 0.001$) and reduced triglycerides ($p = 0.001$)], and a 15 % increase in $VO_2\text{max}$ ($p < 0.001$). Although slight, body composition changes were also favorable, with reductions in body weight ($p < 0.001$), intra-abdominal fat ($p < 0.001$), and total body fat ($p < 0.001$). Moreover, weight loss averaged 1.0 ± 0.2 kg, or approximately 1.2 %, a value consistent with the GERS requirement of body weight maintenance within ± 5 % of baseline body weight.

Table 3-18. Characteristics of study participants at baseline and following aerobic exercise training.

	N	Baseline	After Training
VO ₂ max (mL/kg/min)	115	25.7 ± 0.4	29.6 ± 0.6*
Weight (kg)	116	81.7 ± 1.4	80.7 ± 1.4*
BMI (kg/m ²)	116	27.9 ± 0.4	27.6 ± 0.4
Intra-abdominal fat (cm ²)	103	124 (116-133)	115 (107-124)*
Subcutaneous fat (cm ²)	101	303 ± 11	296 ± 10
Total fat (%)	110	36.5 ± 0.8	35.0 ± 0.8*
Total cholesterol (mg/dL)	92	211 ± 3	208 ± 3
Triglycerides (mg/dL)	91	148 (134-163)	134 (122-148)*
HDL-cholesterol (mg/dL)	90	47 ± 2	51 ± 2*
LDL-cholesterol (mg/dL)	90	130 ± 3	128 ± 3
Fasting glucose (mg/dL)	81	92 ± 1	93 ± 1
Fasting insulin (pmol/L)	69	80 ± 3	72 ± 3*
ISI	66	3.3 (2.9-3.7)	3.8 (3.4-4.2)*
Glucose AUC	79	16355 ± 449	16061 ± 423
Insulin AUC	68	50652 (45300-56637)	39976 (34261-46644)*
Fasting visfatin (ng/mL)	63	20 (18-23)	23 (20-26)*

Data are expressed as mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95% confidence interval). VO₂max, maximal oxygen consumption; BMI, body mass index; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units for glucose AUC are mg/dL x 120 min; measurement units for insulin AUC are pmol/L x 120 min. * indicates significant difference from baseline with aerobic exercise training (via paired t-test).

PBEF1 -4689 G>T (rs2110385)

There were no significant differences between the PBEF1 -4689 genotype groups in any of the outcome variables with 24 weeks of aerobic exercise training (Table 3-19). There were also no significant interactive effects involving the -4689 polymorphism and gender or aerobic exercise training. However, the GG, GT, and TT genotype groups all experienced significant increases in VO₂max ($p < 0.001$) and significant decreases in intra-abdominal ($p = 0.009$, $p < 0.001$, $p = 0.020$) and total body fat ($p < 0.001$, $p < 0.001$, $p = 0.004$) as a result of the training intervention. In addition, both the GG and GT genotype groups significantly decreased weight ($p = 0.009$, $p < 0.001$), BMI ($p = 0.007$, $p < 0.001$), and insulin AUC ($p = 0.001$, $p < 0.001$), and significantly increased HDL-cholesterol ($p = 0.001$, $p < 0.001$) and ISI ($p = 0.006$, $p < 0.001$) values with training. The exercise session attendance rates for the groups also did not differ ($p = 0.690$), averaging 90.3 ± 1.0 , 91.4 ± 1.0 , and 90.1 ± 1.9 % for the GG, GT, and TT groups, respectively.

PBEF1 -1543 C>T

Although there were no significant differences between the PBEF1 -1543 genotype groups for any of the outcome variables following the completion of aerobic exercise training (Table 3-20), the CC and CT+TT genotype groups significantly increased VO₂max (both $p < 0.001$), HDL-cholesterol ($p = 0.002$, $p < 0.001$), and ISI ($p = 0.010$, $p = 0.004$) and significantly decreased weight (both $p = 0.001$), BMI (both $p = 0.001$), total body fat (both $p < 0.001$), fasting insulin ($p = 0.017$, $p = 0.021$), and insulin AUC (both $p < 0.001$) with training. There was also a significant gender by genotype interaction detected for VO₂max (Figure 3-8; $p = 0.043$), but after

Bonferroni adjustment, none of the pairwise comparisons differed. The average attendance for the CC and CT+TT genotype groups did not differ ($p = 0.829$) and was 90.8 ± 0.9 and 90.5 ± 1.0 %, respectively.

Table 3-19. After training characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 -4689 polymorphism.

	GG (n = 23-43)	GT (n = 32-53)	TT (n = 11-18)	P-value
VO ₂ max	29.1 ± 0.4†	30.1 ± 0.4†	29.4 ± 0.7†	0.181
Weight (kg)	80.8 ± 0.3†	80.4 ± 0.3†	80.9 ± 0.5	0.562
BMI (kg/m ²)	27.6 ± 0.1†	27.4 ± 0.1†	27.7 ± 0.2	0.394
IA fat (cm ²)	115 (108-122)†	118 (112-124)†	108 (99-118)†	0.251
SC fat (cm ²)	296 ± 7	294 ± 6	285 ± 11	0.664
Total fat (%)	35.0 ± 0.3†	34.7 ± 0.3†	35.2 ± 0.5†	0.613
TC (mg/dL)	203 ± 3	210 ± 3	209 ± 5	0.315
TG (mg/dL)	132 (121-144)†	139 (129-150)	118 (101-138)	0.167
HDL (mg/dL)	50 ± 1†	51 ± 1†	50 ± 2	0.595
LDL (mg/dL)	124 ± 3	129 ± 3	135 ± 6	0.172
Glucose	93 ± 2	94 ± 1	94 ± 2	0.864
Insulin	72 ± 4†	74 ± 3	65 ± 5†	0.323
ISI	3.7 (3.3-4.1)†	3.8 (3.5-4.2)†	4.0 (3.4-4.6)	0.718
Glucose AUC	16375 ± 441	15923 ± 394	16889 ± 655	0.431
Insulin AUC	43152 (38637-48195)†	40926 (37411-44875)†	42855 (36644-50234)	0.731
Visfatin	22 (19-26)	24 (21-27)†	21 (17-26)	0.623

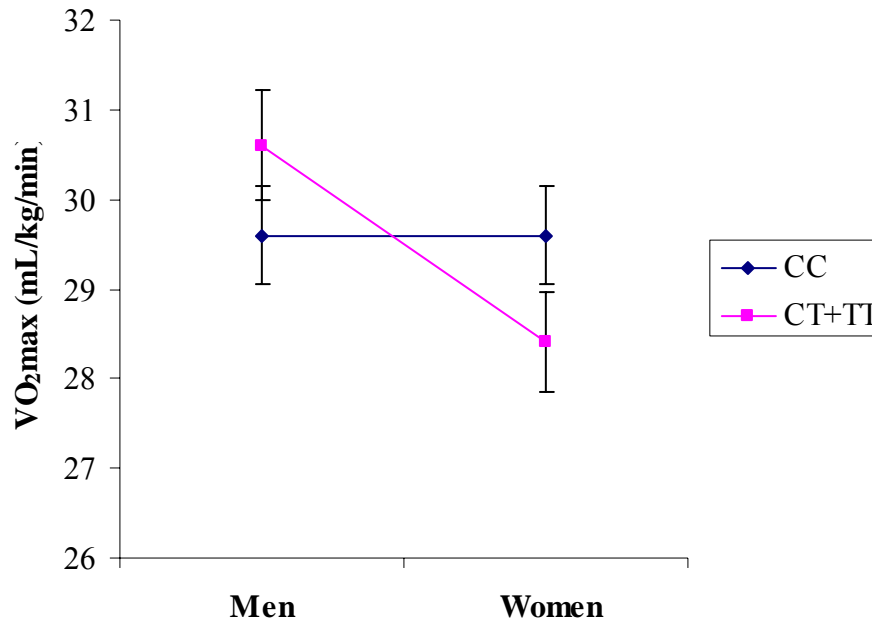
Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95% confidence interval). VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: VO₂max, mL/kg/min; glucose, mg/dL; insulin, pmol/L; glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min; visfatin, ng/mL. P-value is for the main effect of genotype. * indicates significant difference between genotype groups. † indicates significant change within genotype group with aerobic exercise training (via paired t-test).

Table 3-20. After training characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 -1543 polymorphism.

	CC (n = 34-62)	CT+TT (n = 32-53)	P-value
VO ₂ max	29.6 ± 0.4†	29.5 ± 0.4†	0.879
Weight (kg)	80.8 ± 0.3†	80.9 ± 0.3†	0.705
BMI (kg/m ²)	27.6 ± 0.1†	27.7 ± 0.1†	0.714
IA fat (cm ²)	114 (109-120)†	117 (111-124)	0.497
SC fat (cm ²)	296 ± 6	296 ± 7	0.990
Total fat (%)	35.0 ± 0.3†	35.0 ± 0.3†	0.940
TC (mg/dL)	208 ± 3	209 ± 3	0.729
TG (mg/dL)	137 (127-149)	130 (121-141)†	0.325
HDL (mg/dL)	50 ± 1†	51 ± 1†	0.101
LDL (mg/dL)	128 ± 3	129 ± 3	0.663
Glucose (mg/dL)	93 ± 1	94 ± 1	0.754
Insulin (pmol/L)	69 ± 3†	75 ± 3†	0.144
ISI	3.8 (3.5-4.2)†	3.7 (3.4-4.0)†	0.534
Glucose AUC	16431 ± 373	16069 ± 383	0.503
Insulin AUC	41495 (38019-45290)†	42658 (38994-46666)†	0.674
Visfatin (ng/mL)	22 (19-25)	23 (20-27)†	0.431

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95% confidence interval). VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: VO₂max, mL/kg/min; glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of genotype. * indicates significant difference between genotype groups. † indicates significant change within genotype group with aerobic exercise training (via paired t-test).

Figure 3-8. Interaction between gender and the PBEF1 -1543 polymorphism for VO₂max (p = 0.043) following 24 weeks of aerobic exercise training. Data are presented as adjusted mean ± SE.



PBEF1 -1001 T>G (rs9770242)

The aerobic exercise training intervention resulted in significant increases in VO₂max (both p < 0.001) and HDL-cholesterol (both p < 0.001) and significant reductions in weight (p = 0.002, p < 0.001), intra-abdominal fat (p < 0.001, p = 0.033), total body fat (both p < 0.001), triglyceride (p = 0.038, p = 0.006), and insulin AUC (p = 0.007, p = 0.004) values in the GG+GT and TT genotype groups.

However, there were no significant differences between the PBEF1 -1001 genotype groups for any of the outcome variables after training, although fasting insulin neared significance (Table 3-21; p = 0.052). There was a significant gender by genotype interaction detected for fasting glucose (Figure 3-9; p = 0.031), and pairwise comparisons indicated that women with the G allele had significantly higher glucose

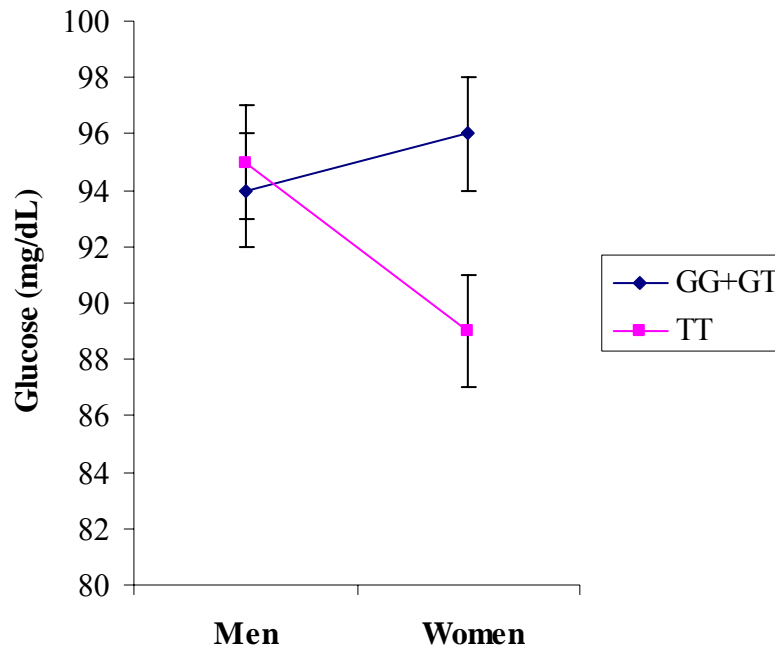
values after 6 months of aerobic exercise training than women with the TT genotype ($p = 0.037$). The exercise session adherence for the GG+GT and TT genotype groups was similar ($p = 0.870$), averaging 90.6 ± 1.1 and 90.8 ± 0.8 %, respectively.

Table 3-21. After training characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 -1001 polymorphism.

	GG+GT (n = 34-62)	TT (n = 32-53)	P-value
VO ₂ max	29.7 ± 0.4†	29.5 ± 0.3†	0.716
Weight (kg)	80.6 ± 0.3†	80.7 ± 0.3†	0.776
BMI (kg/m ²)	27.6 ± 0.6	27.6 ± 0.5	0.937
IA fat (cm ²)	112 (106-119)†	117 (111-123)†	0.265
SC fat (cm ²)	293 ± 7	297 ± 6	0.643
Total fat (%)	34.9 ± 0.3†	35.0 ± 0.2†	0.888
TC (mg/dL)	210 ± 3	206 ± 3	0.342
TG (mg/dL)	132 (121-144)†	135 (126-145) †	0.694
HDL (mg/dL)	51 ± 1†	50 ± 1†	0.372
LDL (mg/dL)	131 ± 3	126 ± 3	0.243
Glucose (mg/dL)	95 ± 1†	92 ± 1	0.087
Insulin (pmol/L)	77 ± 3	68 ± 3†	0.052
ISI	3.6 (3.3-4.0)	3.9 (3.6-4.3)†	0.282
Glucose AUC	16227 ± 528	15928 ± 467	0.681
Insulin AUC	43954 (36058-53456)†	37154 (31189-44157)†	0.215
Visfatin (ng/mL)	23 (20-27)	22 (20-25)†	0.800

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95% confidence interval). VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: VO₂max, mL/kg/min; glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of genotype. * indicates significant difference between genotype groups. † indicates significant change within genotype group with aerobic exercise training (via paired t-test).

Figure 3-9. Interaction between gender and the PBEF1 -1001 polymorphism for fasting glucose ($p = 0.031$) following 24 weeks of aerobic exercise training. Data are presented as adjusted mean \pm SE.



PBEF1 -948 G>T

Several advantageous changes occurred with aerobic exercise training in the PBEF1 -948 GG and GT+TT genotype groups, including increases in $VO_2\max$ (both $p < 0.001$) and HDL-cholesterol ($p < 0.001$, $p = 0.001$) and decreases in intra-abdominal fat ($p = 0.007$, $p = 0.004$) and total body fat ($p < 0.001$, $p = 0.001$) measurements. However, total cholesterol ($p = 0.002$) and LDL-cholesterol ($p = 0.012$) concentrations varied by genotype group with the GG group experiencing decreases ($p = 0.005$, $p = 0.029$) and the GT+TT group experiencing increases ($p = 0.022$, $p = 0.024$) in both variables with training (Table 3-22). As displayed in Figure 3-10, there were significant gender by genotype interaction effects for subcutaneous fat ($p = 0.044$) and HDL-cholesterol ($p = 0.046$). Furthermore, pairwise comparisons

indicated that men with the T allele had lower subcutaneous fat after aerobic exercise training than women with the GG genotype ($p = 0.043$) or T allele ($p = 0.005$).

However, there were no group differences for HDL-cholesterol after Bonferroni adjustment. For subcutaneous fat, the gender main effect was also significant

(women > men; $p = 0.001$). Last, exercise adherence did not differ between the GG and GT+TT genotype groups ($p = 0.668$, 90.5 ± 0.8 versus 91.2 ± 1.4 %).

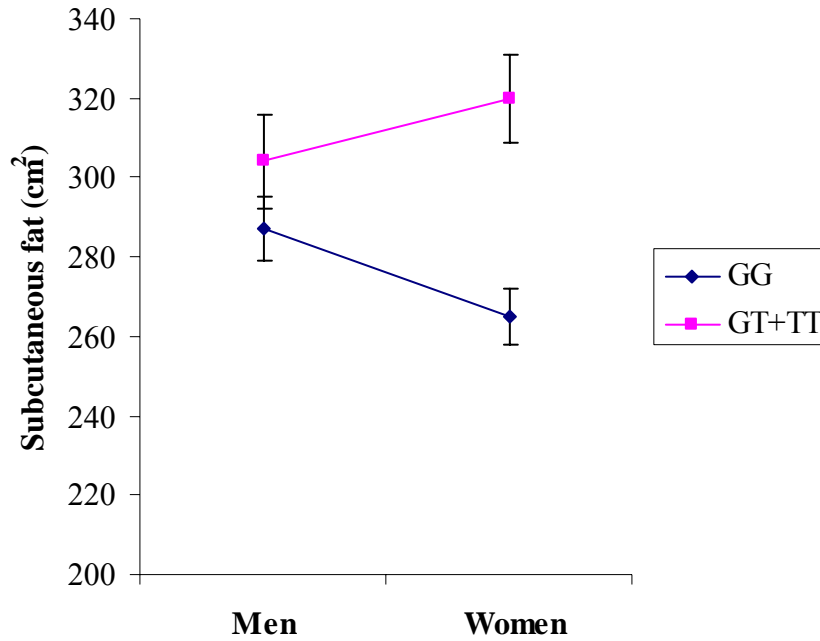
Table 3-22. After training characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 -948 polymorphism.

	GG (n = 45-81)	GT+TT (n = 18-34)	P-value
VO ₂ max	29.5 ± 0.3†	29.8 ± 0.5†	0.609
Weight (kg)	80.5 ± 0.2†	81.1 ± 0.4	0.175
BMI (kg/m ²)	27.8 ± 0.4	27.1 ± 0.7	0.385
IA fat (cm ²)	117 (112-122)†	111 (104-118)†	0.194
SC fat (cm ²)	296 ± 5	292 ± 8	0.724
Total fat (%)	34.9 ± 0.2†	35.1 ± 0.3†	0.641
TC (mg/dL)	204 ± 2†	218 ± 4†	0.002*
TG (mg/dL)	135 (126-144)†	130 (117-145)	0.573
HDL (mg/dL)	50 ± 1†	52 ± 1†	0.115
LDL (mg/dL)	125 ± 2†	137 ± 4†	0.012*
Glucose (mg/dL)	92 ± 1	96 ± 2†	0.080
Insulin (pmol/L)	71 ± 2†	72 ± 4	0.848
ISI	3.9 (3.6-4.2)†	3.5 (3.1-4.0)	0.247
Glucose AUC	15623 ± 404†	17064 ± 626	0.063
Insulin AUC	37670 (32359-43752)†	46666 (36308-59979)	0.157
Visfatin (ng/mL)	23 (21-26)†	22 (18-26)	0.664

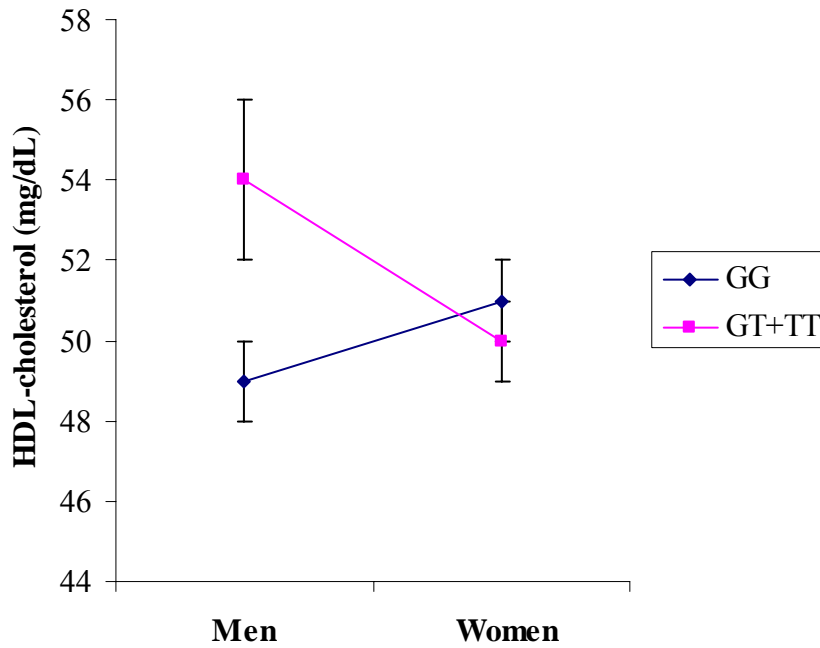
Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95% confidence interval). VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: VO₂max, mL/kg/min; glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of genotype. * indicates significant difference between genotype groups. † indicates significant change within genotype group with aerobic exercise training (via paired t-test).

Figure 3-10. Interaction between gender and the PBEF1 -948 polymorphism for A) subcutaneous fat ($p = 0.044$) and B) HDL-cholesterol ($p = 0.046$) following 24 weeks of aerobic exercise training. Data are presented as adjusted mean \pm SE.

A)



B)



PBEF1 SER301SER C>T (rs2302559)

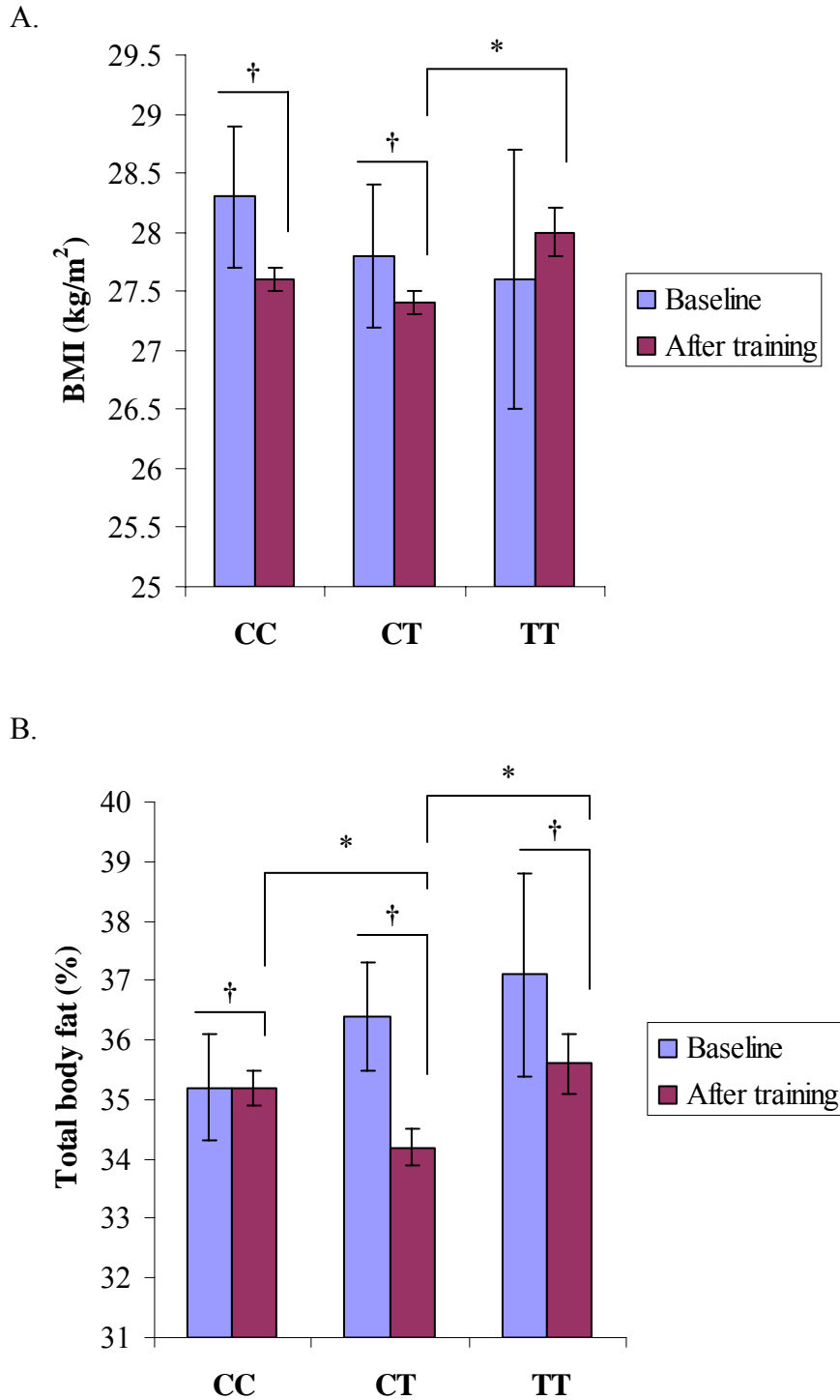
Table 3-23 displays after training characteristics for the participants grouped by PBEF1 SER301SER genotype. With aerobic exercise training, all 3 genotype groups significantly increased VO₂max ($p < 0.001$) and decreased total body fat ($p < 0.001$, $p < 0.001$, $p = 0.030$). In addition, the CC and CT groups significantly increased HDL-cholesterol ($p = 0.001$, $p < 0.001$) and ISI (both $p = 0.006$) and significantly decreased weight ($p = 0.003$, $p < 0.001$), BMI ($p = 0.002$, $p < 0.001$), intra-abdominal fat ($p < 0.001$, $p = 0.018$), total body fat (both $p < 0.001$), triglyceride ($p = 0.006$, $p = 0.016$), and insulin AUC (both $p < 0.001$) measurements. However, the magnitude of change in BMI and total body fat was not consistent between the groups, resulting in significant differences in BMI ($p = 0.030$) and total body fat ($p = 0.011$) values by genotype. Furthermore, the CT group decreased BMI significantly more than the TT group ($p = 0.030$) and decreased total body fat significantly more than the CC and TT groups ($p = 0.037$ and 0.046 , respectively) [Figure 3-11]. Overall, there were no significant interactive effects detected involving the SER301SER polymorphism and gender or aerobic exercise training, and exercise attendance did not differ ($p = 0.689$) between the groups, averaging 90.2 ± 1.0 , 91.4 ± 1.1 , and 90.5 ± 2.0 %, respectively.

Table 3-23. After training characteristics of Gene Exercise Research Study participants genotyped for the PBEF1 SER301SER polymorphism.

	CC (n =26-48)	CT (n = 31-51)	TT (n = 8-14)	P-value
VO ₂ max	29.3 ± 0.4†	30.1 ± 0.4†	29.4 ± 0.8†	0.365
Weight (kg)	81.0 ± 0.3†	80.5 ± 0.3†	82.1 ± 0.6	0.054
BMI (kg/m ²)	27.6 ± 0.1†	27.4 ± 0.1†	28.0 ± 0.2	0.030*
IA fat (cm ²)	114 (108-121)†	116 (110-123)†	116 (104-128)	0.862
SC fat (cm ²)	297 ± 7	287 ± 6	300 ± 12	0.475
Total fat (%)	35.2 ± 0.3†	34.2 ± 0.3†	35.6 ± 0.5†	0.011*
TC (mg/dL)	205 ± 3	208 ± 3	215 ± 6	0.333
TG (mg/dL)	135 (123-147)†	132 (122-144)†	145 (120-175)	0.702
HDL (mg/dL)	49 ± 1†	51 ± 1†	51 ± 2	0.240
LDL (mg/dL)	124 ± 3	130 ± 3	133 ± 7	0.242
Glucose	93 ± 1	93 ± 1	96 ± 2	0.557
Insulin	67 ± 3†	77 ± 3	67 ± 5	0.058
ISI	3.9 (3.5-4.3)†	3.7 (3.4-4.1)†	3.7 (3.1-4.4)	0.838
Glucose AUC	16380 ± 423	15862 ± 400†	17053 ± 684	0.315
Insulin AUC	40087 (36141- 44463)†	41879 (38282- 45709)†	47973 (40644- 56624)	0.216
Visfatin	22 (19-25)	24 (20-27)	22 (18-28)	0.703

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95% confidence interval). VO₂max, maximal oxygen consumption; BMI, body mass index; IA, intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: VO₂max, mL/kg/min; glucose, mg/dL; insulin, pmol/L; glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min; visfatin, ng/mL. P-value is for the main effect of genotype. * indicates significant difference between genotype groups. † indicates significant change within genotype group with aerobic exercise training.

Figure 3-11. Body composition variables grouped by PBEF1 SER301SER genotype. A) BMI and B) total body fat. Data are adjusted mean \pm SE. * indicates significant difference between genotype groups. † indicates significant difference within genotype group with aerobic exercise training.



PBEF1 Haplotypes

GCTGC

Many advantageous changes occurred in the GCTGC haplotype carrier and non-carrier groups with 24 weeks of aerobic exercise training. Furthermore, both haplotype groups significantly increased VO₂max (both $p < 0.001$), HDL-cholesterol (both $p < 0.001$), and ISI ($p = 0.021$, $p = 0.003$) values and significantly decreased weight ($p < 0.001$, $p = 0.006$), intra-abdominal fat ($p = 0.001$, $p = 0.023$), total body fat (both $p < 0.001$), triglyceride ($p = 0.005$, $p = 0.040$), fasting insulin ($p = 0.014$, $p = 0.025$), and insulin AUC ($p = 0.009$, $p = 0.001$) values. As a result of these similar changes, there were no significant differences in glucose and obesity-related outcomes between the GCTGC haplotype groups following the completion of aerobic exercise training (Table 3-24), even though glucose AUC and insulin AUC were different at baseline. Exercise adherence also did not differ ($p = 0.657$), averaging 90.4 ± 0.8 % in the haplotype carrier group and 91.0 ± 1.1 % in the haplotype non-carrier group.

GTTGC

Table 3-25 displays the after aerobic exercise training values for glucose and obesity-related variables grouped by GTTGC haplotype. With aerobic exercise training, VO₂max (both $p < 0.001$), HDL-cholesterol (both $p < 0.001$), and ISI increased ($p = 0.006$, $p = 0.014$), whereas weight ($p = 0.001$, $p < 0.001$), total body fat (both $p < 0.001$), fasting insulin ($p = 0.020$, $p = 0.017$), and insulin AUC ($p < 0.001$, $p = 0.014$) values decreased in the GTTGC haplotype carrier and non-carrier groups, respectively. Including exercise attendance ($p = 0.849$), which averaged 90.8 ± 1.1

and 90.6 ± 0.9 %, there were no significant differences between the GTTGC haplotype groups after training.

Table 3-24. After training characteristics of Gene Exercise Research Study participants grouped by PBEF1 GCTGC haplotype status.

	GCTGC carriers (n = 35-66)	GCTGC non-carriers (n = 28-50)	P-value
VO ₂ max (mL/kg/min)	29.5 ± 0.4†	29.6 ± 0.4†	0.830
Weight (kg)	80.5 ± 0.3†	80.9 ± 0.3†	0.373
BMI (kg/m ²)	27.4 ± 0.5	27.8 ± 0.6	0.661
IA fat (cm ²)	115 (110-121)†	115 (108-121)†	0.879
SC fat (cm ²)	294 ± 6	298 ± 7	0.614
Total fat (%)	34.9 ± 0.2†	35.0 ± 0.3†	0.797
TC (mg/dL)	207 ± 3	210 ± 3	0.426
TG (mg/dL)	138 (129-149)†	128 (118-139)†	0.172
HDL (mg/dL)	50 ± 1†	51 ± 1†	0.189
LDL (mg/dL)	127 ± 3	131 ± 3	0.331
Glucose (mg/dL)	93 ± 1	93 ± 1	0.913
Insulin (pmol/L)	72 ± 3†	71 ± 3†	0.872
ISI	3.7 (3.4-4.0)†	3.9 (3.5-4.2)†	0.543
Glucose AUC	16125 ± 461	15980 ± 519	0.838
Insulin AUC	37497 (31623-44566)†	43251 (35645-52602)†	0.283
Visfatin (ng/mL)	22 (20-25)	23 (20-27)	0.619

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups. † indicates significant change within haplotype group with aerobic exercise training (via paired t-test).

Table 3-25. After training characteristics of Gene Exercise Research Study participants grouped by PBEF1 GTTGC haplotype status.

	GTTGC carriers (n = 24-50)	GTTGC non-carriers (n = 38-66)	P-value
VO ₂ max (mL/kg/min)	29.4 ± 0.4†	29.7 ± 0.4†	0.567
Weight (kg)	80.8 ± 0.3†	80.6 ± 0.3†	0.650
BMI (kg/m ²)	28.0 ± 0.6	27.3 ± 0.5	0.379
IA fat (cm ²)	117 (111-124)	113 (108-119)†	0.341
SC fat (cm ²)	301 ± 7	292 ± 5	0.303
Total fat (%)	35.1 ± 0.3†	34.9 ± 0.2†	0.595
TC (mg/dL)	208 ± 3	208 ± 3	0.888
TG (mg/dL)	131 (121-142)†	136 (126-147)	0.519
HDL (mg/dL)	51 ± 1†	50 ± 1†	0.419
LDL (mg/dL)	127 ± 3	129 ± 3	0.662
Glucose (mg/dL)	94 ± 1	93 ± 1	0.822
Insulin (pmol/L)	73 ± 3†	71 ± 3†	0.553
ISI	3.7 (3.4-4.1)†	3.8 (3.5-4.1)†	0.773
Glucose AUC	16114 ± 525	16021 ± 455	0.895
Insulin AUC	43351 (35481- 52845)†	37670 (31769- 44668)†	0.297
Visfatin (ng/mL)	24 (20-28)†	22 (19-25)	0.441

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups. † indicates significant change within haplotype group with aerobic exercise training (via paired t-test).

TCGTT

With aerobic exercise training, TCGTT haplotype carriers and non-carriers significantly increased VO₂max (both $p < 0.001$) and HDL-cholesterol ($p = 0.001$, $p < 0.001$) levels and significantly decreased intra-abdominal (both $p = 0.005$) and total body fat ($p = 0.002$, $p < 0.001$) measurements. However, TCGTT haplotype carriers had significantly higher total cholesterol ($p = 0.002$) and LDL-cholesterol ($p = 0.018$) concentrations than haplotype non-carriers after the training intervention (Table 3-26). These differences were consistent with the changes that occurred with aerobic exercise training, as both total cholesterol and LDL-cholesterol increased in the haplotype carrier group ($p = 0.017$, $p = 0.037$), but decreased in the haplotype non-carrier group ($p = 0.007$, $p = 0.046$). There were also significant gender by haplotype interaction effects detected for subcutaneous fat ($p = 0.025$), intra-abdominal fat ($p = 0.042$), and HDL-cholesterol ($p = 0.019$) [Figure 3-12]. Furthermore, men with the TCGTT haplotype had significantly lower subcutaneous fat after 24 weeks of aerobic exercise training than women with the TCGTT haplotype ($p = 0.003$) or women without the haplotype ($p = 0.027$), but after Bonferroni adjustment, none of the group comparisons were different for intra-abdominal fat or HDL-cholesterol. Along with the interaction effects, the gender main effect was significant for subcutaneous fat (women > men, $p < 0.001$) and intra-abdominal fat (women > men, $p = 0.040$), and the haplotype main effect was significant for HDL-cholesterol (carriers > non-carriers, $p = 0.039$). Overall, there were no differences ($p = 0.873$) in exercise session adherence between the TCGTT haplotype carrier and non-carrier groups (90.9 ± 1.5 and 90.6 ± 0.8 %, respectively).

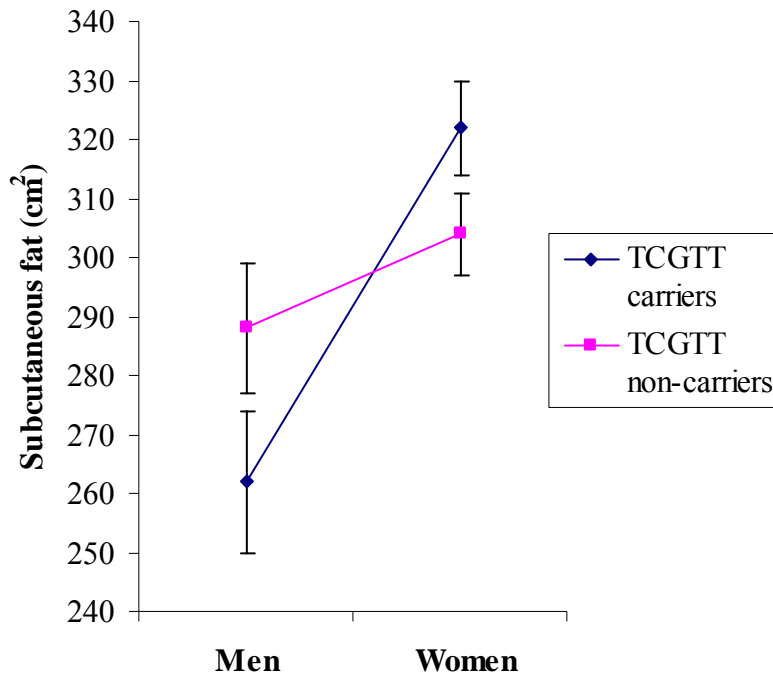
Table 3-26. After training characteristics of Gene Exercise Research Study participants grouped by PBEF1 TCGTT haplotype status.

	TCGTT carriers (n = 24-50)	TCGTT non-carriers (n = 38-66)	P-value
VO ₂ max (mL/kg/min)	29.7 ± 0.5†	29.5 ± 0.3†	0.684
Weight (kg)	81.2 ± 0.4	80.5 ± 0.2†	0.144
BMI (kg/m ²)	27.1 ± 0.7	27.8 ± 0.4	0.407
IA fat (cm ²)	109 (102-117)†	117 (112-122)†	0.102
SC fat (cm ²)	292 ± 8	296 ± 5	0.694
Total fat (%)	35.1 ± 0.4†	34.9 ± 0.2†	0.682
TC (mg/dL)	219 ± 4†	205 ± 2†	0.002*
TG (mg/dL)	129 (115-145)	135 (127-144)†	0.480
HDL (mg/dL)	53 ± 1†	50 ± 1†	0.039*
LDL (mg/dL)	137 ± 4†	126 ± 2†	0.018*
Glucose (mg/dL)	96 ± 2†	92 ± 1	0.080
Insulin (pmol/L)	72 ± 4	71 ± 2†	0.848
ISI	3.5 (3.1-4.0)	3.9 (3.6-4.2)†	0.247
Glucose AUC	17064 ± 626	15623 ± 404†	0.063
Insulin AUC	46666 (36308-59979)	37670 (32359-43752)†	0.157
Visfatin (ng/mL)	22 (18-26)	23 (21-26)†	0.609

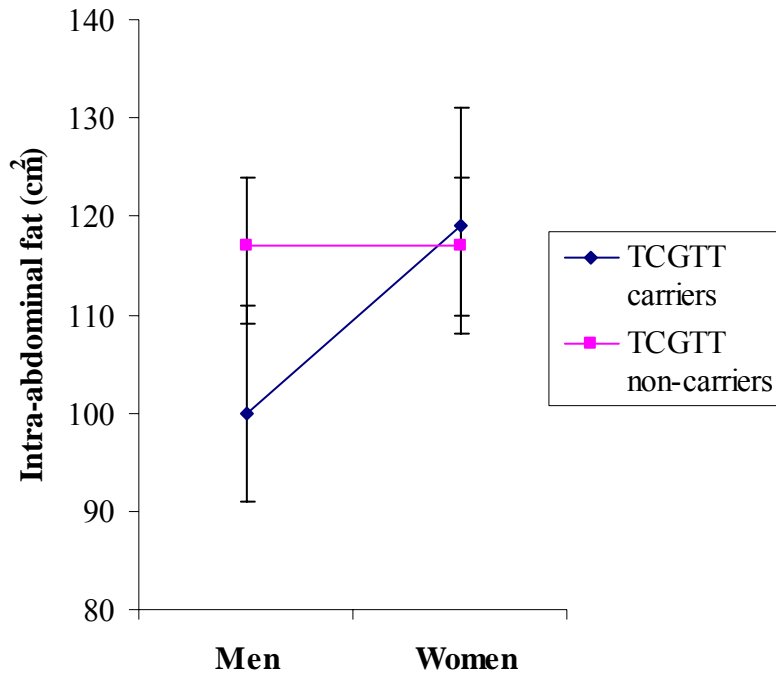
Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups. † indicates significant change within haplotype group with aerobic exercise training (via paired t-test).

Figure 3-12. Interaction between gender and the PBEF1 TCGTT haplotype for A) subcutaneous fat ($p = 0.025$), B) intra-abdominal fat ($p = 0.042$), and C) HDL-cholesterol ($p = 0.039$) following 24 weeks of aerobic exercise training. Data are presented as adjusted mean \pm SE (subcutaneous fat, HDL-cholesterol) or geometric mean \pm 95 % confidence interval (intra-abdominal fat).

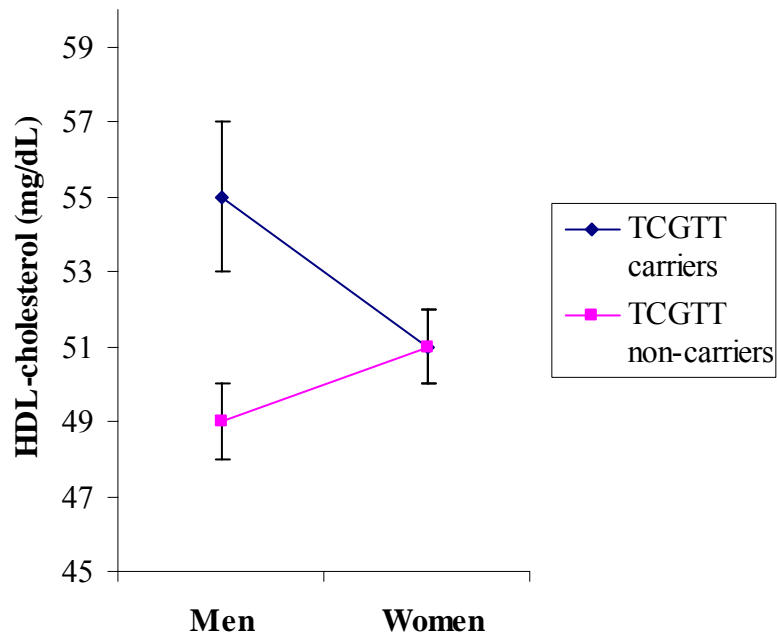
A)



B)



C)



TCTGT

Both the TCTGT haplotype carrier and non-carrier groups increased $VO_2\text{max}$ (both $p < 0.001$) and ISI ($p = 0.020$, $p = 0.004$) and decreased weight ($p = 0.020$, $p < 0.001$), total body fat ($p = 0.002$, $p < 0.001$), and insulin AUC ($p = 0.007$, $p = 0.002$) values with aerobic exercise training. Thus, there were no significant differences in glucose or obesity-related variables between the TCTGT haplotype groups after training (Table 3-27). There were also no differences ($p = 0.143$) in exercise adherence between the haplotype carrier and non-carrier groups, 93.0 ± 1.6 and 90.3 ± 0.7 %, respectively.

Table 3-27. After training characteristics of Gene Exercise Research Study participants grouped by PBEF1 TCTGT haplotype status.

	TCTGT carriers (n = 11-19)	TCTGT non-carriers (n = 55-97)	P-value
VO ₂ max (mL/kg/min)	30.1 ± 0.7†	29.4 ± 0.3†	0.323
Weight (kg)	80.6 ± 0.5†	80.7 ± 0.2†	0.785
BMI (kg/m ²)	26.6 ± 0.9	27.8 ± 0.4	0.258
IA fat (cm ²)	121 (110-133)	114 (109-119)†	0.284
SC fat (cm ²)	304 ± 11	294 ± 5	0.409
Total fat (%)	35.0 ± 0.5†	35.0 ± 0.2†	0.999
TC (mg/dL)	210 ± 5	208 ± 2	0.704
TG (mg/dL)	136 (116-158)	133 (126-141)†	0.830
HDL (mg/dL)	50 ± 2	51 ± 1†	0.646
LDL (mg/dL)	132 ± 6	128 ± 2	0.510
Glucose (mg/dL)	91 ± 2	94 ± 1	0.225
Insulin (pmol/L)	71 ± 5	72 ± 2†	0.912
ISI	4.1 (3.5-4.7)†	3.7 (3.5-4.0)†	0.335
Glucose AUC	15316 ± 888	16194 ± 368	0.367
Insulin AUC	42170 (30269- 58749)†	39537 (34356- 45499)†	0.729
Visfatin (ng/mL)	23 (18-28)	23 (20-25)†	0.947

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. VO₂max, maximal oxygen consumption; BMI, body mass index; IA; intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups. † indicates significant change within haplotype group with aerobic exercise training (via paired t-test).

TCGGT

With 24 weeks of aerobic exercise training, TCGGT haplotype carrier and non-carrier groups significantly increased VO_2 max (both $p < 0.001$) and HDL-cholesterol ($p = 0.036$, $p < 0.001$) values and significantly decreased weight ($p = 0.004$, $p < 0.001$), total body fat (both $p < 0.001$), and insulin AUC ($p = 0.014$, $p = 0.002$) values. However, total cholesterol decreased only in the haplotype carrier group with training ($p = 0.017$), which contributed to differing total cholesterol concentrations by haplotype group after training (Table 3-28; $p = 0.021$). Exercise adherence did not differ ($p = 0.815$), averaging 91.1 ± 1.6 and 90.6 ± 0.8 % for the haplotype carriers and non-carriers, respectively.

Table 3-28. After training characteristics of Gene Exercise Research Study participants grouped by PBEF1 TCGGT haplotype status.

	TCGGT carriers (n = 11-19)	TCGGT non-carriers (n = 52-97)	P-value
VO ₂ max (mL/kg/min)	29.5 ± 0.6†	29.6 ± 0.3†	0.890
Weight (kg)	79.9 ± 0.5†	80.8 ± 0.2†	0.099
BMI (kg/m ²)	28.1 ± 0.9	27.5 ± 0.4	0.540
IA fat (cm ²)	117 (107-127)	115 (110-119)†	0.691
SC fat (cm ²)	292 ± 10†	296 ± 5	0.708
Total fat (%)	34.6 ± 0.4†	35.1 ± 0.2†	0.327
TC (mg/dL)	199 ± 4†	210 ± 2	0.021*
TG (mg/dL)	139 (123-158)	132 (124-140)†	0.464
HDL (mg/dL)	50 ± 1†	51 ± 1†	0.552
LDL (mg/dL)	122 ± 5†	130 ± 2	0.145
Glucose (mg/dL)	94 ± 2†	93 ± 1	0.777
Insulin (pmol/L)	71 (63-80)	68 (64-72)†	0.526
ISI	3.9 (3.4-4.6)	3.7 (3.5-4.0)†	0.500
Glucose AUC	15144 ± 796	16276 ± 376	0.208
Insulin AUC	38905 (28708- 52602)†	40272 (34834- 46452)†	0.841
Visfatin (ng/mL)	23 (18-28)	23 (20-25)†	0.968

Data are expressed as adjusted mean ± SE with the exception of intra-abdominal fat, triglycerides, insulin, ISI, insulin AUC, and visfatin, which are presented as geometric mean (95 % confidence interval), and categorical data, which are presented as count. VO₂max, maximal oxygen consumption; BMI, body mass index; IA, intra-abdominal; SC, subcutaneous; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; ISI, insulin sensitivity index; AUC, total area under the curve; n, sample size. Sample sizes are varied due to the inability to obtain all measurements on all participants. Measurement units: glucose AUC, mg/dL x 120 min; insulin AUC, pmol/L x 120 min. P-value is for the main effect of haplotype. * indicates significant difference between haplotype groups. † indicates significant change within haplotype group with aerobic exercise training (via paired t-test).

Chapter 4: Discussion

In a paper that was later retracted (36), Fukuhara and colleagues initially linked visfatin with glucose metabolism and obesity via its reported insulin-like abilities (activating the insulin receptor, increasing glucose uptake) (35). However, other researchers have suggested that visfatin's connection to glucose and obesity-related variables may be through its role as the NAD biosynthetic enzyme Nampt and NAD's involvement in transcriptional regulation, including that of the sirtuin family (95-97). Regardless of the mechanism, researchers have examined the role of visfatin and visfatin gene (PBEF1) polymorphisms in glucose and obesity-related conditions and visfatin's response to weight loss and medication interventions. Aerobic exercise training has been shown to be an effective way to improve many health outcomes, including glucose tolerance and insulin sensitivity. However, less is known regarding visfatin's response to an aerobic exercise training intervention, and no one, to our knowledge, has examined whether polymorphic variation in the PBEF1 gene affects aerobic exercise training-induced changes in glucose and obesity-related variables.

Thus, the present study sought to determine whether 6 months of aerobic exercise training reduced plasma visfatin levels in generally healthy, middle-to-older aged individuals with impaired glucose tolerance (IGT) or normal glucose tolerance (NGT). In addition, this study investigated the influence of common polymorphisms in the PBEF1 gene on glucose and obesity-related variables and their responses to aerobic exercise training.

The major findings were that 1) plasma visfatin levels were similar in IGT and NGT individuals at baseline and increased similarly in response to 6 months of

aerobic exercise training, 2) there were significant associations at baseline between a number of glucose and obesity-related variables and PBEF1 gene polymorphisms and haplotypes, and 3) PBEF1 gene polymorphisms and haplotypes influenced the aerobic exercise training-induced change in several glucose and obesity-related variables.

Visfatin Levels

Our data indicate no significant differences in the baseline level of fasting visfatin in our generally healthy, but sedentary, middle-to-older aged IGT and NGT participants. In addition, baseline visfatin levels were not significantly correlated with weight, BMI, intra-abdominal fat, subcutaneous fat, total body fat, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fasting glucose, fasting insulin, glucose AUC, insulin AUC, or insulin sensitivity (via ISI) in the combined study population, the IGT group, or the NGT group. Despite previous reports suggesting ties between visfatin and inflammation (9; 54; 79; 86; 119), there were also no significant correlations with C-reactive protein levels at baseline

Jian *et al.* (55), Li *et al.* (73), and Dogru *et al.* (29) have previously compared visfatin levels in IGT and healthy NGT individuals, and similar to our findings, reported no difference between the groups. Also in concordance with our study, Dogru *et al.* found no significant correlations between visfatin and BMI, fasting insulin, fasting glucose, lipoprotein-lipids, insulin sensitivity (via HOMA-IR), or C-reactive protein in either group (29). Based on these data, circulating levels of visfatin do not appear to be related to glucose tolerance or obesity-related variables.

Three previous studies have examined the visfatin response to aerobic exercise training. Haider *et al.* reported a significant reduction in circulating visfatin levels after 2 and 4 months of aerobic exercise training in T1D patients (43). Similarly, Choi *et al.* reported significant decreases in plasma visfatin after 12 weeks of a combined aerobic and resistance exercise training program in overweight and obese Korean women (25). Brema *et al.* also reported significant decreases in visfatin in young obese and T2D patients with 12 weeks of exercise (17). In contrast to these studies, and to our hypothesis, we found a slight but significant increase in plasma visfatin levels with 6 months of aerobic exercise training in generally healthy, IGT and NGT individuals. Furthermore, our data indicate that there were no differences in the magnitude of the aerobic exercise training induced-change in visfatin, as both groups increased levels of the adipokine by approximately 15-20 %.

Weight loss is one explanation for the discrepancy in results between our study and the Choi *et al.* study. Moreover, the participants in the Choi *et al.* study lost ~ 4-5 kg of body weight during the 12 week intervention (25), whereas our participants lost ~ 2 kg in the IGT group and ~ 0.5 kg in the NGT with 24 weeks of aerobic exercise training. Thus, weight loss, independently or in combination with exercise training, may have been the cause of the decreased visfatin levels in the Choi *et al.* study.

In contrast, participants in the Haider *et al.* study did not change their BMI, total cholesterol, LDL-cholesterol, HDL-cholesterol, or fasting glucose levels with 2 or 4 months of aerobic exercise training or 8 months after the cessation of the training program (43). In fact, after 4 months of the exercise training program, the T1D

patients experienced a non-significant decrease in HDL-cholesterol (from 72 ± 17 to 64 ± 13 mg/dL) and a non-significant increase in fasting glucose (from 151 ± 46 to 192 ± 61 mg/dL) (43). Based on the lack of improvement in HDL-cholesterol and since no training variable was measured (*i.e.* $VO_2\text{max}$), it does not appear that a training effect took place. Furthermore, it is possible that the T1D patients were already physically active before the aerobic exercise training intervention began, since regular exercise was only reported as an exclusionary factor in the non-exercising control group. Thus, the change in visfatin levels in the Haider *et al.* study may have been due to a factor other than exercise training.

Participants in the Brema *et al.* study also did not experience improvement in many physiological outcomes with training. In fact, the T2D patients did not change their $VO_2\text{max}$ with 12 weeks of training even though they were bicycling 4 times per week, 1 hour per session, at 75 % $VO_2\text{max}$ intensity (17). It is also possible that the patients were regularly physically active before the aerobic exercise training intervention began, which may explain the lack of improvement. At any rate, it does not appear that a training effect occurred and that the training effect was responsible for the visfatin decrease.

Another possible explanation for the differing results may be the retrospective design of our study, including the analysis of visfatin from plasma samples stored for 2-8 years at -80 degrees Celsius. Although plasma sample age was not related to visfatin levels in our study, it is possible that the viability of this cytokine-like molecule may have been compromised by its time in storage. Moreover, only one study, to our knowledge, has examined the influence of storage on visfatin levels, and

the researchers found that storage at -25 degrees Celsius for 1 month resulted in measurement of 92.7 ± 31.1 % of the initial value (83).

An additional reason for the discrepancy in results may be due to the C-terminal assay used in this study and in the Choi *et al.*, Haider *et al.*, and Brema *et al.* studies to measure circulating visfatin. Moreover, Korner *et al.* compared serum visfatin measures from 3 immunoassays, the C-terminal competitive EIA we used, a competitive radioimmunoassay (RIA, Phoenix Pharmaceuticals), and a sandwich enzyme-linked immunosorbent assay (ELISA, AdipoGen Inc.) and found no correlation between the visfatin measurements (65). The researchers also compared the ability of the assays to detect the visfatin dimer, previously measured at 100-150 kDa, and using size exclusion chromatography serum fractions found that only the ELISA detected a peak in this area (65). Another recent study reported a low correlation ($r = 0.4121$) between the EIA C-terminal assay and the full-length ELISA assay (94). Thus, measurement of full-length visfatin with the newer ELISA may have provided a better indicator of physiologically circulating visfatin levels.

It is also quite possible that the circulating visfatin concentration is not reflective of biologically relevant levels of visfatin. Furthermore, visfatin occurs as an extracellular secreted protein and as an intracellular protein (97). The importance of each of these sources to visfatin biology and to its downstream metabolic effects has not been determined. In addition, Frydelund-Larson *et al.* found 3-fold increases in visfatin abdominal adipose tissue mRNA expression after 3 hours of acute cycling exercise, but found no differences in skeletal muscle mRNA expression or plasma levels (34). However, it is unclear if these acute exercise effects are similar to

exercise training effects. Finally, adipose tissue is only one of the possible sources of visfatin as visfatin mRNA and protein levels have been found in a variety of tissues (97; 102). Thus, the relative contribution of the differing production sites to the total circulating visfatin pool is also not known.

Finally, in the present study, the change in visfatin levels with aerobic exercise training was not significantly correlated with the change in glucose and obesity-related variables in the total study population or in the NGT group. However, in the IGT group, the aerobic exercise training-induced increase in visfatin was correlated with the non-significant decrease in CRP levels. But after accounting for the effect of gender, this relationship was no longer significant. Thus, the aerobic exercise training-induced change in visfatin does not appear to be related to training-induced improvements in glucose and obesity-related variables.

Influence of PBEF1 Polymorphisms & Haplotypes at Baseline

Genetic variation in the PBEF1 gene has previously been associated with a wide range of metabolic phenotypes (11; 16; 64; 115; 132). In agreement with this and consistent with the theme of hypothesis 2, we found significant associations with glucose and obesity-related variables for the PBEF1 -4689, -1001, -948, and SER301SER polymorphisms and the GCTGC, GTTGC, TCGTT, and TCGGT haplotypes.

First, our data show an influence of the -4689 polymorphism on ISI and insulin AUC. Furthermore, the TT genotype group, with lower insulin AUC and higher ISI values, was found to be more insulin sensitive than the GG genotype group. In contrast, Bailey *et al.* reported near significant associations for the PBEF1 -

4689 G>T (rs2302559) polymorphism with fat mass, total apoB, LDL apoB, and LDL-cholesterol, but not with glucose or insulin levels, in the Quebec Family Study (11). Last, the -4689 polymorphism could influence transcriptional activity of the PBEF1 gene, as it is located in the promoter region of the PBEF1 gene. However, no studies, to our knowledge, have investigated this or other functional effects of the -4689 polymorphism.

We found that the -1001 T>G (rs9770242) polymorphism was associated with VO₂max, BMI, fasting insulin, and ISI. Furthermore, the GG+GT group had lower VO₂max, BMI, and fasting insulin, and higher ISI values than the TT genotype group. In concordance with these findings, Bailey *et al.* reported that the G allele was associated with lower fasting insulin and fasting glucose levels in the Quebec Family Study (11), and Korner *et al.* reported higher waist-to-hip ratios in healthy school children with the TT genotype (64). However, Bottcher *et al.* did not find associations with T2D, body composition, or glucose-related variables in Germans (16). Bottcher's group also did not find an influence of the -1001 polymorphism on circulating visfatin levels or visfatin mRNA expression, but they found that subjects homozygous for the G allele had a lower visceral to subcutaneous visfatin mRNA expression ratio (16). With regard to functional significance, a luciferase reporter gene assay found that the G allele did not alter PBEF1 gene expression in lung microvascular endothelial cells (129). However, the PBEF1 -1001 polymorphism is reportedly in complete linkage disequilibrium with rs1319501, another PBEF1 promoter polymorphism, which may be functional (11).

The -948 G>T polymorphism has been associated with fasting insulin, total apoB, and LDL apoB in the Quebec Family Study (11), diastolic blood pressure in obese children (64), and inflammation (CRP and fibrinogen levels) in Italians (132). In addition, in case-control studies, Zhang *et al.* found that the T allele was associated with T2D (132), whereas Bottcher *et al.* found no association with T2D (16). Bottcher *et al.* also reported that non-diabetic German T allele carriers had significantly lower OGTT 2-hour plasma glucose, lower fasting insulin levels, and a lower visceral to subcutaneous visfatin mRNA expression ratio than those with the GG genotype (16). In contrast to our hypothesis, but in agreement with Bottcher *et al.*, we found lower fasting insulin, lower insulin AUC, lower glucose AUC, and higher ISI values in the -948 T allele carriers than in the GG homozygotes. The -948 G>T polymorphism is located in the promoter region of PBEF1, and to date, no one has examined whether the -948 T allele alters PBEF1 transcriptional activity.

To our knowledge, this is the first study to examine the SER301SER C>T (rs2302559) polymorphism in connection with glucose and obesity-related variables. We found that the TT genotype group had significantly lower intra-abdominal fat and significantly higher insulin sensitivity values than the CT and CC genotype groups. The TT genotype group also had lower fasting insulin and lower insulin AUC values than the CC genotype group. As this polymorphism results in a synonymous amino acid change, its functional importance may be via linkage with another SNP.

Although we found significant associations for the PBEF1 -4689, -1001, -948, and SER301SER polymorphisms, we found no influence of the -1543 C>T polymorphism on glucose or obesity-related variables. In agreement with these

findings, Jian *et al.* reported that the -1543 polymorphism was not associated with T2D, fasting glucose, 120 minute OGTT glucose, glucose AUC, total cholesterol, triglycerides, HDL-cholesterol, or LDL-cholesterol in a study involving Chinese NGT, IGT, and T2D patients (55). In contrast, Tokunaga *et al.* reported an association with triglyceride and HDL-cholesterol levels in Japanese individuals; however, they did not adjust for or report the age or gender composition of the genotype groups (115). Tokunaga's group also did not assess diet, physical activity levels, or menopausal status, and differences in these factors could also have influenced the lipoprotein-lipid measures. Ye *et al.* reported that the -1543 polymorphism was functional, with ~ 1.8-fold decrease in luciferase reporter gene expression in human lung microvascular endothelial cells with the T allele promoter (129). However, Tokunaga *et al.* found no differences in luciferase activity for the C versus T promoters in 3T3-L1 adipocytes (115).

As the combination of polymorphisms is often more informative in determining the genetic contribution to phenotypes, we constructed haplotypes from the PBEF1 -4689 , -1543, -1001, -948, and SER301SER polymorphisms using the program PHASE, version 2.1 and investigated their association with glucose and obesity-related outcomes. Overall, we found that 5 PBEF1 haplotypes were commonly occurring in our participant population: GCTGC, GTTGC, TCGTT, TCTGT, and TCGGT. Moreover, carriers of the GCTGC haplotype, composed of the common alleles for the 5 PBEF1 polymorphisms, had higher glucose AUC and higher insulin AUC values than non-carriers. Carriers of the GTTGC haplotype, comprised of the common alleles for the -4689, -1001, -948, and SER301SER polymorphisms

and the variant allele for the -1543 polymorphism, were found to have a higher mean BMI than non-carriers.

In contrast, we found that TCGTT haplotype carriers had significantly lower fasting insulin, lower glucose AUC, lower insulin AUC, and higher ISI values than TCGTT haplotype non-carriers. Furthermore, the TCGTT haplotype contains the variant alleles for the -4689, -1001, -948, and SER301SER polymorphisms and common allele for the -1543 polymorphism.

Haplotype TCTGT, composed of the -4689 and SER301SER variant alleles and -1543, -1001, and -948 common alleles, was not associated with any glucose or obesity-related phenotypes. Last, carriers of the TCGGT haplotype, made up of the variant alleles for the -4689, -1001, and SER301SER polymorphisms and the common alleles for the -1543 and -948 polymorphisms, had greater subcutaneous fat mass, but lower fasting glucose than haplotype non-carriers.

Thus, genetic variations in the PBEF1 gene were found to influence glucose and obesity-related variables at baseline.

Influence of PBEF1 Polymorphisms & Haplotypes After Aerobic Exercise Training

No previous study has investigated the influence of PBEF1 polymorphisms and haplotypes on the response of glucose and obesity-related variables to aerobic exercise training. Our data indicate no significant differences after training for any of the outcome variables by the PBEF1 -4689 G>T, -1543 C>T, and -1001 T>G genotype groups. In the -4689 and -1001 groups this is due to the disadvantaged group at baseline experiencing greater advantageous changes with training, resulting

in no differences after training between the groups. In contrast, there were no differences at baseline between the -1543 genotype groups, and the groups changed similarly with training. However, we found that total cholesterol and LDL-cholesterol levels varied by the -948 genotype after training due to the GG group decreasing and the GT+TT group increasing these variables with aerobic exercise training. In addition, we found significant SER301SER genotype effects on BMI and total body fat after training. Moreover, the CT group decreased BMI significantly more than the TT group and decreased total body fat significantly more than the CC and TT groups.

In PBEF1 haplotype analyses, we found no significant differences in glucose and obesity-related exercise training outcomes between the GCTGC, GTTGC, and TCTGT haplotype carriers and non-carriers. However, the TCGTT haplotype carriers had significantly higher after training total cholesterol, HDL-cholesterol, and LDL-cholesterol levels than TCGTT haplotype non-carriers. These differences were a result of significant increases in total cholesterol and LDL-cholesterol with aerobic exercise training in the TCGTT haplotype carrier group and significant decreases with training in the TCGTT haplotype non-carrier group. In addition, HDL-cholesterol increased in both the TCGTT haplotype carrier and non-carrier groups, but the magnitude of the increase was greater in the haplotype carrier group. Finally, in the TCGGT haplotype group, total cholesterol decreased only in the haplotype carriers, resulting in differing total cholesterol levels by haplotype after training.

The mechanism linking PBEF1 polymorphic variation to differential responses in glucose and obesity-related variables after aerobic exercise training is

unknown. The initial step may involve alterations in visfatin/Nampt mRNA and protein levels. Moreover, there are hypoxia (10; 85; 105) and stress responsive elements (85) in the PBEF1 promoter region that may affect PBEF1 transcription in response to aerobic exercise training. Furthermore, it is possible that one or more of the promoter polymorphisms examined in this study affects transcription factor binding at or near these sites, thereby altering PBEF1 transcription. However, more research is needed to clarify these speculations.

Thus, PBEF1 polymorphisms and haplotypes influence aerobic exercise training-induced changes in glucose and obesity-related variables.

Gender & Genotype/Haplotype Interaction Effects

In addition to the significant PBEF1 polymorphism and haplotype effects in the present study, numerous gender by genotype and gender by haplotype interaction effects were detected. All of the women in our study were postmenopausal, and in all instances, HRT use did not differ by genotype group. Furthermore, Ognjanovic *et al.* did not include any sex hormone specific response elements in their list of putative PBEF1 transcription factor binding sites (85). Thus, it is unclear as to why the response of men and women differed by genotype. Future studies investigating PBEF1 genetic variation must at least account for the moderating effect of gender and may want to consider analyzing men and women separately to avoid this variability.

PBEF1 Genotype & Haplotype Results Summary

Overall, we found that the variant allele group for the -4689, -1001, -948, and SER301SER polymorphisms had better glucose and obesity-related outcomes,

including greater insulin sensitivity, than the common allele group at baseline. These similar results may be due to the moderate pairwise linkage disequilibrium between the polymorphisms. However, the functional significance of the polymorphisms remains to be determined, as does the effect that leads to alterations in glucose and obesity-related phenotypes. Thus, research in these areas is needed.

Our data also show PBEF1 haplotype effects on glucose and obesity-related variables at baseline. Consistent with the single SNP analyses, the TCGTT haplotype, composed of the variant alleles for the -4689, -1001, -948, and SER301SER polymorphisms and the -1543 common allele, was associated with higher insulin sensitivity measures. Furthermore, the GCTGC haplotype (comprised of the polymorphism common alleles) and GTTGC haplotype (comprised of polymorphism common alleles and the -1543 variant allele) were both associated with more disadvantageous phenotypes. Despite these interesting results, future research needs to clarify the functional impact of the different haplotypes and identify the mechanism responsible for alterations in glucose and obesity-related phenotypes.

We also report, for the first time, significant influences of PBEF1 polymorphisms and haplotypes on glucose and obesity-related variables following the completion of 24 weeks of aerobic exercise training. Moreover, the PBEF1 -948 variant, TCGTT haplotype, and TCGGT haplotype were associated with differential changes in lipoprotein-lipid levels with training. In addition, the SER301SER polymorphism influenced changes in BMI and total body fat. However, the reason for these differential aerobic exercise training responses needs to be elucidated.

Chapter 5: Conclusion

Adipokines, such as visfatin, may help to connect diabetes and obesity, and previous research has linked visfatin and visfatin gene polymorphisms with glucose and obesity-related conditions. Furthermore, aerobic exercise training has often been used as an effective means to improve many health outcomes, including insulin sensitivity. Although we report that plasma visfatin levels increased similarly in response to an aerobic exercise training intervention in IGT and NGT individuals, we found no relationships between the aerobic exercise training-induced change in circulating visfatin levels and the training-induced changes in glucose and obesity-related variables. Thus, alterations in circulating visfatin levels are likely not related to or responsible for the beneficial improvements in glucose and obesity-related variables with aerobic exercise training. However, we found that polymorphic variation in the visfatin gene (PBEF1) influenced glucose and obesity-related outcomes at baseline and modulated their aerobic exercise training-induced responses. The mechanism responsible for these differential changes is unknown. Thus, future studies need to address the functional significance of PBEF1 polymorphisms and haplotypes, clarify mechanisms connecting visfatin to glucose and obesity-related phenotypes, and verify the influence of PBEF1 polymorphisms and haplotypes on glucose and obesity-related variables and their responses to aerobic exercise training.

Chapter 6: Literature Review

Visfatin History

In 2004, Fukuhara and colleagues used the term visfatin to describe a seemingly novel adipokine that they had isolated from the visceral fat of humans and mice (35). However, visfatin had previously been isolated from lymphocytes in the early 1990s and identified as pre-B cell colony-enhancing factor (PBEF) (102). Furthermore, PBEF was a cytokine-like molecule, enhancing the effects of interleukin-7 and stem cell factor on pre-B-lymphocyte colony formation, which was also present in skeletal muscle, liver, and bone marrow (102). Additionally, Jia and colleagues reported that PBEF was an inflammatory cytokine since it was upregulated in neutrophils and acted to inhibit/delay neutrophil apoptosis in experimental inflammation and sepsis (54). PBEF was also reported to function as a nicotinamide phosphoribosyltransferase (100) known as Nampt, active in nicotinamide adenine dinucleotide (NAD) salvage pathways and suggested to be a regulator of mammalian health and longevity (127). Thus, visfatin, PBEF, and Nampt are the same enzyme.

Protein Structure & Secretion

The human visfatin protein reportedly shares over 95 % sequence homology with rat and mouse visfatin (11). Kim and colleagues reported on the crystal structure of rat visfatin in apo form, in complex with nicotinamide mononucleotide (NMN), and in complex with FK-866 (a nicotinamide phosphoribosyltransferase inhibitor) (59). They found that 2 visfatin monomers (491 residues each) combine to form a

homodimer (59). Furthermore, the researchers discovered that both of the monomers were needed for the formation of visfatin's active site (located near the interface of the subunits) and nicotinamide phosphoribosyltransferase activity (59). Khan and colleagues found similar results for mouse and human visfatin (58), and Wang and colleagues added that mouse visfatin was a type II phosphoribosyltransferase (121). Lastly, Takahashi and colleagues characterized the crystal structure of human visfatin in its free form and also found that it was dimeric (111).

Visfatin mRNA and protein expression have been detected in a variety of tissues. In fact, Revollo and colleagues found that mouse brown adipose tissue, liver, and kidney had the highest levels; mouse heart had intermediate levels; mouse white adipose tissue, lung, spleen, testis, and muscle had low levels; and mouse brain and pancreas had no visfatin protein expression levels (97). Furthermore, Fukuhara and colleagues reported that visfatin also occurs as a secreted protein, detectable in the circulation (35). However, visfatin's peptide sequence does not have a typical signal sequence for secretion (102), and this has led researchers to question whether visfatin is a secreted protein or a substance released during cell death. An early study by Kitani and colleagues suggested that visfatin was an intracellular rather than secreted protein with growth-phase dependent changes in subcellular localization (61). However, more recent studies have shown that visfatin occurs intracellularly and as an extracellular secreted protein (97). Moreover, its release was found to occur via a non-classical pathway in adipocytes (97; 114).

Thus, visfatin is a highly conserved, intracellular and extracellular dimeric protein.

Visfatin, Obesity, & Glucose Metabolism

Visfatin: Insulin-mimetic Effects

Via a series of studies involving cell culture, rodent models, and humans, Fukuhara and colleagues extended the realm of visfatin to include obesity and glucose metabolism (35).

With regards to adiposity, Fukuhara *et al.* found increased visfatin mRNA expression and protein secretion during 3T3-L1 adipocyte differentiation *in vitro*. Furthermore, in 101 men and women, plasma visfatin levels were positively and significantly correlated with visceral and subcutaneous fat content ($r = 0.68$ and 0.22 , respectively). In a mouse model of obesity and T2D (KKAy mice), plasma visfatin and visceral fat visfatin mRNA levels were found to increase during the growth period associated with obesity development in the mice. Similar results were reported in c57BL/6J mice fed a high fat diet. Thus, visfatin was related to adiposity. (35)

Fukuhara and colleagues also investigated the biological and physiological effects of visfatin and found relationships with glucose regulation. Acute administration of recombinant visfatin in c57BL/6J mice decreased fasting plasma glucose in a dose-dependent manner, independent of insulin changes. Furthermore, injecting visfatin into obese, insulin resistant KKAy mice and into mice made insulin deficient via streptozotocin treatment decreased plasma glucose, effects similar to insulin injections in the mice. Also, chronic administration of visfatin via an adenovirus vector increased visfatin levels and reduced glucose and insulin levels in c57BL/6J and KKAy mice. (35)

Fukuhara's group also studied visfatin transgenic mice. Visfatin gene knockout mice were found to die during embryonic development, whereas visfatin heterozygous mice were viable, with plasma visfatin levels two-thirds those of wildtype mice. In addition, visfatin heterozygotes were not significantly different from wildtype mice with regard to growth, food intake, body weight, organ and tissue weight, plasma insulin, or insulin sensitivity during an insulin tolerance test. However, plasma glucose levels under fasting and feeding conditions and after 60 and 120 minutes of a glucose tolerance test were found to be elevated in the heterozygous mice as compared to wildtype mice. Based on this work, visfatin was believed to have an insulin-like effect. (35)

Fukuhara's group then further examined visfatin's insulin-like actions. Similar to insulin treatment, visfatin treatment was found to increase glucose uptake in 3T3-L1 adipocytes and L6 myocytes and prevent glucose release from H4IIEC3 hepatocytes. Also similar to insulin, visfatin injection in mice and visfatin treatment in cultured 3T3-F442A adipocytes, L6 myocytes, and H4IIEC3 hepatocytes was found to induce phosphorylation of the insulin receptor, insulin receptor substrate (IRS)-1, and IRS-2. Additionally, the cell culture studies detected phosphatidylinositol 3-kinase binding to IRS-1 and IRS-2 and phosphorylation of Akt and mitogen-activated protein kinase. Further studies found that the binding dissociation constants for visfatin and insulin were similar, although insulin and visfatin were found to bind to the insulin receptor in slightly different ways. The results of these studies again supported visfatin having insulin-like effects. However, the researchers cautioned that although visfatin had physiological effects, its overall

impact was likely to be small due to its low concentration (~ 10 % that of insulin).

(35)

Fukuhara and colleagues' paper was considered seminal in this area, until its retraction in 2007. In the retraction, the researchers indicated that questions raised by the Osaka University Graduate School of Medicine Committee for Research Integrity dealt with their experiments involving visfatin interactions with the insulin receptor. In brief, after publication of the original paper, the researchers later determined that not all of their visfatin preparations bound with or activated the insulin receptor. However, the researchers indicated that they believed in their earlier conclusions and cited a report by Xie and colleagues (126) which found that visfatin had insulin-mimetic effects in cultured osteoblasts. (36)

An earlier article also indicated that the Osaka University Graduate School of Medicine questioned the exclusion of results that did not support the researchers' conclusions. Specifically, the researchers tried to create male and female visfatin heterozygous knockout mice. The male transgenic mice were viable and had half of the visfatin found in a wildtype mouse; however, the females did not experience as much visfatin lowering. Moreover, only the results from the male mice were included in the paper. (82)

Providing support to Fukuhara and colleagues' report of visfatin's insulin-mimetic effects, Xie and colleagues studied the effects of visfatin on human primary osteoblasts. First, they found that human osteoblasts did not express visfatin mRNA, but osteoblast lysates were found to express insulin receptor protein. Next, the researchers examined the actions of visfatin on insulin receptor signaling and found

that visfatin treatment in human osteoblasts led to insulin receptor, IRS-1, and IRS-2 tyrosine phosphorylation, effects similar to those of insulin treatment at the same concentrations. Furthermore, pre-treatment with an inhibitor of insulin receptor tyrosine kinase activity inhibited visfatin and insulin activation of insulin signaling. The researchers then examined whether visfatin influenced glucose transport in osteoblasts and found that visfatin increased glucose uptake at concentrations of 10 and 100 ng/mL, effects similar to those of 10 and 100 ng/mL of insulin. Visfatin also increased osteoblast proliferation, type 1 collagen mRNA expression, type 1 collagen secretion, and osteoblast mineralization; effects similar to those of insulin. (126)

Although Xie and colleagues reported similar insulin-like visfatin effects (126), other researchers have not been able to replicate these findings (97). Furthermore, Revollo and colleagues have surmised that visfatin's connection to obesity and glucose-related variables is via its role as the NAD biosynthetic enzyme Nampt (95; 97). Moreover, the researchers proposed a mechanism linking Nampt to NAD biosynthesis and glucose metabolism via the maintenance of NMN and NAD levels. Studies leading to a description of this mechanism will be briefly discussed next.

Visfatin as Nampt: Linking NAD Biosynthesis & Glucose Metabolism

NAD is a coenzyme in oxidation/reduction reactions and its biosynthesis can be initiated 3 ways: the de-novo pathway from tryptophan, from nicotinic acid, or most commonly, from nicotinamide (95). Moreover, nicotinamide is converted to NMN by Nampt and then into NAD by nicotinamide/nicotinic acid mononucleotide adenylyltransferase (Nmnat). Revollo and colleagues found that Nampt was the rate-

limiting enzyme in the NAD pathway starting from nicotinamide since Nampt had a higher affinity for nicotinamide and was less catalytically efficient than Nmnat (96). In addition, the researchers found that overexpressing Nampt in mouse cell lines increased total NAD levels, whereas overexpressing Nmnat did not increase NAD levels (96).

Revollo and colleagues also characterized intracellular and extracellular Nampt using a series of *in vitro* and *in vivo* experiments. They examined the production of intracellular and extracellular Nampt in pre-adipocytes during differentiation and found that the differentiation of both brown and white (3T3-L1) adipocytes increased intracellular and extracellular Nampt levels, with greater amounts produced by brown adipocytes. Furthermore, they examined the production of extracellular Nampt by different cell types and found that COS-7 (a transformed monkey kidney cell line) and Chinese hamster ovary (CHO) cells produced detectable amounts of extracellular Nampt when Nampt was overexpressed. However, COS-7, CHO, HEK293 (a human embryonic kidney cell line), and NIH3T3 (a mouse fibroblast cell line) cell lines did not naturally produce extracellular Nampt. Next, they investigated the enzymatic activity of intracellular and extracellular Nampt and determined that both were highly active with dimerization necessary for Nampt's activity. (97)

Then, the researchers tried to replicate the findings of Fukuhara and colleagues [*i.e.* insulin-mimetic effects (35)], but were unable to as they found that Nampt did not induce expression of adipocyte differentiation markers, did not affect glucose uptake in adipocytes, did not phosphorylate the insulin receptor or Akt, and

did not reduce blood glucose levels. The researchers also generated Nampt transgenic mice. Similar to the results of Fukuhara and colleagues (35), Revollo's group found that Nampt null mice were embryonically lethal, but that Nampt heterozygous mice were viable. Moreover, the heterozygous mice had normal body weight, normal islet area, normal fasting glucose levels, reduced intracellular Nampt, reduced brown adipose tissue NAD levels, and normal liver NAD levels as compared to wild-type mice. They also found that extracellular Nampt was reduced by ~ 50 % in female Nampt heterozygous knockout mice, but not in male mice. As mentioned previously, Fukuhara and colleagues found ~ 50 % reduced levels of visfatin in male mice (35); furthermore, the possible difference may be because the researchers used different techniques and mouse strains to create the transgenic creatures. Regardless, the female Nampt heterozygous mice had reduced insulin secretion and impaired glucose tolerance during intraperitoneal glucose tolerance tests (IPGTT), but no differences as compared to wild-type mice during insulin tolerance tests. (97)

Next, the researchers confirmed their *in vivo* results with *in vitro* studies of isolated islets. They found that glucose-stimulated insulin secretion was reduced in isolated primary islets from Nampt heterozygous females, and that NAD levels also were ~ 40 % reduced in the animals when nicotinamide was added to the culture media overnight. The researchers also found that the IPGTT glucose tolerance and insulin secretion defects in the heterozygous Nampt mice and isolated islets could be corrected by administering NMN, a product of the Nampt reaction. Furthermore, the researchers found that NMN was also present in plasma, and reduced by ~ 35 % in female heterozygous Nampt mice. Last, the researchers examined the effects of a

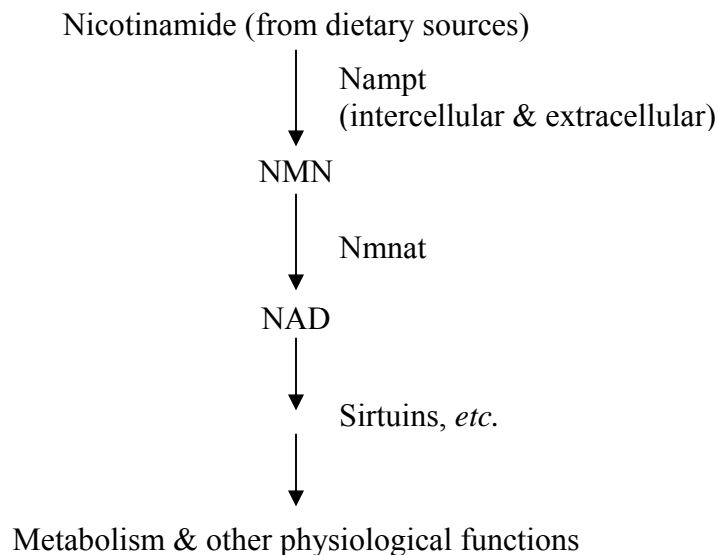
Nampt inhibitor (FK866) and found that the inhibitor reduced NAD biosynthesis by ~ 80 % and glucose-stimulated insulin secretion by ~ 60 % in the isolated islets. (97)

These results suggest that Nampt is a NAD biosynthetic enzyme and that the NAD activity of Nampt is important for glucose regulation. Furthermore, Nampt did not exert insulin-mimetic effects as reported previously by Fukuhara and colleagues. Thus, visfatin's effects on glucose metabolism may be related to its function as Nampt, rather than its suggested function as an adipokine. Moreover, the mechanism proposed by Revollo and colleagues, and outlined in Figure 6-1, links Nampt to metabolic processes via the regulation of NAD-dependent factors such as the sirtuin family. First, nicotinamide absorbed from the diet undergoes distribution to the tissues via the circulation. If taken up by cells, nicotinamide conversion to NMN would take place by intracellular Nampt. If not removed from the circulation, extracellular Nampt could convert nicotinamide to NMN, which would then be transported to the tissues for uptake. Once inside of cells, Nmnat would complete the reaction to produce NAD. Then, via its involvement in transcriptional regulation, NAD could regulate metabolism. (95; 97)

One such target of NAD is the sirtuin family, as NAD cleavage is required for their deacetylase and ADP-ribosylation reactions. Furthermore, Revollo and colleagues found that the Nampt-regulated NAD biosynthesis pathway regulated mammalian sirtuin activity (96). In fact, the researchers found that increasing Nampt dosage led to increased transcriptional repressive activity of the mouse Sir2 α catalytic domain (96). Moreover, the sirtuin family has been implicated in the regulation of genes affecting aging, calorie restriction, and metabolism.

There are 7 mammalian sirtuins (SIRT1-7); moreover, SIRT1 and SIRT3 have been implicated in metabolism via their deacetylase activity, whereas SIRT4 has been connected to metabolism via its role as an ADP-ribosyltransferase. SIRT1 has been shown to regulate genes involved in pancreatic beta cell insulin secretion (14; 80), beta cell survival (60), adipogenesis (91), lipolysis (38; 91; 99), gluconeogenesis (98; 99), and cholesterol degradation (99). In contrast, Hallows and colleagues reported that SIRT3 activates acetyl-coenzyme A synthetase (47), an enzyme important in the formation of acetyl coenzyme A, which is involved in the citric acid cycle, cholesterol synthesis, and fatty acid synthesis. Finally, Haigis and colleagues linked SIRT4 to amino acid-stimulated insulin secretion as it was shown to ADP-ribosylate and inhibit glutamate dehydrogenase (46). Thus, the sirtuin family may help to connect Nampt, NAD biosynthesis, and metabolic function. In addition, Nampt and the sirtuin family have been linked in studies involving stress resistance (128) and smooth muscle cell longevity (118).

Figure 6-1. Mechanism linking Nampt, NAD biosynthesis, and physiological function as proposed by Revollo and colleagues (95; 97).



Visfatin Studies

Regardless of the mechanism, numerous investigators have examined visfatin's potential role in glucose and obesity-related conditions. The studies can be organized into those involving associations with circulating visfatin levels, visfatin mRNA expression, and visfatin gene polymorphisms. Additionally, 1 study has examined the response of visfatin to acute exercise and 3 studies have assessed exercise training's impact on visfatin.

Circulating Visfatin Studies

Type 1 Diabetes & Type 2 Diabetes

Haider and colleagues compared fasting plasma visfatin levels in insulin dependent diabetes mellitus (T1D) patients (n = 18) and healthy individuals (n = 14). The groups differed in terms of age, fasting glucose, and visfatin levels. Moreover, T1D patients had greater plasma visfatin levels than the healthy controls. In addition, there were no correlations found between visfatin and body mass index (BMI) or age. Although the participants were comparable in terms of BMI, lipoprotein-lipid measures, and physical activity status, the researchers did not adjust for differences in age and gender. In addition, some of the patients were receiving medications for comorbidities, including hypertension and cholesterol medication, and these were not taken into account in the analyses. Last, the researchers did not assess diet, and differences in diet could potentially influence the outcome measures. (43)

Lopez-Bermejo and colleagues investigated the relationship between serum visfatin and insulin secretion in 118 non-diabetic men, 64 men and women with type 2 diabetes (T2D), and 58 men and women with T1D. In the non-diabetic subjects,

circulating visfatin was negatively correlated with the acute insulin response to glucose (*i.e.* insulin secretion, $r = -0.27$), fasting insulin ($r = -0.20$), and 30-minute oral glucose tolerance test (OGTT) insulin ($r = -0.24$), and positively correlated with insulin sensitivity ($r = 0.19$). However, only the acute insulin response to glucose was found to be a significant predictor of serum visfatin in multiple linear regression analysis. The researchers also found circulating levels of visfatin to be higher in T2D patients as compared to non-diabetic patients and highly correlated with glycosylated hemoglobin ($r = 0.49$). However, when comparing newly diagnosed T2D patients to the non-diabetics, there was no difference in visfatin levels. Last, visfatin levels were increased in the T1D patients as compared to both the T2D patients and the non-diabetics. Thus, this study suggests that T1D, and possibly beta-cell dysfunction, are associated with increased visfatin levels. However, the study did not take the varying types of medication used by the T2D patients into account. The study also did not account for the effects of diet, physical activity status, or female menopausal status, all of which may have influenced the outcome measures. (74)

Takebayashi and colleagues investigated the association between plasma visfatin and vascular endothelial function in patients with T2D. Overall, the investigators reported no differences in the plasma visfatin concentration between healthy control individuals ($n = 28$) and diabetic patients ($n = 80$). In addition, they found no differences in plasma visfatin between patients receiving and not receiving antihypertensive medications and between smokers and non-smokers. The researchers also looked at the effects of 12 weeks of pioglitazone or insulin treatment on plasma visfatin levels and found no differences from baseline measures. The

investigators did however detect significant correlations between the visfatin concentration and creatinine clearance ($r = -0.2750$), aldosterone ($r = -0.2432$), flow-mediated vasodilation ($r = -0.2672$), and urinary albumin excretion ($r = 0.2305$). (112)

However, the results from the Takebayashi and colleagues study are somewhat difficult to interpret (especially in the diabetics) because of the varying comorbidities and therapies reported. In addition, the diabetic patients were recruited after hospitalization for glycemic control and/or diabetes education, and these issues in these individuals (along with their duration of disease) could potentially influence the results. Furthermore, the investigators provided very brief subject characteristic data in the form of a table and some of the data seems to be entered into the wrong columns. For example, the non-diabetic subjects have significantly higher fasting plasma glucose, glycosylated hemoglobin (HbA1c), and BMI than the diabetic subjects. Also, a dash (rather than a 0) is provided as to the number of smokers and antihypertensive medication takers in the control group. Thus, the control group may not have been queried as to these variables. Finally, there was no indication as to dietary or physical activity status differences in the groups. However, the researchers indicated that 3 of the diabetic patients were using diet alone as their diabetic therapy. Thus, several extraneous factors may have affected the outcome variables. (112)

Dogru and colleagues reported higher levels of plasma visfatin in T2D patients ($n = 22$) as compared to healthy controls ($n = 40$). However, no differences were detected between T2D patients and participants with impaired glucose tolerance (IGT; $n = 18$) or between participants with IGT and the healthy controls. In addition,

there were no detectable differences in visfatin levels between men and women, and visfatin levels were not correlated significantly with BMI, blood pressure, insulin, C-reactive protein (CRP), glucose, lipids, or homeostasis model assessment insulin resistance (HOMA-IR). One of the positive aspects of this study was the detailed inclusion/exclusion criteria. However, the researchers did not assess diet, physical activity status, or female menopausal status in the participants, all of which could have influenced the results. (29)

Chen and colleagues compared visfatin plasma concentrations in 61 Chinese T2D patients and 59 sex and age matched non-diabetic controls. The researchers found that the visfatin concentration was independently and significantly associated with T2D. Moreover, the plasma visfatin concentration was elevated in the T2D patients as compared to the healthy controls even after adjusting for age, gender, BMI, and smoking status. Plasma visfatin was also found to be associated with age, waist-to-hip ratio (WHR), fasting plasma insulin, adiponectin, and HOMA-IR in simple regression models. These results suggest that visfatin may play a role in T2D pathogenesis. However, the researchers did not determine if differences existed with regard to diet, physical activity status, and female menopausal status among the participants, and these factors may have affected the measurements. (22)

Retnakaran and colleagues compared serum visfatin levels in Thai T2D outpatients (n = 50) and healthy controls (n = 79) using a new ELISA kit designed to target full-length visfatin (as opposed to using the C-terminal assay). The researchers found that visfatin levels were significantly higher in the T2D patients as compared to the healthy controls, even after adjustment for age, gender, BMI, waist circumference,

systolic blood pressure, LDL-cholesterol, HDL-cholesterol, triglycerides, fasting glucose, and HOMA-IR. In addition, the researchers found that resistin was the only variable that correlated significantly with visfatin. Although this study had very specific entrance criteria for the Thai T2D patients, the authors failed to assess and account for potential differences in diet, physical activity, and female menopausal status among the participants. Furthermore, these factors may have affected the outcome variables and metabolic covariates. (94)

Sandeep and colleagues investigated relationships between serum visfatin and obesity and diabetes-related traits in 150 T2D (n = 75 men, n = 75 women) and 150 non-diabetic (n = 75 men, n = 75 women) Asian Indians. They found significantly higher levels of visfatin in T2D versus non-diabetic individuals, and this relationship remained significant after adjusting for age and gender, but was no longer significant after additionally adjusting for BMI (p = 0.067) or waist circumference (p = 0.057). Visfatin levels were also associated with obesity, visceral fat, and HOMA-IR, but not with subcutaneous fat. Furthermore, the association between visfatin levels and obesity remained significant, with an odds ratio of 1.060, after adjusting for age, gender, and diabetes status. The association between visfatin levels and visceral fat also remained significant after adjustment for age, gender, diabetes status, and BMI, and the relationship between visfatin levels and HOMA-IR remained significant after adjustment for age and gender, but not after additionally adjusting for diabetes status. This suggests that visfatin levels are associated with obesity and that associations with diabetes may occur via visfatin's connection with obesity. Besides this study's limitation of being cross-sectional, no mention was made regarding potential

differences in diet or physical activity levels between the groups. In addition, no indication was made as to whether co-morbidities existed and were being treated in the diabetic patients. (104)

Fernandez-Real and colleagues assessed whether an interaction existed between visfatin and parameters of iron metabolism (ferritin and prohepcidin) in 95 healthy Spanish men with normal glucose tolerance (NGT) and in 43 otherwise-healthy Spanish men with altered glucose tolerance (IGT, impaired fasting glucose (IFG), or undiagnosed diabetes). The researchers found that serum visfatin, ferritin, and prohepcidin were increased in men with previously undiagnosed diabetes as compared to the NGT or IGT/IFG groups of men. In the entire group, relationships among visfatin, insulin sensitivity, and parameters of iron metabolism were modulated by the obesity status and glucose tolerance of the men. Furthermore, in nonobese men, insulin sensitivity was found to contribute to visfatin variance, whereas only the serum soluble transferrin receptor contributed to visfatin variance in obese men after adjusting for BMI, age, prohepcidin, insulin sensitivity, and glucose tolerance. The researchers concluded that these results may indicate that visfatin is upregulated in connection with increased iron stores and proposed a mechanism connecting oxidative stress with insulin, iron overload, and visfatin levels. However, this study was cross-sectional in nature and both longitudinal and confirmatory studies are needed to validate many of the steps in the proposed mechanism. This study also did not control for diet or physical activity status, both of which could possibly influence parameters of iron metabolism and visfatin levels. (31)

Li and colleagues investigated plasma visfatin levels in NGT (n = 36), IGT (n = 26), and T2D (n = 30) patients and found lower fasting and 2-hour OGTT visfatin levels in T2D patients as compared to the NGT controls, even after adjustment for age, gender, and BMI. Furthermore, fasting plasma visfatin was positively correlated with BMI (r = 0.27) and WHR (r = 0.42) and negatively correlated with 2-hour OGTT glucose levels (r = -0.33) and hemoglobin A(1c) (r = -0.25) (73). Despite the positive findings, the researchers did not address whether the different treatments used in the T2D group affected visfatin levels. The researchers also did not assess menopausal status or hormone replacement use in the female study participants, even though the average ages of the NGT, IGT, and T2D groups were 51 ± 9 , 55 ± 9 , and 56 ± 10 yrs. Last, diet and physical activity status differences were not accounted for, even though some of the T2D patients were reportedly using diet treatment. (73)

Jian and colleagues found that serum visfatin levels were similar in Chinese adults with NGT (n = 61), IGT (n = 65), and T2D (n = 115). However, visfatin levels were significantly lower in obese (n = 43) versus normal weight (n = 93) individuals and obese (n = 43) versus overweight (n = 105) individuals. Serum visfatin was also similar in males and females, and there were no significant correlations between visfatin and BMI, waist-to-hip ratio (WHR), or OGTT-related variables. Since the group comparisons involved t-tests, adjustment was not made for the statistically significant differences in body composition between the NGT, IGT, and T2D groups. The researchers also did not assess diet, physical activity status, or menopausal status in the participants, and these factors could have influenced the results. (55)

Tsiotra and colleagues compared visfatin expression in peripheral monocyte-enhanced mononuclear cells from 24 women with T2D and 26 healthy women. Both groups of women were further subdivided by BMI into lean or overweight, thus there were 4 subject groups. All women were pre-menopausal and aged 21-48 years. Although plasma visfatin tended to be higher in the overweight T2D patients as compared to the other groups, it was not statistically significant. Plasma TNF- α also did not differ, although plasma adiponectin was lower in the T2D women and IL-6 was higher in the overweight T2D women. There were also no significant correlations found for visfatin with BMI, waist circumference, fasting glucose, fasting insulin, HOMA-IR, triglycerides, or HDL-cholesterol. Thus, this study suggests that circulating levels of visfatin are not elevated in mononuclear cells from diabetic women. However, there were a few problematic aspects involving this study which may have impacted the results. First, different treatments were being used in the T2D patients (15 were on special diets, 5 were on oral hypoglycemic agents, and 2 were on combined insulin and hypoglycemic therapy), and these were not examined as potential confounding variables. In addition, the duration of diabetes, the physical activity status, and the average age for each of the groups were not provided and were not examined as potential confounding variables in the analyses. Last, beyond fasting, there was no mention of the blood drawing conditions. Thus, the time course of medication usage in the T2D participants was not provided. (116)

Gestational Diabetes Mellitus

Lewandowski and colleagues examined the relationship between visfatin and glucose tolerance in pregnant women (n = 51). The women were grouped based on

their responses to a glucose challenge test (GCT) and OGTT. Moreover, the control group (n = 20) responded normally to both tests, the intermediate group (n = 15) had an elevated GCT and normal OGTT, and the gestational diabetes (GDM) group (n = 16) had elevated results for both tests. Overall, the groups were similar in terms of age and BMI and had no pregnancy-related complications. The researchers found that there was a statistically significant elevation in serum visfatin levels in the GDM group as compared to the control group and an almost significant increase in the intermediate group as compared to the control group ($p = 0.07$) and GDM group ($p = 0.08$). In addition, significant correlations were detected for visfatin and insulin ($r = 0.38$), 120 minute insulin ($r = 0.39$), 120 minute glucose ($r = 0.035$), HOMA-IR ($r = 0.35$), and an insulin sensitivity index ($r = 0.32$). This suggests that visfatin is increased in pregnant women with abnormal glucose tolerance and may be related to insulin sensitivity. However, weight gain, diet, and physical activity status were not examined in the study and differences in these factors may have influenced the results. (72)

In a study by Krzyzanowska and colleagues comparing women with GDM (n = 64) to healthy pregnant women (n = 30), GDM was associated with increased plasma visfatin levels. No association was found for visfatin levels and fasting glucose, insulin, BMI, or HOMA-IR in the diabetic women. Furthermore, in a subgroup of 24 women with GDM, visfatin levels were found to increase during pregnancy (comparing weeks 28-30 with weeks 38-40) and after delivery (comparing 2 weeks after delivery with weeks 38-40). One of the positive aspects of this study was the examination of medication usage (diet versus insulin) in the participants with

GDM. However, diet was not examined in the healthy pregnant women, and physical activity status was not assessed in either group; moreover, these factors could have influenced the outcome variables. (68)

In contrast, Chan and colleagues reported decreased plasma visfatin levels in Chinese women with GDM (n = 20) as compared to healthy pregnant women (n = 20) (20). Moreover, they detected significant correlations between visfatin levels and maternal age, first trimester body weight, and first trimester BMI (r = -0.399, -0.350, and -0.336, respectively). The researchers assessed many potentially influential factors in the study including first trimester weight, first trimester BMI, GCT weight, GCT BMI, and gestational age at glucose challenge. The researchers also excluded participants with multiple pregnancies, fetal abnormalities, chronic diseases, pre-existing hypertension, or pre-existing diabetes. However, the researchers did not assess diet or physical activity status in the subjects, and they did not mention whether cigarette smoking status was exclusionary. These factors may have impacted the results. (20)

Haider and colleagues reported similar results when they compared plasma visfatin levels from OGTTs of 10 women with GDM to plasma visfatin levels from 10 healthy pregnant, age-matched women. Besides having lower fasting visfatin levels, the women with GDM also had higher fasting glucose and lower insulin sensitivity than the healthy controls. However, fasting insulin and the insulin responses during the glucose challenge were similar in the groups. In response to the glucose challenge, plasma levels of visfatin increased in both groups. However, the glucose-induced increase in visfatin, measured by visfatin area under the curve, was

lower in the GDM patients than in the controls. This suggests that impaired glucose tolerance in women with GDM may contribute to a blunted oral glucose challenge visfatin response. However, since glucose induces visfatin release (and the women with GDM had higher glucose levels), the lower visfatin levels in the women with GDM must be related to something other than glucose and insulin, and the researchers suggested cytokines. Although the cause of the lower visfatin levels in the women with GDM was not determined, some of the positive aspects of this study were that none of the women were on a special diet, none were taking medication, all were in weeks 24-28 of pregnancy, and both groups had similar anthropometric and lipid measurements. (40)

Obesity, Body Composition, & Weight Loss

Ingelsson and colleagues investigated whether plasma visfatin would be positively related to diabetes, obesity, and visceral adiposity in 374 participants from the Framingham Third Generation Cohort. Participants with computer tomography scans were chosen randomly for inclusion in the study, with over-sampling of participants from low and high BMI quartiles. Variables assessed included age, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol, triglycerides, fasting glucose, visceral adipose tissue, subcutaneous adipose tissue, metabolic syndrome status, low LDL-cholesterol status, diabetes status (based on fasting glucose), and smoking status. Thus, the researchers did not assess insulin sensitivity or perform an OGTT for determination of diabetes status. When analyzed as continuous variables, age and triglyceride levels had borderline significance with plasma visfatin (both $p = 0.07$). When analyzed

categorically, hypertension was found to be associated with plasma visfatin; however, the presence of hypertension was found to be lower for both the lowest and highest quintiles of plasma visfatin as compared to the middle three quintiles. This study suggests that plasma visfatin may not be a marker for metabolic traits. Besides its cross-sectional nature, this study did not assess whether diet or physical activity status differed among the subject population. In addition, the average age of the participants was 45 ± 6 yrs, and no indication was made as to the menopausal status of the female participants (53 % of the group). These factors could have influenced the study's major outcome variables. (53)

Choi and colleagues compared fasting plasma visfatin levels in healthy, non-diabetic obese ($n = 36$) and overweight ($n = 12$) Korean women. The women were 30-55 yrs of age, sedentary (< 20 minutes of exercise twice per wk), free of cardiovascular disease, and not taking laboratory test result-altering medications. Overall, the obese women had higher plasma visfatin levels than the overweight women. However, the researchers did not assess or account for differences in demographic variables or diet between the groups, and these factors may have influenced the outcome measures. (25)

In a study by Zahorska-Markiewicz and colleagues, serum levels of visfatin were determined in 21 obese, but otherwise healthy, women and compared with those of 16 normal weight women. Overall, the obese women had significantly higher levels of serum visfatin and insulin than the normal weight women. In addition, significant correlations were found for serum visfatin and insulin levels ($r = 0.51$) in the normal weight women and with serum visfatin and glucose levels in the obese

women ($r = 0.52$). However, no statistically significant correlations were detected between body composition measures and serum visfatin. Overall, physical activity and diet were not considered in the experiment, which is a limitation of the study since these factors may have influenced the results. Moreover, differences in physical activity and/or diet may explain why the normal weight women had significantly higher HDL-cholesterol levels than the obese women (61.2 ± 11.6 vs. 50.3 ± 8.5 mg/dL), since all other lipid measures did not differ between the groups. (131)

Pagano and colleagues analyzed the plasma visfatin concentrations of 30 normal weight and 39 obese men and women. They also compared visfatin levels in subcutaneous and visceral fat and in free fatty acid-induced insulin resistance. Plasma visfatin levels were lower in obese individuals as compared to normal weight individuals; moreover, plasma visfatin correlated negatively with BMI in the obese participants ($r^2 = 0.14$). Plasma visfatin was also positively correlated with subcutaneous visfatin mRNA expression ($r^2 = 0.32$). Last, plasma levels of visfatin were not different between men and women and were not influenced by free fatty acid-induced insulin resistance. Overall, the subjects had an unlimited diet and were recruited from hospital staff or outpatients. However, this study did not address physical activity status, female menopausal status, or medication usage as potential influential factors. (88)

Jin and colleagues examined serum visfatin concentrations in generally healthy, obese ($n = 72$) and normal weight ($n = 76$) Chinese adolescents. Even after adjustment for age, gender, and Tanner stage, the researchers found a higher median visfatin concentration in the obese adolescents as compared to the non-obese

adolescents. In obese adolescents, significant correlations were found for visfatin and age ($r = -0.280$), HDL-cholesterol ($r = 0.251$), Tanner stage ($r = -0.298$), and an early insulin secretion index ($r = -0.284$). Furthermore, age and HDL-cholesterol were significant predictors of visfatin in multiple regression analysis. Although the researchers excluded adolescents who were dieting, had secondary obesity, or IGT from study participation, they did not query participants on behavioral factors such as physical activity or cigarette smoking status. Moreover, differences in these factors may have influenced the outcome variables. (56)

Haider and colleagues found almost 2-fold higher plasma visfatin levels in 83 non-diabetic obese children as compared to 40 lean, healthy children. However, there were no significant associations detected for visfatin with CRP, lipids, BMI, or age. Overall, the groups of children did not differ in terms of age, gender composition, or pubertal status. However, the potential influence of diet or physical activity status on the outcome variables was not measured. (41)

Araki and colleagues also studied children and reported that plasma visfatin levels were a diagnostic marker for visceral fat accumulation in obese Japanese children. The obese children ($n = 56$; 37 boys, 19 girls) were age-matched, but not pubertal status-matched, with non-obese children ($n = 20$; 12 boys, 8 girls), and no mention was made as to the dietary or physical activity status of the participants. Overall, visfatin levels were higher in the obese children as compared to the non-obese children, but did not vary by gender. In simple correlation analyses, age, height, weight, waist circumference, visceral adipose tissue, subcutaneous adipose tissue, triglycerides, insulin, and HOMA-IR were all significantly and positively

correlated with plasma visfatin levels in the obese children. However, after adjustment for age and gender, only visceral adipose tissue remained significantly correlated with visfatin levels ($r = 0.347$). The researchers also grouped the children based on the presence of metabolic syndrome and found higher plasma visfatin levels in the obese children with metabolic syndrome ($n = 7$) as compared to both the obese children without metabolic syndrome ($n = 49$) and to the non-obese controls ($n = 20$). (7)

Malavazos and colleagues studied epicardial fat in connection with visfatin levels. Forty two obese but otherwise healthy women ($n = 27$ severely obese) and 15 normal weight women had epicardial fat thickness measured via echocardiograph and circulating levels of visfatin, plasminogen activator inhibitor-1 (PAI-1), and other inflammatory markers assessed. The obese women had thicker epicardial fat and higher visfatin and PAI-1 levels than the normal weight women. In addition, visfatin was correlated with epicardial fat thickness, even after adjustment for body fat. Thus, visfatin may be related to epicardial fat thickness, possibly via its role as an inflammatory adipokine. (76)

Korner and colleagues assayed visfatin levels in serum samples stored from a previous study (13) using a new ELISA. They compared 10 subcutaneously obese, 10 viscerally obese, and 10 lean controls and found no significant differences between the groups with regard to serum visfatin levels, although visfatin tended to be higher in the viscerally obese group. The researchers also found no relationship between serum visfatin and BMI, waist circumference, hip circumference, waist-to-hip ratio, body fat, fasting glucose, fasting insulin, glucose infusion rate, 120 minute

OGTT blood glucose, plasma lipids, or visfatin mRNA expression. Moreover, with the exception of BMI (which was shown as a scatterplot), no numerical correlations were presented for any of these measures. It is also important to note that the participants chosen did not differ in terms of glucose tolerance, as there were no differences between the lean and obese groups with regard to fasting glucose or 120 minute OGTT glucose. Thus, this study did not address whether visfatin levels differ in those with varying degrees of glucose tolerance. In addition, the statistical analysis involved t-tests, even though the gender composition of the groups was different (4 females in the lean group, 8 females in the subcutaneous obese group, and 7 females in the visceral obese group). (65)

Garcia-Fuentes and colleagues investigated the change in plasma visfatin concentrations in severely obese patients ($BMI = 54.4 \pm 6.8 \text{ kg/m}^2$) before and after weight loss due to bariatric surgery. Plasma visfatin levels were higher in the severely obese individuals at baseline as compared to healthy individuals ($BMI = 26.8 \pm 3.8 \text{ kg/m}^2$) not undergoing surgery. After grouping the severely obese individuals as normal, impaired, or diabetic based on fasting glucose levels, the researchers found that severely obese individuals in the IFG and T2D groups had significantly higher levels of plasma visfatin than the healthy controls. Seven months after bariatric surgery, plasma visfatin levels were higher in the severely obese individuals as compared to their pre-operative values and as compared to the healthy controls. Moreover, the increase in post-operative visfatin levels was not related to the type of bariatric surgery or to the pre-operative fasting glucose level. However, the post-operative visfatin concentration was significantly correlated to the post-

operative leptin concentration ($r = 0.39$) and waist reduction percentage ($r = -0.36$). No statistically significant correlations were found with glucose or insulin sensitivity. These results suggest that visfatin is increased in severely obese individuals with IFG and T2D and increases after bariatric surgery regardless of surgery type or fasting glucose. A major limitation of this study is that dietary and physical activity habits were not accounted for before or after the intervention, and both could have influenced the 7 month measurements of weight loss, waist circumference, lipids, and adipokines. (37)

Haider and colleagues found that plasma visfatin levels decreased in obese patients 6 months after gastric banding. Thirty one participants (3 males, 28 females) who were overall healthy but had a BMI ≥ 40 kg/m² were studied before and 6 months after bariatric surgery. Their data were compared to those of 14 healthy controls (7 males, 7 females) with a BMI < 25 kg/m². As compared to the healthy controls, visfatin and leptin concentrations were increased in the obese patients before surgery, whereas adiponectin was lower. There were no significant correlations at baseline involving visfatin, although leptin and adiponectin were both positively correlated with HOMA-IR. After surgery, 30 of the 31 patients lost weight. Overall, weight decreased from 133 ± 20 kg to 116 ± 20 kg, and BMI decreased from 46 ± 5 kg/m² to 40 ± 5 kg/m². In addition, plasma visfatin and leptin levels decreased, whereas adiponectin levels increased. In the surgery patients, changes in visfatin were found to correlate positively with changes in BMI ($r = 0.69$) and weight ($r = 0.64$) and negatively with changes in waist circumference ($r = -0.43$), insulin ($r = -0.42$) and HOMA-IR ($r = -0.43$). Moreover, changes in leptin and adiponectin were

not correlated with any of the changes in body composition or glucose and insulin metabolism. Of interest, fasting glucose levels, fasting insulin levels, and HOMA-IR were not statistically changed by the gastric banding. Multiple linear regression analysis indicated that the best predictors of change in visfatin were change in insulin, change in BMI, and change in HOMA-IR. There are several limitations in this study which could have influenced the results including non-assessment of diet, physical activity status, and female menopausal status. In addition, gender and age were not considered as possible influential factors in the statistical analyses. (45)

In contrast, Krzyzanowska and colleagues found that plasma visfatin levels increased in obese patients 14 months after gastroplastic surgery. Thirty six participants (28 females, 8 males) who were overall healthy but had a BMI > 40 kg/m² were studied before and 14 months after gastroplastic surgery with vertical banded gastroplasty. After the surgery, weight decreased from 128 kg to 94 kg, and BMI decreased from 44.3 kg/m² to 31.9 kg/m². Furthermore, fasting insulin, fasting glucose, interleukin-6, CRP, and HOMA-IR decreased, whereas visfatin changes were variable with an increase occurring in most patients. Prior to the surgery, fasting insulin, HOMA-IR, and triglycerides were found to be correlated with visfatin concentration ($r = -0.35$, -0.36 , and -0.33 , respectively). After the surgery, changes in visfatin concentration were negatively correlated with changes in insulin ($r = -0.35$) and HOMA-IR ($r = -0.45$). Moreover, HOMA-IR was an independent predictor of pre-surgery visfatin concentration, but not visfatin concentration after the surgery. One of the many positive aspects of this study was its analysis of diet, which was found to be comparable pre- and post-operation. (69)

Botella-Carretero and colleagues conducted a study that also examined morbidly obese women undergoing bariatric surgery (n = 41; 28 premenopausal, 12 postmenopausal). The researchers reported that the women had increased levels of serum visfatin post-surgery, measured after attainment of weight loss of at least 15% of initial weight (14 ± 9 months post-surgery). Overall, there were no differences in serum visfatin between the different surgery types (biliopancreatic diversion or laparoscopic gastric bypass), between premenopausal and postmenopausal women, and between hyperandrogenic and non-hyperandrogenic premenopausal women. A multiple regression model indicated that weight loss, previous diabetic status, and change in waist circumference were the main predictors of the change in visfatin following bariatric surgery. Although this study did account for the reproductive and diabetic status of the participants, it did not examine diet and physical activity status, and both of these factors could have influenced many of the variables measured. (15)

Manco and colleagues detected a slight decrease in visfatin levels 24 or 36 months (the abstract and methods differ) after bilio-pancreatic diversion in women losing > 20 % of their initial BMI (n = 9), but not in the total study population of women undergoing bilio-pancreatic diversion (n = 10). This study had the most specific subject inclusion criteria, including no medication usage, no alcohol consumption, overall healthiness, and no regular physical activity. The study also required measurement during the same menstrual phases. However, these specifics make the study participants far from representative of the general population. (77)

Varma and colleagues investigated the relationships between visfatin and insulin sensitivity, obesity, intramyocellular lipid levels, and inflammation in 15

subjects undergoing elective abdominal surgery and in 75 healthy subjects willing to undergo muscle and adipose tissue biopsies. Considerable variation existed within the groups; moreover, age (24-62 yrs), BMI (29-76 kg/m²), and type of surgery (gastric bypass, gastric restriction, cholecystectomy, hysterectomy, hernia repair, *etc.*) were wide-ranging in the surgery group and BMI (19-55 kg/m²), percent body fat (15.5-54.1 %), and insulin sensitivity (0.62-26.8 x 10⁻⁵ x min⁻¹/pM) were variable among the biopsy participants. Plasma visfatin levels were not correlated with BMI, insulin sensitivity, or intramyocellular lipid levels. In a subgroup of BMI-matched IGT and NGT women, plasma visfatin was not related to insulin sensitivity. Moreover, the researchers surmised that visfatin plasma levels may not reflect differences in body composition or insulin sensitivity, possibly because adipose tissue is only one source of circulating visfatin. However, diet and physical activity status were not assessed in the groups, and both of these factors could have influenced many of the assessed variables. (119)

Berndt and colleagues found that plasma visfatin concentration correlated positively with visceral visfatin mRNA expression ($r^2 = 0.17$), BMI ($r^2 = 0.04$), percent body fat ($r^2 = 0.04$), and negatively with subcutaneous visceral fat mRNA expression ($r^2 = 0.19$) in 163 men and women with a range of body fat and glucose tolerance. However, the researchers detected no relationship between plasma visfatin concentration and fasting insulin levels, fasting plasma glucose levels, glucose infusion rate during a euglycemic-hyperinsulinemic clamp, 2-hour OGTT plasma glucose, or computed tomography measures of visceral fat mass. Overall, the subjects ranged in age (24 yrs to 86 yrs) and BMI (20.8 kg/m² to 54.1 kg/m²) and

were undergoing abdominal surgery for gastric banding, cholecystectomy, appendectomy, weight reduction surgery, injury, or laparotomy. Although sex, BMI, and WHR subgroups were analyzed separately, age and surgery-type groups were not. In addition, diet, physical activity status, and female menopausal status were not assessed. Thus, these factors could have influenced the results. (13)

Polycystic Ovary Syndrome

Chan and colleagues compared plasma visfatin levels in 26 Chinese women with PCOS and 26 healthy Chinese women. They found that women with PCOS had higher fasting plasma visfatin levels than healthy women, and that plasma visfatin was positively correlated with BMI in the PCOS patients ($r = 0.396$). However, plasma visfatin was not correlated with age, fasting insulin, thyroid hormones, or reproductive hormones in either group. Thus, this study suggests that women with PCOS have higher visfatin levels than healthy women. Although the PCOS and healthy women were similar in age and BMI, and the researchers excluded those with other various metabolic and chronic diseases, the researchers did not assess diet or physical activity status. Differences in either of these variables could have influenced visfatin levels. (19)

Kowalska and colleagues examined visfatin levels in women with PCOS. Seventy (23 lean and 47 obese) women with PCOS were compared to 45 (25 lean and 20 obese) healthy women. The women with PCOS had lower insulin sensitivity and higher serum visfatin levels as compared to the healthy women. However, when compared separately, only the lean PCOS patients were found to have increased visfatin levels. In the entire study group, serum visfatin was correlated with BMI ($r =$

0.23), waist girth ($r = 0.24$), insulin sensitivity ($r = -0.27$), 120 minute glucose ($r = 0.20$), free fatty acid levels ($r = 0.20$), HDL-cholesterol ($r = -0.27$), and numerous gender-related hormone levels. Thus, visfatin levels in lean PCOS patients appear to be related to insulin sensitivity and hyperandrogenism-related variables. Although participants in the study were non-smokers and taking no anti-inflammatory or carbohydrate and lipid affecting medications, diet and physical activity status were not assessed. Moreover, these factors may have influenced the results. (66)

Tan and colleagues compared visfatin levels in 8 women with PCOS to 8 healthy women with unexplained infertility. Visfatin mRNA expression in subcutaneous and omental adipose tissue and in subcutaneous adipocytes was greater in the PCOS patients than in the controls. In addition, visfatin mRNA expression in the PCOS patients was higher in omental fat than in subcutaneous fat. Similar findings were reported for visfatin protein and visfatin plasma levels. Furthermore, plasma visfatin levels were positively correlated with visfatin protein levels, visfatin mRNA levels, insulin, HOMA-IR, testosterone, 17-beta estradiol, and negatively correlated with sex hormone binding globulin in the total study population. However, plasma visfatin was not correlated with BMI or WHR, and visfatin protein levels were not correlated with BMI or WHR. Some of the more important findings from this study indicate that visfatin plasma levels are related to adipose tissue visfatin protein levels and mRNA levels and that visfatin plasma levels are elevated in women with PCOS. Unfortunately, correlation coefficients were not reported for the associated variables in this study, just probability values. In addition, diet and physical activity status were not examined for their potential influence on study

variables. However, medication usage, cigarette smoking, and the presence of additional metabolic and cardiovascular diseases were taken into account as exclusionary criteria. (113)

Chronic Kidney Disease

Axelsson and colleagues found that serum visfatin levels were higher in stage 5 chronic kidney disease (CKD) patients (n = 149) than in stage 3-4 patients (n = 40) or healthy controls (n = 30). Univariate analyses also indicated differences in visfatin levels in patients with versus without diabetes and in patients with versus without cardiovascular disease. Furthermore, visfatin was negatively correlated with glomerular filtration rate (r = -0.22), apolipoprotein B (r = -0.15), and serum albumin (r = -0.28) and positively correlated with IL-6 (r = 0.17), CRP (r = 0.14), and soluble vascular cell adhesion molecule 1 (r = 0.39), but not with fat mass or markers of insulin resistance. Although the researchers did adjust some of their analyses for age, gender, and factors significantly associated with visfatin, and they assessed nutritional status, they did not account for female menopausal status, medication usage differences, or physical activity status. Moreover, these factors could have influenced the outcomes. (9)

Yilmaz and colleagues compared the serum visfatin concentration in 406 non-diabetic patients with different stages of CKD to those of 80 healthy controls. Overall, visfatin levels were increased in all but stages 1 and 2 of CKD. In addition, visfatin was positively correlated with HOMA-IR (r = 0.11) and CRP (r = 0.56) and negatively correlated with estimated glomerular filtration rate (r = -0.62), flow-mediated dilatation (r = -0.53), and nitroglycerine mediated dilatation (r = -0.35).

Interestingly, similar correlations were reported for adiponectin. Thus, visfatin levels may be related to endothelial dysfunction in non-diabetic CKD patients. Although the groups were similar in terms of age, gender composition, and BMI, the researchers did not account for female menopausal status, length of disease, or dietary differences in their analyses. Furthermore, these variables may have influenced several of the outcome measures. (130)

Metabolic Syndrome

Filippatos and colleagues examined whether visfatin levels in obese and overweight individuals with metabolic syndrome varied from obese and overweight individuals without metabolic syndrome. Twenty-eight individuals with metabolic syndrome and 28 age- and gender-matched individuals without metabolic syndrome served as the study population. All participants were free of cardiovascular disease, T2D, impaired renal function, impaired hepatic function, and elevated thyroid stimulating hormone levels. In addition, any lipid-lowering medications were discontinued for at least 6 weeks prior to the start of the study. However, antihypertensive medications were permitted, and there was no mention of or correction for diet, physical activity status, and hormone replacement therapy status differences. Despite these limitations, after adjustment for age, gender, and BMI, the researchers found that overweight and obese individuals with metabolic syndrome had significantly higher plasma visfatin levels than individuals without metabolic syndrome. Significant correlations were found for plasma visfatin and age ($r = 0.32$), waist circumference ($r = 0.31$), triglyceride ($r = 0.59$), glucose ($r = 0.33$), and HDL-

cholesterol ($r = -0.38$). This work suggests that plasma visfatin levels are elevated in those with the metabolic syndrome. (32)

Chen and colleagues investigated the relationships between visfatin and parameters of metabolic syndrome. Study participants were 244 males and 256 females from a community-based metabolic syndrome study in Taiwan. Overall, there was no significant difference in visfatin levels between males and females. Furthermore, in males, the researchers found no significant relationships between visfatin and age, waist circumference, hip circumference, WHR, blood pressure, fasting plasma glucose, fasting serum insulin, HOMA-IR, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, total cholesterol to HDL-cholesterol ratio, and uric acid. However, visfatin was negatively and significantly correlated with BMI ($r = -0.128$). Results were slightly different in women; moreover, total cholesterol ($r = -0.132$), HDL-cholesterol ($r = 0.170$), LDL-cholesterol ($r = -0.146$), and the total cholesterol to HDL-cholesterol ratio ($r = -0.260$) were found to be statistically correlated with visfatin. The correlations remained significant after adjustment for waist circumference and after adjustment for age plus waist circumference. However, this cross-sectional study did not take into account potential confounding variables such as diet and physical activity status. Moreover, the reported age range of the participants was 39.89 to 85.17 yrs and menopausal status and hormone replacement therapy use were not reported for the female participants, which could have confounded some of the results. (21)

Zhong and colleagues compared visfatin levels in 139 metabolic syndrome patients and 105 healthy, age- and sex-matched controls. In addition, they further

characterized the metabolic syndrome cohort as to the presence or absence of carotid plaques. Regardless of carotid plaque status, serum visfatin levels were higher in those with metabolic syndrome than in the healthy controls. In addition, visfatin levels were higher in metabolic syndrome patients with carotid plaques as compared to those without carotid plaques. However, within the metabolic syndrome group, there were no differences in visfatin levels between men and women or between those with 3 versus 4 metabolic syndrome components. Last, visfatin was correlated with total cholesterol ($r = 0.192$) and LDL-cholesterol ($r = 0.219$) in the metabolic syndrome cohort, and remained associated with LDL-cholesterol in multiple regression analysis. Despite the significant findings, this study did not account for potential differences in diet, physical activity, or female menopausal status among the participants. Moreover, these factors may have influenced several of the metabolic and outcome variables. (133)

Cholesterol, Hypertension, & Coronary Heart Disease

Wang and colleagues assessed plasma visfatin levels in relation to adiposity, insulin resistance, and lipids in 40 non-diabetic Caucasian males ($n = 21$) and females ($n = 19$) from the familial combined hyperlipidemia (FCHL) spouses databank versus 35 non-diabetic Caucasian male ($n = 15$) and female ($n = 20$) FCHL family members. Visfatin did not differ between the groups. It also did not differ by gender. Furthermore, in both groups and in the entire study population, visfatin was not related to BMI, percent body fat, subcutaneous fat, total cholesterol, LDL-cholesterol, or ApoB. However, visfatin was negatively correlated with visceral fat size in the FCHL spouses group ($r = -0.345$) and in the total study population ($r = -0.269$), with

HOMA-IR score in the total study population ($r = -0.245$), with triglyceride levels in all groups ($r = -0.402, -0.465, \text{ and } -0.408$, respectively) and positively correlated with HDL-cholesterol in all groups ($r = 0.469, 0.403, \text{ and } 0.439$, respectively). Multiple linear regression modeling determined that HDL-cholesterol and group identity were predictors of plasma visfatin. Thus, this work suggests a possible connection between visfatin and lipid metabolism. Limitations of this study include its cross-sectional nature, lack of dietary control, lack of information regarding hormone replacement therapy status in women, and lack of information regarding physical activity status. These factors could significantly influence lipid levels and potentially visfatin levels. (120)

Dogru and colleagues reported that plasma visfatin levels did not differ ($p = 0.06$) in young, newly diagnosed, male hypertensive patients ($n = 33$) as compared to healthy males ($n = 33$) (30). In addition, plasma visfatin levels were not correlated with blood pressure, BMI, lipid levels, fasting glucose, fasting insulin, CRP, or HOMA-IR in either group of males. This study was cross-sectional in nature with the diagnosis of hypertension preceding the measurement of visfatin. In addition, diet and physical activity status were not examined in this study, which may have influenced the results. (30)

Choi and colleagues compared visfatin levels in 49 patients with coronary heart disease (CHD) to 42 age- and gender-matched, healthy controls. The researchers reported no difference in visfatin between the groups; however, lipocalin-2 levels were higher in the CHD patients. Furthermore, there were no significant correlations reported between visfatin and age, height, weight, waist circumference,

systolic blood pressure, diastolic blood pressure, HDL-cholesterol, triglycerides, fasting glucose, fasting insulin, or HOMA-IR. Thus, this study suggests that visfatin may not be related to metabolic traits. However, the authors failed to account for potential subject differences in diet, physical activity, and female menopausal status, which may have affected several of the metabolic and outcome variables. (26)

Inflammation, Arthritis, & Bone Turnover

Moschen and colleagues compared visfatin levels in human inflammatory conditions to test whether their *in vitro* proinflammatory results applied *in vivo*. They found that patients with ulcerative colitis or inflammatory bowel disease had higher visfatin serum levels than those of healthy controls. Thus, *in vitro* and *in vivo* evidence suggests that visfatin is a proinflammatory adipokine. (79)

Oki and colleagues investigated the associations between serum visfatin and markers of systemic inflammation (IL-6 and CRP) and insulin resistance (HOMA-IR) in Japanese Americans (126 men, 169 women) living in Hawaii. The participants were part of an epidemiological study started in 2002 which looked at risk factors for diabetes, hypertension, and atherosclerosis. The average age of the participants was 68.7 ± 14.9 years, and no additional demographic information was provided in the paper. The researchers found positive correlations between visfatin and IL-6 and CRP. Furthermore, visfatin was significantly correlated with IL-6 in both men ($r = 0.303$) and women ($r = 0.265$), but with CRP only in men ($r = 0.232$). After adjusting for age, gender, BMI, percent body fat, and waist girth, visfatin was still correlated with IL-6 and CRP. No significant trend was found for tertiles of visfatin and HOMA-IR. This work suggests that visfatin may be related to inflammation status.

However, diet and physical activity status were not taken into account in this study and neither was hormone replacement therapy status. These factors may have influenced measurement of visfatin and the markers of inflammation and insulin resistance. (86)

Otero and colleagues examined adipokine levels, including visfatin, in rheumatoid arthritis patients ($n = 31$) as compared to healthy people ($n = 18$). Higher plasma levels of adiponectin, leptin, and visfatin were detected in the rheumatoid arthritis patients as compared to the healthy controls, but no difference was found for resistin levels. Furthermore, CRP was significantly correlated with adiponectin, leptin, and visfatin ($r = 0.59$, $r = 0.71$, and $r = 0.63$, respectively), but not with resistin. This suggests that adipokines, including visfatin, may influence inflammation in rheumatoid arthritis patients. However, this study did not examine whether the type of medication the patients were taking influenced the measurement outcomes. It also did not mention the sampling conditions for measurement of the adipokines. Other factors that were not examined in the study which could also have influenced the results include diet, physical activity status, and menopausal status in the females. (87)

Brentano and colleagues examined visfatin's relationship with rheumatoid arthritis using synovial fibroblasts, monocytes, synovial fluid, and serum. They found that administration of rheumatoid arthritis-related cytokines to fibroblasts increased visfatin expression, whereas visfatin administration activated cytokines and matrix metalloproteinases (MMP). Synovial fluid and serum visfatin levels were also elevated in rheumatoid arthritis patients as compared to osteoarthritis patients and

correlated with CRP levels ($r = 0.92$ and 0.73 , respectively) and disease severity ($r = 0.73$ and $r = 0.74$, respectively). Thus, visfatin may be a proinflammatory factor in rheumatoid arthritis. (18)

Peng and colleagues examined relationships between adipocytokines, including visfatin, and bone mineral density and bone turnover markers in 232 healthy Chinese men aged 20-80 years. They found no relationship between serum visfatin and fat mass, bone mineral density, or bone turnover markers in the men (adjusted for age and BMI). The study had strict inclusion/exclusion criteria, resulting in a population with very little variability in terms of body composition. The other major variables also may not have varied enough for relationships to be detected, but means were not provided for any of the bone-related variables to assess this. Furthermore, diet could impact body composition and bone-related variables. However, dietary information was not examined in the study. Interestingly, smoking status was not an exclusionary factor in the study, but was instead used as a potential predictor of bone mineral density in the multivariate analyses. Last, on a positive note, habitual physical activity level was recorded and used as a potential predictor in the multivariate analyses. (89)

Medication Treatment

Hsieh and colleagues reported that neither slow-release nor regular-form metformin treatment altered insulin resistance, adipokine levels (including visfatin), or inflammatory markers in individuals with T2D taking part in a 12 week, randomized, double-blind, parallel, active medication-control study (51).

Hammarstedt and colleagues found that circulating levels of visfatin did not change in response to 3 weeks of thiazolidinedione treatment in newly diagnosed, untreated T2D patients (n = 2 males, n = 5 females) or in non-diabetic males (n = 6) with reduced insulin sensitivity. Unfortunately, diet, physical activity status, female menopausal status, and the presence of co-morbidities were not assessed in this study, and these factors may have affected the results. (48)

Pfutzner and colleagues assessed whether 3 months of pioglitazone, simvastatin, or combined pioglitazone and simvastatin treatment affected adiponectin and visfatin levels in non-diabetic patients with metabolic syndrome. Seventy eight females and 47 males took part in the prospective, randomized clinical trial. Although pioglitazone and the combined treatment improved insulin sensitivity and increased adiponectin levels, simvastatin treatment decreased adiponectin levels. In contrast, visfatin levels did not change with any of the treatments. After adjusting for differences in insulin levels, glucose levels, and BMI, the results did not change. Furthermore, baseline visfatin levels were not correlated with fasting glucose or insulin levels and the change in visfatin levels was not correlated with the change in HOMA-IR. Thus, visfatin levels are not influenced by pioglitazone or simvastatin treatment. However, the researchers did not report female menopausal status, hormone replacement therapy usage, physical activity status, or diet, and these factors may have influenced several of the outcome variables. (90)

Last, Haider and colleagues gave 16 healthy young males thiazolidinedione treatment for 3 weeks and found that their plasma visfatin levels increased (42).

Other Interventions

Haider and colleagues found that plasma visfatin concentrations increased in response to glucose infusion in healthy men ($n = 9$). Glucose concentrations of both 8.3 and 11.2 mmol/L were found to significantly increase baseline levels of visfatin. Moreover, insulin co-infusion prevented the increase in visfatin concentration. Similar increases in visfatin were found in subcutaneous and visceral adipocytes exposed to glucose *in vitro*. (44)

Sun and colleagues found baseline serum visfatin levels to be positively correlated with baseline serum triglycerides ($r = 0.36$) in healthy young men ($n = 61$). Furthermore, after short-term overfeeding, serum visfatin decreased by an average of 19 % in the men. Diet and physical activity levels were both assessed in this study; moreover, physical activity levels could not vary by more than 15 % from baseline during the overfeeding component of the study. In addition, participants had to be healthy, weight stable for 6 months, and not taking lipid lowering medication. (110)

Gender, Age, & Race

Seo and colleagues found that visfatin levels correlated positively with percent body fat ($r = 0.206$), IL-6 ($r = 0.269$), CRP ($r = 0.233$), triglycerides ($r = 0.192$), total cholesterol ($r = 0.199$), and diastolic blood pressure ($r = 0.273$) in non-diabetic Korean women. However, none of these variables were significantly associated with visfatin levels in Korean men. In multiple stepwise regression analyses, only diastolic blood pressure and IL-6 were related to visfatin levels in Korean women. However, female menopausal status, diet, and physical activity status were not

reported, and these factors could have affected measurement of the outcome variables. (106)

Kanda and colleagues assessed plasma visfatin levels in 154 bedridden geriatric and nursing home patients (n = 39 males, n = 115 females) from Osaka, Japan. The average age of the participants was 80.3 ± 13.6 yrs, and the average bed confinement period was 45.5 ± 40.3 months. A variety of clinical characteristics were assessed for characterization of potential relationships with plasma visfatin, including blood pressure, total cholesterol, adiponectin, and health complications (pneumonia, asthma, diabetes, dementia, renal failure, *etc.*). The researchers found that plasma visfatin levels were associated with diastolic blood pressure, adiponectin, and CRP in the elderly subjects. No other clinical parameters were associated with visfatin, thus the results suggest that visfatin may be a marker of inflammation in elderly bedridden patients. However, only univariate analyses were used to assess relationships between the variables, and information such as means and counts were not provided for any of the clinical characteristics that were analyzed. As the results were presented in the form of a letter, detailed information regarding other important information such as medication usage was also not provided. (57)

Smith and colleagues compared serum visfatin levels in Asian Indians and Caucasians living in Canada. They detected no differences in visfatin levels between the races, although the difference between Asian Indian and Caucasian women neared significance ($p = 0.07$). In the Asian Indian men, serum visfatin was positively correlated with HDL-cholesterol ($r = 0.41$) and apo A1 ($r = 0.41$). Similar results were reported for the Asian Indian women ($r = 0.46$ and 0.61 , respectively), who also

had significant correlations with percent body fat ($r = 0.58$) and BMI ($r = 0.52$). However, there were no significant correlations involving visfatin in the Caucasian men and women. This study did not take any of the significant differences between the groups (including smoking status and body composition) into account in the analyses. In addition, factors like diet, physical activity status, and female menopausal status were also not considered. Moreover, these factors could have influenced many of the outcome measures. (107)

Summary: Circulating Visfatin Studies

Tables 1-1 and 1-2 (pages 12 and 13) summarize the results of studies comparing circulating visfatin levels in disease conditions. Although the results have not been entirely consistent, most studies have found increased circulating levels of visfatin in T1D (43; 74), T2D (22; 29; 31; 74; 94; 104), GDM (68; 72), obesity (7; 25; 37; 41; 45; 56; 76; 131), PCOS (19; 66; 113), CKD (9; 130), and metabolic syndrome (7; 32; 133) patients as compared to healthy or less-diseased individuals.

As detailed in Table 6-1, the majority of studies investigating changes in visfatin levels with various diabetes treatments, in response to OGTTs, and in response to weight loss surgery have found no statistical differences or no consistent differences. In addition, no differences in visfatin levels were reported in studies comparing men and women (13; 21; 88; 112; 120) or Asian Indians and Caucasians (107).

As shown in Table 1-3 (page 14), several studies have also found significant correlations between circulating visfatin and glucose and obesity-related variables including fasting insulin (69; 72; 74; 113; 131), insulin sensitivity [assessed via

homeostasis model assessment-insulin resistance; HOMA-IR; (69; 113; 120)], fasting glucose (32; 131) visceral fat (7; 120), body mass index [BMI; (13; 19; 22; 73; 88)], and triglyceride levels (32; 69; 120). There have also been correlations reported between the surgical weight loss-related change in visfatin levels and the change in fasting insulin (45; 69) and insulin sensitivity (45; 69). However, these relationships have not always been consistent (Table 1-3).

Part of the disparity in the results may be due to a lack of dietary and/or physical activity control in the studies. Moreover, only 10 studies assessing visfatin levels also mentioned some sort of subject diet and/or physical activity status criteria; moreover, 5 of these studies included diet information (17; 40; 56; 69; 88), 4 included physical activity information (25; 43; 77; 89), and 1 study included both diet and physical activity information (110). In addition, few of the studies involving women reported menopausal status or hormone replacement therapy usage, either of which could also have influenced many of the variables. Thus, studies assessing visfatin levels that account for diet, physical activity status, and menopausal status would be useful.

Taken together, the results of plasma and serum visfatin studies support a connection to glucose and obesity-related phenotypes.

Table 6-1. Circulating visfatin in intervention studies.

	Result	Comparison	Sample	Source
Medication	NC	12 wks pioglitazone in T2D vs baseline	plasma	(112)
	NC	T2D after 12 wks insulin vs baseline	plasma	(112)
	NC	T2D after metformin vs baseline	serum	(51)
	NC	T2D w/4 wks pioglitazone vs baseline	plasma?	(48)
	ND	T2D w/hypertension medication vs w/o Pioglitazone, simvastatin, or combined therapy vs baseline	plasma	(112)
	NC	NGT w/3 wks pioglitazone vs baseline	?	(90)
	NC	NGT w/3 wks rosiglitazone vs baseline	plasma?	(48)
	↑	NGT w/3 wks rosiglitazone vs baseline	plasma	(42)
OGTT	↓	T2D vs NGT	plasma	(73)
	NC	T2D, IGT, NGT 2-hr values vs baseline	plasma	(73)
	↓	GDM vs healthy pregnant	plasma	(40)
Weight loss	↑	Severely obese after bariatric surgery vs healthy	plasma	(37)
	↓	Obese after bariatric surgery vs baseline	plasma	(45)
	↑	Severely obese after bariatric surgery vs baseline	plasma	(37)
	↑	Severely obese after bariatric surgery vs baseline	serum	(15)
	↑	Obese after gastroplastic surgery vs baseline	plasma?	(69)
	↓	After bilio-pancreatic diversion in women losing > 20 % BMI vs baseline	serum?	(77)
	Exercise	NC	Healthy w/acute exercise vs baseline	plasma
↓		Healthy w/12 wks training vs baseline	plasma	(25)
↓		T1D w/2 months training vs baseline	plasma	(43)
↓		T1D w/4 months training vs baseline	plasma	(43)
↓		T2D w/12 wks training vs baseline	plasma	(17)
↓		Obese w/12wks training vs baseline	plasma	(17)
Other	↑	Glucose infusion vs baseline	plasma	(42)
	NC	FFA-induced IR in healthy vs baseline	plasma	(88)
	NC	Lipid infusion vs baseline	plasma	(42)
	↓	Short term overfeeding vs baseline	serum	(110)

OGTT, oral glucose tolerance test; T2D, type 2 diabetes; wks, weeks; vs, versus; w/, with; w/o, without; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; GDM, gestational diabetes mellitus; FFA, free fatty acid; IR, insulin resistance; NC, no change; ND, no difference; ↑, increased; ↓, decreased; ?, not clearly stated.

Visfatin mRNA & Gene Expression Studies

In contrast to Fukuhara's rodent visfatin mRNA expression studies (described earlier) (35), Kloting and Kloting found no differences in visfatin gene expression in adipocytes from WOKW rats, a model of metabolic syndrome, as compared to control strains of rats (62).

Pagano and colleagues investigated visfatin mRNA expression in 30 normal weight and 39 obese men and women. Subcutaneous adipose tissue visfatin mRNA expression was reduced in obese subjects as compared to normal weight controls; however, the visceral adipose tissue of obese participants had higher visfatin mRNA expression than the healthy controls. Furthermore, BMI was positively correlated with visceral adipose tissue visfatin mRNA expression ($r^2 = 0.35$). Overall, the subjects had an unlimited diet and were recruited from the hospital staff or outpatients. However, this study did not address physical activity status, female menopausal status, or medication usage as potential influential factors. (88)

Varma and colleagues investigated the relationships between visfatin and insulin sensitivity, obesity, intramyocellular lipid levels, and inflammation in 15 subjects undergoing elective abdominal surgery and in 75 healthy subjects willing to undergo muscle and adipose tissue biopsies. Substantial variation existed within the groups, with age (24-62 yrs), BMI (29-76 kg/m²), and type of surgery (gastric bypass, gastric restriction, cholecystectomy, hysterectomy, hernia repair, *etc.*) ranging in the surgery group and BMI (19-55 kg/m²), percent body fat (15.5-54.1 %), and insulin sensitivity ($0.62-26.8 \times 10^{-5} \times \text{min}^{-1}/\text{pM}$) variable among the biopsy participants. Overall, no significant differences were found in visceral and subcutaneous adipose

tissue visfatin mRNA expression, although visceral visfatin mRNA expression was positively associated with BMI ($r = 0.75$), whereas subcutaneous visfatin mRNA expression was negatively associated with BMI ($r = -0.48$). Furthermore, subcutaneous visfatin mRNA expression correlated with insulin sensitivity ($r = 0.50$), type I and type II intramyocellular lipid levels ($r = -0.58$ and -0.66 , respectively), and markers of inflammation, such as tumor necrosis factor- α ($r = -0.62$). In contrast, skeletal muscle visfatin mRNA expression was not correlated with BMI, insulin sensitivity, or intramyocellular lipid levels. In a subgroup of BMI-matched IGT and NGT women, visfatin expression was found to be lower in the IGT participants. These findings suggest that visfatin may play a role in obesity and insulin resistance. However, diet and physical activity status were not assessed in the groups, and both of these factors could have influenced many of the assessed variables. (119)

Berndt and colleagues found that plasma visfatin concentration correlated positively with visceral visfatin mRNA expression ($r^2 = 0.17$) and negatively with subcutaneous visfatin mRNA expression ($r^2 = 0.19$) in 163 men and women with a range of body fat and glucose tolerance. Furthermore, in 189 participants there were no differences in visfatin gene expression between visceral and subcutaneous adipose tissue; however, visceral visfatin gene expression was positively correlated with BMI ($r^2 = 0.09$) and percent body fat ($r^2 = 0.06$) in women. Overall, this study was a retrospective cross-sectional study involving subjects who were undergoing abdominal surgery for gastric banding, cholecystectomy, appendectomy, weight reduction surgery, injury, or laparotomy. Besides the aforementioned metabolic and potential disease etiology differences, the participants also ranged in age (24-86 yrs).

Moreover, sex, BMI, and WHR subgroups were analyzed separately, but age and surgery-type groups were not. In addition, diet, physical activity status, and female menopausal status were not assessed. Thus, these factors could also have influenced the results. (13)

Tsiotra and colleagues compared visfatin expression in peripheral monocyte-enhanced mononuclear cells from 24 women with T2D and 26 healthy women. Both groups of women were further subdivided by BMI into lean or overweight, thus there were 4 subject groups. All women were pre-menopausal and aged 21-48 years. Visfatin mRNA levels were found to be almost 3-fold higher in both the lean and overweight T2D patients than in the healthy women. Thus, this study suggests that mRNA expression of visfatin is elevated in mononuclear cells from diabetic women. However, there were a few problematic aspects involving this study which may have impacted the results. First, differing treatments were being used to treat T2D (15 were on special diets, 5 were on oral hypoglycemic agents, and 2 were on combined insulin and hypoglycemic therapy), and these were not examined as potential confounding variables. In addition, the duration of diabetes, the physical activity status, and the average age for each of the groups were not provided and were not examined as potential confounding variables in the analyses. Last, beyond fasting, there was no mention of the blood drawing conditions. Thus, the time course of medication usage in the T2D participants was not provided. (116)

Poulain-Godefroy and colleagues examined the expression of inflammation and adipocyte differentiation-related genes in omental and subcutaneous adipose tissue samples from 6 lean women and 18 obese women undergoing abdominal

surgery. Furthermore, the obese women were having bariatric surgery, whereas the lean women were having surgery for benign diseases. The obese group was further subdivided by glucose tolerance status into normoglycemic, IGT, or T2D groups for analysis. Overall, there were no differences in PBEF1 expression in omental versus subcutaneous fat or between any of the groups. (92)

Tan and colleagues compared visfatin levels in 8 women with PCOS to 8 healthy women with unexplained infertility. They looked at the mRNA expression of visfatin in subcutaneous and omental adipose tissue and subcutaneous adipocytes, visfatin protein levels in adipose tissue, and visfatin plasma levels. Visfatin mRNA expression in subcutaneous and omental adipose tissue and in subcutaneous adipocytes was greater in the PCOS patients than in the controls. In addition, visfatin mRNA expression in the PCOS patients was higher in omental fat than in subcutaneous fat. Furthermore, subcutaneous and omental fat visfatin mRNA levels were correlated positively with BMI and WHR, and they were also correlated with each other. Last, visfatin mRNA levels did not correlate with visfatin protein levels in either subcutaneous or omental fat. Some of the more important findings from this study indicate that visfatin plasma levels are related to adipose tissue visfatin protein levels and mRNA levels and that visfatin mRNA, protein, and plasma levels are elevated in women with PCOS. Unfortunately, correlation coefficients were not reported for the associated variables in this study, just probability values. In addition, diet and physical activity status were not examined for their potential influence on the study variables. However, medication usage, cigarette smoking, and the presence of

additional metabolic and cardiovascular diseases were taken into account as exclusionary criteria. (113)

In contrast to the plasma results of Haider and colleagues (42), Hammarstedt and colleagues found that visfatin mRNA expression did not increase in response to 3 weeks of thiazolidinedione (TZD) treatment in newly diagnosed, untreated T2D patients (n = 2 males, n = 5 females) or in non-diabetic males (n = 6) with reduced insulin sensitivity (48). They did however confirm visfatin's role as an adipokine, with mRNA expression in both adipose tissue and isolated adipocytes. Unfortunately, diet, physical activity status, female menopausal status, and the presence of co-morbidities were not assessed in this study, and these factors may have affected the results. (48)

Using microarray, Dahl and colleagues found that visfatin gene expression was increased ~ 2-3 fold in carotid plaques from symptomatic (n = 14) as compared to asymptomatic (n = 7) endarterectomy patients. Moreover, they confirmed their results by finding increased visfatin mRNA levels and increased visfatin protein levels in samples from the symptomatic patients. The researchers also localized visfatin to the plaque's lipid-rich core in symptomatic patients and to lipid-rich regions of the lesion in asymptomatic patients. The group also examined visfatin expression during plaque rupture in coronary artery disease patients and found results similar to those of the endarterectomy patients. Visfatin gene expression was also found to be significantly increased in the THP-1 monocytic cell line following stimulation with or 6 and 24 hour incubation with oxidized LDL, tumor necrosis factor- α (TNF- α), or a combination of the two. Lastly, addition of recombinant

human visfatin to the THP-1 monocytes and to peripheral blood mononuclear cells was found to significantly increase levels of matrix metalloproteinase-9 in the monocytes and TNF- α and IL-8 in the peripheral blood mononuclear cells, effects that were abolished with the addition of an inhibitor of insulin receptor signaling. This work supports a role of visfatin as an inflammatory mediator. (27)

Building on the work of Dahl *et al.*, Adya and colleagues examined whether visfatin could activate nuclear factor- κ B (NF- κ B). In human vascular endothelial cells transfected with a plasmid containing 5 NF- κ B binding sites connected to a luciferase reporter gene, visfatin increased the transcriptional activity of NF- κ B. Addition of a NF- κ B inhibitor reduced visfatin's induction of MMP-2 and MMP-9 mRNA, protein levels, and activity. Thus, visfatin may be involved in the process of vascular inflammation. (4)

Ognjanovic and colleagues found that visfatin mRNA levels were increased in fetal membranes from patients with severe infection-related pre-term labor as compared to controls. Treatment of amniotic epithelial cells with various cytokines, including IL-6, TNF- α , and IL-1 β , was also found to increase PBEF expression. Thus, these lines of evidence suggest cytokine-like properties for visfatin. (85)

Hufton and colleagues reported that the visfatin gene was one of the genes overexpressed in primary colorectal cancer (52). Koczan and colleagues found similar results in psoriasis patients (63). Turpaev and colleagues found similar results in nitric-oxide exposed monocytic cells (117).

Kralisch and colleagues examined the hormonal regulation of visfatin in 3T3-L1 adipocytes. As compared with *in vivo* epididymal fat, visfatin mRNA expression

in vitro was increased 6-fold in 3T3-L1 adipocytes and 3-fold during adipogenesis. Kralisch *et al.* also found that hormones known to induce insulin resistance *in vitro* and *in vivo* affected visfatin levels. Moreover, dexamethasone treatment upregulated visfatin mRNA expression, whereas growth hormone, TNF- α , and isoproterenol treatments downregulated visfatin mRNA expression. Insulin did not affect visfatin mRNA expression. These results suggest that the differential hormonal regulation of visfatin may influence the pathogenesis of obesity and insulin resistance. (67)

MacLaren and colleagues investigated factors that regulated visfatin mRNA expression in adipocytes and pre-adipocytes. Visfatin mRNA expression increased during 3T3-L1 adipocyte differentiation and during dexamethasone treatment in pre-adipocytes and adipocytes. In contrast, treatment of pre-adipocytes with insulin, progesterone, testosterone, palmitate, and oleate decreased mRNA expression. In adipocytes, insulin, TNF- α , and TZD treatment decreased mRNA expression. Thus, multiple hormones regulate visfatin mRNA expression in adipocytes and pre-adipocytes, which suggests a physiological role for visfatin. (75)

Wen and colleagues determined the effects of oleate [monounsaturated free fatty acid (FFA)] and palmitate (saturated FFA) on adipocyte visfatin mRNA expression and glucose transport in an attempt to examine the role of visfatin in FFA-induced insulin resistance. Cultured 3T3-L1 adipocytes and pre-adipocytes were incubated overnight with 0.125, 0.5, or 1.0 mmol/L of either oleate or palmitate. Basal glucose transport was not affected by oleate or palmitate, but insulin-stimulated glucose transport was inhibited in both cases. Furthermore, the inhibition was dose-dependent. Visfatin mRNA expression increased more than 1.5-fold during adipocyte

differentiation, but was significantly decreased with oleate or palmitate. Again, this decrease was dose-dependent. The researchers concluded that FFA-induced glucose transport/insulin sensitivity reductions in adipocytes may be a consequence of oleate's and palmitate's ability to decrease visfatin mRNA expression. (123)

Hector and colleagues incubated visceral adipose tissue from lean non-diabetic patients undergoing abdominal surgery with TNF- α for 24, 48, and 72 hours. They reported a significant reduction in the mRNA expression (97 %, 91 %, and 96 %, respectively) and protein expression (42 %, 28 %, and 39 %, respectively) of adiponectin (n = 4), whereas the mRNA level of visfatin (n = 6) increased dramatically (255 %, 335 %, and 341 %, respectively). This suggests that the increased expression of TNF- α , common in obesity and insulin resistance, may contribute to the increased levels of visfatin often reported in conditions of obesity and/or insulin resistance. (49)

Choi and colleagues examined the effects of peroxisome proliferator activated receptor (PPAR)- α and PPAR- γ agonists (fenofibrate and rosiglitazone, respectively) on visfatin expression in male Otsuka Long-Evans Tokushima fatty (OLETF) rats during the early to advanced diabetic stages. As compared to rats without treatment, rats receiving the fenofibrate or rosiglitazone treatment had lower glucose and insulin levels and higher visceral fat visfatin mRNA expression. Thus, PPAR- α and PPAR- γ agonists improve glucose homeostasis in OLETF rats, results which may be related to the PPAR- α and PPAR- γ agonist-induced increases in visfatin mRNA expression. (24)

Choi and colleagues examined whether a PPAR- δ agonist affected adipokine gene expression levels in rat visceral adipose tissue and in cultured adipocytes, since activation of PPAR- δ has been shown to improve metabolic derangements in animal models. Although body weight was similar at the start of the study (6 weeks of age in the rats), rats fed a high-fat diet plus a PPAR- δ agonist for 4 weeks gained significantly less weight than rats fed a high-fat diet for 4 weeks. In addition, visceral adipose tissue from rats fed both the high fat diet and PPAR- δ agonist had significantly greater mRNA levels of visfatin, adiponectin, and PPAR- δ as compared to rats not receiving the agonist. Similar results were found in cultured 3T3-L1 adipocytes having PPAR- δ agonist added to their differentiation medium. Thus, the addition of a PPAR- δ agonist helped to prevent weight gain in rats fed a high fat diet and increased the gene expression of adipokines, including visfatin, in rats and cultured adipocytes. (23)

Yang and colleagues found that cell stress and nutrient restriction increased visfatin/Nampt expression. Furthermore, visfatin/Nampt levels were 1.5-fold to 2-fold higher than controls in human fibrosarcoma HT1080 cells exposed to serum-free media, livers from rats fasted for 48 hours, cardiomyocytes exposed to hypoxia, and cardiomyocytes exposed to serum-free media. The researchers also found that visfatin/Nampt protected against genotoxic stress-induced cell death since cells overexpressing it were more resistant to methylmethane sulfonate, and cells with less of it were more sensitive to methylmethane sulfonate. Thus, this work suggests that visfatin/Nampt may have an important function in cell survival. (128)

Summary: Visfatin mRNA & Gene Expression Studies

Overall, the results of these studies suggest that no differences exist in visfatin mRNA expression in visceral and subcutaneous fat depots (13; 119), although this may not be the case in obese individuals (88), individuals with altered glucose tolerance (116; 119), or PCOS patients (113). Moreover, visceral visfatin mRNA expression has been positively correlated with BMI (13; 88; 113; 119), percent body fat (13), and plasma visfatin (13), whereas subcutaneous visfatin mRNA expression has been negatively correlated with markers of inflammation (119) and plasma visfatin (13) and positively correlated with insulin sensitivity (119). Lastly, visfatin mRNA expression has been found to increase during adipogenesis (67; 75) and to be influenced by hormones and other factors known to influence insulin sensitivity (23; 24; 49; 67; 75; 123). Thus, the results of visfatin mRNA studies support a connection to glucose and obesity-related phenotypes.

Visfatin Polymorphism Studies

The human visfatin protein reportedly shares over 95 % sequence homology with rat and mice visfatin (11). Besides its high degree of conservation, an important functional role for visfatin may be inferred from the finding that visfatin gene homozygous knockout mice die during embryonic development (35).

Ognjanovic and colleagues reported on the organization of the visfatin gene (PBEF1) and found that it had 11 exons, 10 introns, putative regulatory elements, and no sequence homology with any known cytokines (85). Furthermore, its transcription factor binding sites were suggested to include locations for Sp1, activator protein-1 (AP-1), activator protein-2 (AP-2), glucocorticoid responsive elements, corticotropin

releasing factor, cyclic adenosine monophosphate (cAMP) response element binding protein, nuclear factors (NF-1, NF-IL6, and NF- κ B), hepatic nuclear factors, and liver factor-1 (85).

PBEF1 is located on chromosome 7q22. This chromosomal region has previously been reported to have linkage with the insulin response to aerobic exercise training in Caucasians (70), insulin resistance syndrome phenotypes in Mexican-Americans (8), and BMI in the National Heart, Lung, and Blood Institute's Family Blood Pressure Program (125). Thus, based on its chromosomal location, the visfatin gene may be a candidate for glucose and obesity-related phenotypes.

Over 52 single nucleotide polymorphisms (SNPs) have been reported in PBEF1, with several studies conducting genotype and haplotype association studies. This brief review will focus on polymorphisms and haplotypes with reported frequencies > 0.05 .

PBEF1 Promoter Polymorphisms

-4689 G>T (rs2110385), -4191 A>G (rs1737358), & -3186 C>T (rs11977021)

Bailey and colleagues examined the -4689 G>T, -4191 A>G, and -3186 C>T polymorphisms with obesity and glucose-related phenotypes in participants from the Quebec Family Study. Overall, no associations were significant at the $p < 0.05$ level for the -4689 and -4191 polymorphisms, although fat mass ($p = 0.062$), total apoB ($p = 0.067$), LDL apoB ($p = 0.074$), and LDL cholesterol ($p = 0.092$) neared significance for the -4689 polymorphism. In contrast, total cholesterol ($p = 0.03$) and LDL cholesterol ($p = 0.049$) were significantly associated with the -3186 polymorphism, assuming an additive model of inheritance. The frequencies of the minor alleles were

reported as 0.41, 0.42, and 0.17, respectively. Positive features of this study include its large sample size (n = 136 male founders, n = 173 female founders, n = 261 male nonfounders, n = 348 female nonfounders) and outcome measurements. In addition, the analyses accounted for the influence of age, gender, relatedness (identity by descent probabilities), and mode of inheritance. However, there was no mention of menopausal status for the female participants or medication usage, diet, or physical activity status for the entire group of participants. Moreover, these factors could have influenced the obesity and glucose-related outcomes. (11)

Concerning function, promoter region polymorphisms can conceivably alter transcription factor activity and visfatin gene expression. Although no information was provided for the -4689 G>T or -4191 A>G polymorphisms, the -3186 C>T polymorphism is reportedly located in a nuclear hormone response element binding sequence (11).

-2657 G>A

In single SNP analyses, Zhang and colleagues found that the -2657 G>A polymorphism was not significantly associated with T2D. Their study compared 2 different sets of T2D patients [n = 479 (cardiovascular complications of diabetes study) and n = 335 (randomly selected Joslin Clinic patients)] with non-diabetic controls (n = 320; unrelated non-diabetic spouses or parents of T1D patients), all recruited from the Boston, MA area by the Joslin Clinic. Unfortunately, no subject characteristic data pertaining to co-morbidities, medication usage, diet, physical activity status, or female menopausal status was provided. Furthermore, all of the alleles studied in single SNP analyses by Zhang and colleagues were those that they

determined to be haplotype-tagging. The minor allele frequency was reported as 0.06. (132)

-2423 A>G (rs7789066)

Bailey and colleagues also examined the -2423 variant in the Quebec Family Study and found that it was significantly associated with HDL triglycerides ($p = 0.032$) and very low density lipoprotein (VLDL) apoB ($p = 0.032$) using a dominance parameter. Moreover, VLDL apoB remained significant in an additive model ($p = 0.012$), whereas HDL triglycerides and VLDL triglycerides neared significance (both $p < 0.07$). The minor allele frequency was reported as 0.08. (11)

Zhang and colleagues also examined the rs7789066 variant (labeling it -2422 A>G) as a haplotype tagging SNP and found no association with T2D in their case-control study involving patients from the Boston Joslin Diabetes Center (132). The minor allele frequency was reported as 0.09 (132).

With regard to function, this polymorphism is reportedly located within a nuclear factor of activated-T cells (NFAT) binding sequence (11). Thus, a possible functional effect could occur via NFAT's postulated involvement in adipogenesis (50) and insulin gene transcription (71).

-1543 C>T (also known as -1535 C>T)

Ye and colleagues conducted a case-control SNP association study in white patients with sepsis-associated acute lung injury and severe sepsis. The frequency of the T allele was significantly lower in the acute lung injury group ($n = 8$; 20 %) as compared to the healthy control subjects ($n = 8$; 31 %). However, only a weak association ($p < 0.06$) was found for the PBEF1 -1543 C>T genotype and acute lung

injury after controlling for age, gender, and co-morbidities in multiple logistic regression analysis. Furthermore, the researchers reported a ~ 1.8-fold decrease in luciferase reporter gene expression in human lung microvascular endothelial cells with the T allele as compared to the C allele. Unfortunately, no subject characteristics were provided in the paper besides disease status and race. Thus, group comparability is impossible to ascertain. It is interesting that significant and nearly significant findings were detected with such small sample sizes, and the reduction in transcription does suggest a potential functional effect for this variant. It is also important to note that the polymorphism designated by Ye and colleagues as -1543 is actually located 1535 basepairs upstream of the PBEF1 ATG site. (129)

Bajwa and colleagues investigated the -1543 polymorphism in 375 acute respiratory distress syndrome patients and 787 at-risk controls. They found that the polymorphism did not increase the risk of acute respiratory distress syndrome, but that it was associated with a reduced risk of acute respiratory distress syndrome in septic shock patients. In the acute respiratory distress syndrome patients, the -1543 T allele was also associated with better mortality and ventilation outcomes. (12)

Jian and colleagues examined the PBEF -1535 C>T SNP and glucose and obesity-related variables in NGT (19 men, 42 women), IGT (26 men, 39 women), and newly diagnosed T2D (66 men, 49 women) patients in China. The -1535 C>T SNP was not associated with T2D, fasting glucose, 120 minute OGTT glucose, glucose AUC, total cholesterol, triglycerides, HDL-cholesterol, or LDL-cholesterol. Moreover, the T allele frequency was reported as 0.53 in the NGT participants. Analyses were adjusted for age, gender, and glucose tolerance status. In addition, all

participants were > 40 years of age and were taking no lipid- or glucose-altering medications. However, cigarette smoking status, female menopausal status, diet, and physical activity status were not reported; moreover, all of these factors could have affected measurement of the outcome variables. (55)

Axelsson and colleagues investigated the polymorphism in stage 5 chronic kidney disease patients (n = 135) and reported no differences in visfatin levels. The minor allele frequency was ~ 0.258. In addition, the researchers reported that the polymorphism was in linkage disequilibrium with the -1001 and -423 variants (both $r^2 = 0.11$). (9)

Tokunaga and colleagues studied the -1535 SNP in healthy, non-diabetic (191 men, 142 women) and T2D (245 men, 203 women) Japanese individuals. They reported that it was the only promoter polymorphism detected in their cohort. In the case-control portion of their study, the frequency of the C allele did not differ between the non-diabetic and T2D groups (0.44 vs. 0.45). In genetic association studies involving the non-diabetic participants, there were no differences reported for BMI, fasting glucose, fasting insulin, or HOMA-IR by -1535 genotype group. However, the -1535 polymorphism was associated with triglyceride and HDL-cholesterol levels. Moreover, the TT group had lower triglycerides and higher HDL-cholesterol levels than the CC group. In the group with T2D, there were no significant genotype effects found for the lipid measures, and there was no mention of the glucose and insulin-related variables. The researchers also conducted a reporter gene assay in mouse 3T3-L1 adipocytes and found no differences in luciferase

activity for the C versus T promoters, in contrast to the Ye *et al.* study (129) involving human lung microvascular endothelial cells. (115)

It is important to note that menopausal status, hormone therapy usage, diet, and physical activity status were not accounted for in the study by Tokunaga and colleagues. Furthermore, these factors could have influenced many of the outcome variables. In addition, the age and gender make-up of the genotype groups was not reported, and one-way ANOVA was used to compare differences in the genotype groups. Thus, adjustment for the possible influence of age and gender on the outcome variables did not take place. (115)

-1001 T>G (rs9770242)

Bailey and colleagues reported that the -1001 T>G polymorphism was associated with glucose and insulin phenotypes in the Quebec Family Study population (11). Moreover, the G allele was associated with lower fasting insulin and glucose levels and occurred at a frequency of 0.26 (11).

Bottcher and colleagues studied the -1001 polymorphism in Germans and found that it was not associated with T2D in a case-control study. Moreover, in 626 non-diabetics, the polymorphism was also not associated with body composition or glucose-related variables. It was also not associated with plasma visfatin, subcutaneous fat visfatin mRNA expression, and visceral fat mRNA expression in a subgroup of abdominal surgery patients. However, the -1001 polymorphism was associated with a lower ratio of visceral to subcutaneous visfatin mRNA expression in the surgery patients. The case-control study involved 503 T2D patients (250 men, 253 women) and 476 non-diabetics (236 men, 240 women), and adjustment was made

for age, gender, and BMI differences between the groups. The subgroup of non-diabetics consisted of 308 men and 318 women. However, no information regarding overall characteristics, diet, physical activity status, medication usage, or female menopausal status was provided. Thus, these factors may have affected the outcome variables. Last, the subgroup of 189 individuals (95 men, 94 women) with abdominal tissue samples were undergoing surgery for weight loss, explorative laparotomy, or abdominal injury repair. In addition, these individuals varied in terms of age, BMI, and insulin sensitivity. Although analyses accounted for age, gender, and body composition differences, no information regarding other co-morbidities, diet, physical activity status, medication usage, or female menopausal status was provided. Moreover, these factors could have influenced measurement of visfatin levels. Last, the G allele frequency was reported as 0.31. (16)

Axelsson and colleagues found significant -1001 genotype effects for visfatin levels in stage 5 chronic kidney disease patients, but after adjusting for age, sex, glomerular filtration rate, and serum albumin the visfatin differences in the stage 5 chronic kidney disease patients were no longer significant. However, the -1001 polymorphism was found to be a significant predictor of visfatin levels in stage 5 chronic kidney disease patients in multivariate regression models, accounting for 4 % of the variation. The minor allele frequency was ~ 0.25. (9)

Korner and colleagues examined the -1001 polymorphism in connection with obesity and insulin resistance in children. The researchers studied 167 (89 boys, 78 girls) obese and 508 (243 boys, 265 girls) healthy German children. In the healthy children, a significantly higher WHR was detected in T homozygotes as compared to

GT heterozygotes and G homozygotes after adjustment for age and gender. However, pubertal status was not taken into account in the single SNP analyses. Diet and physical activity levels also were not assessed in the study, and these factors could also have influenced many of the metabolic outcomes. The frequency of the G allele was 0.25 in the school children. (64)

Last, in case-control studies, the G allele was associated with increased odds of acute respiratory distress syndrome (12), acute lung injury (129), and sepsis (129).

With regard to functional significance, a luciferase reporter gene assay found that the -1001 G allele did not significantly alter PBEF gene expression in lung microvascular endothelial cells (129). However, the -1001 polymorphism is in complete linkage disequilibrium with rs1319501, another PBEF1 promoter SNP. Thus, it has been suggested that the functional variant may actually be rs1319501 (11). Furthermore, since the -1001 polymorphism is reportedly in moderate linkage disequilibrium ($r^2 = 0.47$) and proximity (53 basepairs) with the -948 polymorphism, the 2 may be acting as a functional SNP's marker (132). Bottcher and colleagues also reported moderate linkage disequilibrium for the -1001 polymorphism with the rs4730153 ($r^2 = 0.53$) and rs11553095 ($r^2 = 0.52$) polymorphisms (16). Finally, Axelsson and colleagues reported that the polymorphism was in linkage disequilibrium with the -1535 polymorphism ($r^2 = 0.11$), too (9).

-948 G>T (also known as C>A)

In case-control studies, Zhang and colleagues found that the -948 T allele was significantly associated with T2D (132), whereas Bottcher and colleagues did not find the polymorphism to be associated with T2D (16). The T allele frequency was

reported as 0.12 (132) and 0.19 (16), respectively. A possible difference between these studies involves subject characteristics. For example, in the Zhang *et al.* study, the non-diabetic cases and T2D controls were of similar age (61 ± 15 yrs versus 63 ± 7 yrs) and from the Boston, MA area, whereas the non-diabetic controls and T2D cases in the Bottcher *et al.* study varied in terms of age (47 ± 0.7 yrs versus 59 ± 0.4 yrs) and were German.

Zhang and colleagues also conducted a study in 630 healthy Italians and reported a significant association for the -948 T allele with higher plasma CRP and fibrinogen levels, suggesting a possible connection with low-grade inflammation. However, no associations were detected with fasting glucose or insulin levels. Moreover, the researchers suggested that the healthy, young, lean participants in the study were likely not ideal subjects for studies of insulin resistance. Although the participants were not taking medication, the researchers did not provide information regarding diet or physical activity status, and differences in these 2 factors could have influenced the outcome variables. The T allele frequency was ~ 0.13 . (132)

In contrast, the -948 G>T polymorphism was associated with fasting insulin, total apoB, and LDL apoB in the Quebec Family Study's French-Canadian population (11), and the G allele was associated with increased diastolic blood pressure in obese children (64). Furthermore, non-diabetic German PBEF1 -948 T allele carriers had significantly lower OGTT 2-hour plasma glucose and fasting insulin levels than GG homozygotes, and TT homozygotes tended to have a lower percent body fat ($p = 0.06$) and higher clamp glucose infusion rate ($p = 0.09$) than G allele carriers (16). These findings again contrast with the results of the Zhang *et al.* study possibly

because of subject differences. Last, in 189 Caucasians undergoing abdominal surgery, a lower ratio of visceral to subcutaneous visfatin mRNA expression was found for the -948 T allele (16). The frequency of the T allele was reported as 0.14 (11; 64) and 0.19 (16) in these studies, respectively.

The -948 G>T polymorphism is located in the promoter region of PBEF1 and may influence gene expression via altering transcription factor activity.

Alternatively, the -948 G>T polymorphism may be in linkage disequilibrium with a functional SNP. Bottcher and colleagues reported moderate linkage disequilibrium values for the -948 G>T polymorphism and -1001 T>G ($D' = 0.99$, $r^2 = 0.51$) and -423 A>G ($D' = 0.99$, $r^2 = 0.53$) polymorphisms (16). In addition, Zhang and colleagues reported moderate linkage disequilibrium for the SNP with -1001 T>G ($r^2 = 0.47$), rs6971643 ($r^2 = 0.42$) and rs17314507 ($r^2 = 0.69$) (132).

-423 A>G (rs1319501)

Bailey and colleagues found significant associations for the -423 A>G polymorphism and fasting glucose and fasting insulin levels in the Quebec Family Study participants. Moreover, GG homozygotes had lower fasting glucose and insulin values. In addition, the variant showed complete linkage disequilibrium with the -1001 T>G polymorphism. The frequency of the G allele was 0.26. (11)

Axelsson and colleagues also reported that this polymorphism was in complete linkage disequilibrium with the -1001 polymorphism. Moreover, they found significant -423 genotype effects on visfatin levels until adjustment for age, gender, glomerular filtration rate, and serum albumin level in stage 5 chronic kidney disease patients. The frequency of the minor allele was ~ 0.25. (9)

Rs1319501 is located within a nuclear hormone response element recognized by the nuclear receptor family that includes peroxisome proliferator-activated receptors and the vitamin D receptor (11; 39).

PBEF1 Intronic & Exonic Polymorphisms

rs1319313, rs6947766, rs3801268, & rs3801272

Zhang and colleagues examined the rs1319313 (position 2807, G>A), rs6947766 (position 14664, C>T), rs3801268 (position 26700, G>C), and rs3801272 (position 28803, G>A) polymorphisms individually and as part of a haplotype analysis in a case-control study involving T2D patients. There were no associations with T2D for any of the individual polymorphisms. Moreover, the minor allele frequencies were 0.27, 0.24, 0.42, and 0.35, respectively. The polymorphism locations were reported as intron 1 for rs1319313, intron 4 for rs6947766, intron 8 for rs3801268, and intron 8 for rs3801272. (132)

rs10953502 & rs2058539

Jian and colleagues examined the rs10953502 (T>C) and rs2058539 (A>C) SNPs in connection with glucose and lipid variables in NGT, IGT, and T2D Chinese individuals. Individuals homozygous for the rs10953502 C allele had higher fasting plasma glucose, OGTT 2-hour glucose, and glucose AUC values than individuals homozygous for the T allele. The researchers also found that individuals homozygous for the rs2058539 A allele had higher fasting glucose and glucose AUC values than rs2058539 CC homozygotes. All of the significant findings were adjusted for participant age, gender, and glucose tolerance status. In addition, all participants were > 40 years of age and were taking no lipid- or glucose-altering

medications. However, cigarette smoking status, female menopausal status, diet, and physical activity status were not reported; moreover, all of these factors could have affected measurement of the outcome variables. The rs10953502 C allele frequency was ~ 0.20 in the NGT participants, and the rs2058539 C allele frequency was ~ 0.39. The polymorphisms are reportedly located in intron 1 and intron 9, respectively. (55)
rs4730153

The A allele of the rs4730153 (c. 744 -87 G>A) polymorphism was associated with a lower ratio of visceral to subcutaneous visfatin mRNA expression in Germans and had a frequency of 0.48 (16). This intron 6 SNP also had strong linkage disequilibrium with rs11553095 ($r^2 = 0.80$) and moderate linkage disequilibrium with the -423 A>G ($r^2 = 0.5$) and -1001 T>G ($r^2 = 0.53$) polymorphisms (16).

rs2302559

This polymorphism (C>T) is located in exon 7 of the visfatin gene and results in a synonymous amino acid change at position 301 (SER301SER). It has not been examined previously in association studies, but has a reported minor allele frequency of ~ 0.41 (132).

PBEF1 Haplotypes

Results from 4 PBEF1 haplotype association studies have been published. Moreover, 2 studies investigated haplotypes derived from the rs9770242 (-1001), -948, and rs4730153 polymorphisms (16; 64), 1 study looked at haplotypes created from the -2647, rs7789066, -948, rs1319313, rs6947766, rs3801268, and rs3801271 polymorphisms (132), and 1 study examined haplotypes constructed from the rs9770242 (-1001) and -1543 polymorphisms (129).

Bottcher and colleagues examined haplotypes derived from the -1001 T>G polymorphism, -948 G>T polymorphism, and rs4730153 G>A polymorphism. The researchers examined 4 commonly occurring haplotypes in their study population of non-diabetic Germans (n = 626), the TGG, TGA, GGA, and GTA haplotypes. Furthermore, carriers of the TGA haplotype had higher 2-hour OGTT glucose levels and higher glucose infusion rates during euglycemic, hyperinsulinemic clamps than haplotype non-carriers. The researchers also found that non-carriers of the GTA haplotype had higher fasting plasma insulin levels and higher 2-hour glucose levels than individuals with 1 or 2 copies of the GTA haplotype. Interestingly, the GTA haplotype was the only commonly occurring haplotype containing the -948 T allele, and similar results were found in this same study for single SNP analyses involving the -948 polymorphism. (16)

Korner and colleagues also examined haplotypes from the -1001 T>G polymorphism, -948 G>T polymorphism, and rs4730153 G>A polymorphism. They studied 2 different cohorts of children, obese children and school children, and found no haplotype associations with BMI, WHR, glucose, insulin, or lipid measures. However, the TGA haplotype was associated with increased diastolic blood pressure in both the school children and obese children. (64)

Zhang and colleagues examined 8 common PBEF1 haplotypes, tagged by 7 SNPs, in a case-control study involving T2D patients. The 7 SNPs were the -2657 G>A, rs7789066 A>G, -948 G>T, rs1319313 G>A, rs6947766 C>T, rs3801268 G>C, and rs3801271 T>C. The GATACGT haplotype, the only commonly occurring haplotype with a T at -948, was associated with an increased risk of T2D (Haploscore

= 2.365, $p = 0.018$), whereas the GAGACGT haplotype was associated with a decreased risk of T2D (Haploscore = -2.61, $p = 0.009$). (132)

Finally, Ye and colleagues examined haplotypes composed of the -1001 T>G and -1543 C>T polymorphisms in a case-control study involving patients with sepsis-associated acute lung injury and severe sepsis. They found that the GC haplotype significantly increased the risk of acute lung injury 7.7-fold and the risk of sepsis 4.8-fold. The researchers also found that the TT haplotype was protective, reducing the risk of acute lung injury 0.8-fold; however, this reduction in risk was not significant. There were no differences in risk found for the GT or TC haplotypes. (129)

Summary: Visfatin Polymorphism & Haplotype Studies

Tables 6-2 and 6-3 summarize the results from association studies involving PBEF1 polymorphisms. Taken together, the results of genetic association studies suggest a possible role for visfatin gene polymorphisms and haplotypes in glucose and obesity-related phenotypes.

Table 6-2. Results from PBEF1 intronic and exonic polymorphism studies.

SNP	Genotype Associations	Other	Source
Rs1319313 Intron 1 2807 G>A	Not independently with type 2 diabetes		(132)
Rs2058539 Intron 1 A>C 8693	Fasting plasma glucose Glucose area under the curve	AA ↑ AA ↑	(55)
Rs6947766 Intron 4 C>T 14664	Not independently with type 2 diabetes		(132)
Rs4730153 Intron 6 c.744 -87 G>A C>T 21179	Log vis/subcut visfatin mRNA No obesity or glucose-related traits	A ↓	(16) (64)
Rs3801268 Intron 8 G>C 26700	Not independently with type 2 diabetes		(132)
Rs3801272 Intron 8 G>A 28803	Not independently with type 2 diabetes		(132)
Rs10953502 Intron 9 T>C 32899	Fasting plasma glucose OGTT 120 minute glucose Glucose area under the curve	CC ↑ CC ↑ CC ↑	(55)

Genotype associations are significant unless indicated otherwise. Log, common logarithm; vis, visceral fat; subcut, subcutaneous fat; mRNA, messenger RNA; OGTT, oral glucose tolerance test; ↓, decreased; ↑, increased.

Table 6-3. Results from PBEF1 promoter polymorphism studies.

SNP	Genotype Associations	Other	Source
Rs2110385 -4689 G>T	Fat mass, total apoB (p < 0.07)		(11)
Rs1737358 -4191 A>G	No glucose or obesity-related traits		(11)
Rs11977021 -3186 C>T	Total cholesterol, LDL-cholesterol		(11)
-2657 G>A	Not independently with type 2 diabetes		(132)
	VLDL apoB		
Rs7789066 -2423 A>G	VLDL triglycerides (p = 0.067; 0.069) HDL triglycerides (p = 0.061; 0.032)	Additive; dominant	(11)
	Not independently with type 2 diabetes		(132)
	Acute lung injury (p = 0.059) Luciferase reporter/promoter activity	T ↓ risk T ↓	(129)
-1543 C>T also known as -1535 C>T	Acute respiratory distress syndrome	T ↓ risk	(12)
	No glucose or lipid-related traits		(55)
	Not with visfatin levels in CKD		(9)
	Not with glucose, insulin, or BMI HDL-cholesterol	TT ↑	(115)
	Triglycerides	TT ↓	
	Luciferase reporter/promoter activity	=	
	Fasting glucose, fasting insulin	GG ↓	(11)
	Acute lung injury, sepsis Luciferase reporter/promoter activity	G ↑ risk =	(129)
Rs9770242 -1001 T>G	Log vis/subcut visfatin mRNA		
	No glucose or obesity-related traits	G ↓	(16)
	Not with visfatin levels		(9)
	Not with visfatin levels in CKD		(9)
	Waist-to-hip ratio	TT ↑	(64)
	Acute respiratory distress syndrome	G ↑ risk	(12)
	Fasting insulin, total apoB, LDL apoB		(11)
-948 G>T also known as C>A	Fasting insulin, 2-hour OGTT glucose	T ↓	
	Log vis/subcut visfatin mRNA	T ↓	(16)
	Percent body fat (p = 0.06)	T ↓	
	Type 2 diabetes	A ↑ risk	(132)
	C-reactive protein, fibrinogen levels	A ↑	
	Diastolic blood pressure	G carrier ↑	(64)
Rs1319501 -423 A>G	Fasting glucose, fasting insulin	GG ↓	(11)
	Not with visfatin levels		(9)

Genotype associations are significant unless indicated otherwise. Apo, apolipoprotein; OGTT, oral glucose tolerance test; log, logarithm; vis, visceral fat; subcut, subcutaneous fat; mRNA, messenger RNA; CKD, chronic kidney disease; ↓, decreased; ↑, increased; =, equal.

Visfatin & Exercise

Many beneficial physiological changes occur with aerobic exercise training, including improvements in glucose tolerance and insulin sensitivity. Furthermore, adipokines such as adiponectin are responsive to exercise training. However, the response of visfatin to acute exercise and to aerobic exercise training has only been investigated minimally.

Acute Exercise

Only 1 study, thus far, has examined visfatin's response to acute exercise in humans. Frydelund-Larsen and colleagues studied visfatin mRNA expression in 15 healthy men, approximately 25 yrs of age, with a BMI of $\sim 25 \text{ kg/m}^2$. The 15 men were divided into an exercise group ($n = 8$) and a control group ($n = 7$). The exercise group cycled on a cycle ergometer for 3 hours at 60 % of their maximal oxygen consumption (VO_2max) and then underwent 6 hours of recovery. In contrast, the control group rested for 9 hours. Muscle and adipose tissue biopsies were taken immediately before exercise (0 hour), immediately after the exercise bout (3 hours), and during the recovery period (4.5 hours, 6 hours, 9 hours, 24 hours) in the exercisers, with similarly timed biopsies taken in the control subjects. Venous blood samples were drawn at time points 0, 1, 2, 3, 4.5, 6, 9, and 24 hours, respectively. The researchers found that as compared to the control participants and as compared to baseline, abdominal adipose tissue visfatin mRNA expression increased 3-fold in response to exercise, with significant increases at the 3, 4.5, and 6 hour time points. However, skeletal muscle visfatin mRNA expression and plasma visfatin levels did not significantly change with acute exercise. (34)

Aerobic Exercise Training

Only 3 studies, thus far, have examined visfatin's response to aerobic exercise training in humans. Haider and colleagues examined the effects of aerobic exercise training on plasma visfatin levels in 18 (11 women and 7 men, 42 ± 10 yrs of age) T1D patients. The participants were compared to healthy, non-exercising controls (7 women and 7 men, 29 ± 5 yrs of age). The exercise training program consisted of 4 months of stationary cycling for 1 hour, at an intensity of 60-70 % of heart rate reserve. For the initial 2 weeks of the program, exercise sessions took place twice a week, and then for the remainder of the program exercise sessions were held 3 times per week. At baseline, the T1D patients had greater fasting plasma glucose and visfatin levels than the controls. Moreover, there was no correlation between visfatin and BMI or age at baseline. After both 2 and 4 months of exercise training, visfatin levels decreased significantly in the T1D patients (from 64.1 ± 12 ng/mL to 27.8 ± 2.6 ng/mL and 17.5 ± 3.4 ng/mL, respectively) and remained lower 8 months after the cessation of exercise training. However, BMI, fasting glucose, glycosylated hemoglobin, and lipoprotein-lipids were unchanged with the exercise training. In addition, the researchers did not measure VO_2 max or a similar training effect variable, and to be included in the study, participants only had to attend > 60 % of the exercise sessions. Thus, it is difficult to determine if an actual aerobic exercise training effect occurred. Furthermore, some of the patients were receiving medications for co-morbidities, including hypertension and cholesterol medication, and these were not taken into account in the analyses. Lastly, the researchers did not

assess diet, and differences in diet could potentially influence the outcome measures.

(43)

Choi and colleagues evaluated the effect of a combined aerobic and resistance exercise training program on plasma visfatin levels in non-diabetic Korean women. Healthy, overweight or obese women (n = 48) aged 30-55 yrs were recruited for the study. The participants had to be sedentary (< 20 minutes of exercise twice per week) and underwent a graded exercise test to screen for cardiovascular disease. In addition, to be eligible for the study, participants could not be taking laboratory test result-altering medications. The 12 week exercise training intervention consisted of approximately 45 minutes of aerobic exercise and 20 minutes of resistance exercise, 5 times per week. The intensity of the aerobic exercise started at 40 % of age-predicted maximal heart rate and progressed to 60-75 % of age-predicted maximal heart rate by week 12. All but 2 of the women were premenopausal, and 36 of the women were obese. At baseline, the obese women had higher plasma visfatin levels than the overweight women. Plasma visfatin levels were associated with body weight, BMI, and waist circumference in simple regression analyses and BMI in multiple regression analyses. After 12 weeks of exercise training, the participants had lost ~ 4-5 kg of weight, decreased BMI, waist circumference, blood pressure, percent body fat, fasting glucose, HOMA-IR, and visfatin levels. Thus, a combined aerobic and resistance exercise training program and weight loss significantly decreased plasma visfatin levels in healthy, overweight or obese Korean women. (25)

Although, Choi and colleagues did not measure VO_2 max or a similar training effect variable and did not report exercise training session attendance, the changes in

the other variables measured may be consistent with a training effect. However, diet was not assessed in the study and part of the weight loss was likely due to dietary changes since the caloric cost of the exercise training was estimated by the researchers as ~ 400 kcal per session. In addition, there is no indication that the training changes were adjusted for age within the obese and overweight groups or for age and obesity status within the combined group. Moreover, a graph showing visfatin changes with aerobic exercise training depicts a high degree of variability. Furthermore, data from several subjects appears to have been excluded as outliers. Also, it appears that participants in the overweight group may not have changed their visfatin levels with exercise training, but raw data is only provided for the total group. Thus, it is difficult to assess whether changes in visfatin levels occurred in non-obese individuals with exercise training and whether the exercise training or weight loss caused the reported differences. (25)

Finally, Brema and colleagues compared plasma visfatin levels in 15-30 yr old Caucasian age- and BMI-matched obese and T2D patients before and after the completion of an aerobic exercise training intervention. The training program lasted 12 weeks and consisted of 4 supervised sessions per week at an intensity of 75 % VO_2 max and a duration of 1 hour. Dietary intake reportedly remained unchanged during the program, as did nearly every physiological variable measured, with the exception of waist circumference in the T2D group and VO_2 max in the obese group. Furthermore, visfatin levels were comparable in the obese and T2D patients before the intervention and decreased in both groups with training, with a greater decrease occurring in the T2D patients. Also, in the T2D patients, visfatin was correlated with

percent body fat ($r = -0.933$) and HDL-cholesterol ($r = -0.893$) at baseline. As VO_2max did not change in the T2D patients and most outcomes did not change with the program, it is difficult to assess if a training effect occurred. Furthermore, there was no indication made as to whether the participants were sedentary before the program started. In addition, gender was not accounted for and differences between the groups could have affected many of the outcome variables. (17)

Summary: Visfatin & Exercise

Thus, acute exercise seems to increase visfatin mRNA expression but not visfatin plasma levels in healthy, young men. In contrast, plasma visfatin levels seem to be responsive to exercise training. However, no research has been reported regarding the independent effects of 6 months of aerobic exercise training on plasma visfatin levels in NGT and IGT individuals. In addition, previous studies in T1D patients and non-diabetic Korean women have not controlled for the effects of diet, and all 3 previous studies have not provided evidence that an actual training effect occurred. Thus, studies taking these factors into account would help to further elucidate the effects of aerobic exercise training on plasma visfatin levels.

Aerobic Exercise Training Responses & Their Genetic Link

Coincidentally, the response of many variables to aerobic exercise training tends to be highly variable. In fact, part of the variation in the response of glucose and obesity-related phenotypes to aerobic exercise training has been attributed to common genetic polymorphisms (93). With its links to glucose and obesity-related phenotypes, its polymorphisms associated with glucose and obesity-related phenotypes, and its responsiveness to exercise training, visfatin is a candidate gene

for aerobic exercise training-induced changes in glucose and obesity-related phenotypes.

Appendix A: Delimitations & Limitations

Delimitations

1. Subjects in this study took part in the Gene Exercise Research Study (GERS) at the University of Maryland, College Park, MD.
2. As members of the GERS, the subjects were recruited from the University of Maryland and Washington D.C. metropolitan areas.
3. As participants of the GERS, the subjects had to meet specific study inclusion criteria. Briefly, the participants had to be 50-75 yrs of age, non-smoking, non-diabetic, sedentary, healthy (with the exception of having a National Cholesterol Education Program lipid abnormality and/or elevated blood pressure), not taking medications known to affect glucose levels, postmenopausal (if female), and have a BMI < 37 kg/m². The results, therefore, are most applicable to populations with similar characteristics.

Limitations

1. The participants were recruited as part of the GERS and may not be representative of the general population.
2. The GERS participants self-reported many initial health and lifestyle factors such as physical activity habits, dietary habits, and medication regimens. Thus, it is possible that inaccurate self-reports may affect the results of the proposed study.
3. There was no control group in this study.

4. The frequencies of genotypes were not controlled, so rare allele carriers were grouped together in the analyses when sample sizes were inadequate.
5. Due to a lack of available data and differences in allele and genotype frequencies between ethnic groups, only data from GERS Caucasian participants was examined in this project.
6. Due to the lack of existing data, some of the hypotheses were not directional in nature.
7. This study involved a retrospective analysis of data and used banked plasma and DNA samples.

Appendix B: Definitions

Adipokine – A substance (hormone, cytokine, *etc.*) produced by adipose tissue.

Allele – An alternative form of a gene.

Diabetes mellitus – A group of diseases resulting from defects in insulin action and/or insulin secretion which affect the maintenance of appropriate glycemia.

Genotype – The genetic make-up of an individual.

Glucose – A monosaccharide containing six-carbon atoms that is used as a major energy substrate in the body.

Haplotype – A set of closely linked genetic markers present on 1 chromosome that tend to be inherited together.

Impaired fasting glucose – An intermediate stage in the pathogenesis of type 2 diabetes where glucose levels are above normal but below the diagnostic level for diabetes. In this case, the plasma glucose level is 100-125 mg/dL (5.6-6.9 mmol).

Impaired glucose tolerance – An intermediate stage in the pathogenesis of type 2 diabetes where glucose levels are above normal but below the diagnostic level for diabetes. In this case, plasma glucose concentration is 140-199 mg/dL (7.8-11.0 mmol) after a 2-hour, 75-gram oral glucose tolerance test.

Insulin – A hormone secreted by the islets of Langerhans involved in the control of blood glucose levels by promoting the uptake and storage of glucose in the body.

Insulin resistance – A condition in which the body has a reduced physiological response to circulating insulin.

Linkage disequilibrium – The nonrandom association of 2 or more alleles; the occurrence of alleles at 2 or more loci more frequently than expected.

Nicotinamide adenine dinucleotide – A coenzyme that functions as a carrier of hydrogen atoms in oxidation-reduction reactions.

Nicotinamide – A form of nicotinic acid (vitamin B₃).

Obesity – Excess body fat; often defined as body mass index ≥ 30 kg/m².

Oral glucose tolerance test – A diagnostic assessment measuring the ability of the body to maintain euglycemia in response to a glycemic challenge.

Polymorphism – A base or section of DNA that is variable among individuals.

Promoter – A nucleotide sequence in DNA to which transcription factors and RNA polymerase bind to initiate transcription.

Sedentary – A term used to describe individuals who are not physically active. In this project, individuals who, on average, complete less than 20 minutes of aerobic exercise less than twice per week.

Single nucleotide polymorphism – DNA sequence variation involving the substitution of 1 purine or pyrimidine base (A, T, G, or C) by another purine or pyrimidine base (A, T, G, or C).

TaqMan – A high through-put method for genotyping single nucleotide polymorphisms.

Transcription – A process where the genetic information in 1 strand of DNA is copied into its complementary messenger RNA sequence.

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