ABSTRACT

Title of Thesis: THE EFFECTS OF LAMIN A/C C1908T POLYMORPHISM ON BODY COMPOSITION, PLASMA LIPOPROTEIN-LIPID PROFILE AND INSULIN SENSITIVITY CHANGES WITH AEROBIC EXERCISE TRAINING

Faith Melissa Augrom, Master of Arts, 2005

Thesis directed by: James Hagberg
Professor
Department of Kinesiology

Obesity, lipoprotein subclass concentrations and insulin resistance are predictors of cardiovascular disease (CVD). The Lamin A/C (LMNA) C1908T polymorphism has been associated with obesity-related anthropometric and biochemical traits, higher fasting triglyceride, lower HDL-cholesterol concentrations and the metabolic syndrome. Aerobic exercise training has been shown to generate favorable changes in body composition, lipoprotein-lipid profile and insulin sensitivity and to reduce CVD risk. The purpose of this project was to examine the effect of the C1908T polymorphism on percent body fat, plasma lipoprotein-lipid profile and insulin sensitivity in sedentary subjects before, and their change with, 24 weeks of exercise training. Following IRB approval, 144 sedentary

adults underwent dietary stabilization, body composition scans, a 3-hour oral glucose tolerance test (OGTT) and plasma lipoprotein-lipid profile analysis. Genotyping was performed using standard techniques. Following baseline testing, 104 subjects completed six months of aerobic exercise training and then underwent a final body composition scan, 3-hour OGTT and plasma lipoprotein-lipid profile analysis. Comparisons were made between C-allele carriers and TT homozygotes; ANOVA and ANCOVA procedures were utilized to examine the effects of the C1908T polymorphism on percent body fat, lipoprotein-lipid profile and insulin sensitivity before, and their changes with, 24-weeks of aerobic exercise training. Statistical significance was set at $p \leq 0.05$ level. Overall, genotype frequencies were as predicted by Hardy-Weinberg equilibrium. There were no significant differences between the genotype groups regarding body composition, lipoprotein-lipid profile or OGTT variables at baseline, nor were there significant differences in the change with exercise training of these variables between the two genotype groups. This study suggests that components of the metabolic syndrome and certain CVD risk factors may not be dependent on LMNA A/C genotype. However, a larger and more balanced study population across genotype groups may be needed to reach a definitive conclusion.

THE EFFECTS OF LAMIN A/C C1908T POLYMORPHISM ON BODY COMPOSITION, PLASMA LIPOPROTEIN-LIPID PROFILE AND INSULIN SENSITIVITY CHANGES WITH EXERCISE TRAINING

by

Faith Melissa Augrom

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Master of Arts
2005

Advisory Committee:

James Hagberg Professor, Chair/Advisor
Michael D. Brown, Associate Professor
Stephen M. Roth, Assistant Professor

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. James Hagberg, for his assistance and guidance throughout the Master's program and in the completion of this thesis. I would also like to thank Dr. Michael Brown and Dr. Stephen Roth for their assistance, time, and support in serving on my thesis committee. This project would not have been possible without the members of the GERS team, particularly Jennifer McKenzie, Tina Ellis and Dr. Dana Phares, as their assistance was greatly appreciated. I would also like to extend a special thanks to all of the participants of the GERS study for their hard work and dedication.

Finally, without the loving support of my parents, friends, and especially Neil, this project would not have been possible. They have been able to help me through these stressful times and offer unending encouragement.

TABLE OF CONTENTS

LIST OF TABLES iv

LIST OF ABBREVIATIONS v

CHAPTER 1: INTRODUCTION 1
Research Question
Hypotheses

CHAPTER 2: METHODS 5
Subjects and Sampling
Experimental Design and Variables
Instrumentation
Data Collection Procedures
Statistical Analysis

CHAPTER 3: RESULTS 13
Baseline Population Comparisons
Comparison of Training Adaptations

CHAPTER 4: DISCUSSION 25

CHAPTER 5: CONCLUSION 33

APPENDIX A: PROPOSAL INFORMATION 35
Statement of the problem
Experimental hypotheses
Delimitations
Limitations
Operational definitions

APPENDIX B: LITERATURE REVIEW 38
The Metabolic Syndrome
Heritability of the Metabolic Syndrome and its Components
Effects of Exercise Training
Heritability of Exercise Training Adaptations
Nuclear Lamina
Lamins A & C
The C1908T Polymorphism
Summary
References – Literature Review

APPENDIX C: INFORMED CONSENT FORM 58

REFERENCES 63

LIST OF TABLES

Table 1. Allele and Genotype Frequencies for LMNA C1908T Polymorphism– Baseline Comparison Population 15

Table 2. Subject Characteristics as a Function of LMNA C1908T Polymorphism Genotype Groups – Baseline Comparison Population 15

Table 3. Body Composition Variables as a Function of LMNA C1908T Polymorphism– Baseline Comparison Population 16

Table 4. Plasma Lipoprotein-Lipid Variables as a Function of LMNA C1908T Polymorphism Genotype Groups– Baseline Comparison Population 17

Table 5. OGTT Variables as a Function of LMNA C1908T Polymorphism Genotype Groups– Baseline Comparison Population 18

Table 6. Allele and Genotype Frequencies for LMNA C1908T Polymorphism– Exercise Training Population 21

Table 7. Subject Characteristics as a Function of LMNA C1908T Polymorphism Genotype Groups – Exercise Training Population 21

Table 8. Exercise Training Induced Changes in Body Composition Variables as a Function of LMNA C1908T Polymorphism Genotype Groups – Exercise Training Population 22

Table 9. Exercise Training Induced Changes in Plasma Lipoprotein-Lipid Variables as a Function of LMNA C1908T Polymorphism Genotype Groups – Exercise Training Population 23

Table 10. Exercise Training Induced Changes in OGTT Variables as a Function of LMNA C1908T Polymorphism Genotype Groups – Exercise Training Population 24

Table 11. Allele and genotype frequencies 30

LIST OF ABBREVIATIONS

AVF	abdominal visceral fat
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AD-EDMD	autosomal dominant Emery-Dreifuss muscular dystrophy
BMI	body mass index
CVD	cardiovascular disease
DCM	dilated cardiomyopathy
FPLD	Dunnigan's familial partial lipodystrophy
EDMD	Emery-Dreifuss muscular dystrophy
EDTA	ethylenediaminetetraacetic acid
FPG	fasting plasma glucose
FPI	fasting plasma insulin
FM	fat mass
HDL-C	high-density lipoprotein
HRT	hormone replacement therapy
INM	inner nuclear membrane
ISI	insulin sensitivity
LMNA	lamin A/C
LM	lean mass
LGMD1B	limb girdle muscular dystrophy type 1B
LDL	low-density lipoprotein
MAD	Mandibuloacral Dysplasia

NE	nuclear envelope
OGTT	oral glucose tolerance test
%FM	percentage of fat mass
SNP	single nucleotide polymorphism
TC	total cholesterol
TG	triglycerides
VLDL	very-low-density lipoprotein

CHAPTER 1: INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death among Americans, accounting for 38% or 1 out of every 2.6 deaths in the United States in 2002 (3). More than 70 million Americans have one or more types of CVD and 27 million of those affected are over the age of 65. The estimated cost of CVD in the US is $393.5 billion in the year 2005. Risk factors for CVD include diabetes, obesity, dyslipidemia, hypertension and physical inactivity (3).

A risk factor of CVD, type 2 diabetes mellitus, is caused by a combination of insulin resistance and insufficient insulin secretion by the pancreas. Insulin resistance, the result of obesity and physical inactivity, is accompanied by dyslipidemia and increased prothrombotic factors (15). The combination of these factors, which are the predecessors of type 2 diabetes and risk factors for CVD, is called the metabolic syndrome. The metabolic syndrome, its components, and type 2 diabetes have all been shown to be the result of both environmental and genetic factors (8; 31).

Many genome-wide scans have been conducted to determine specific chromosomal loci that, along with environmental factors, pre-dispose individuals to type 2 diabetes and related phenotypes. Susceptibility loci for type 2 diabetes have been identified on chromosome 1q21-24 in French Whites, British Whites, Chinese and Old Order Amish populations (37; 40; 43; 20). Lamin A/C (LMNA), a gene located in this region, is associated with familial partial lipodystrophy, a condition characterized by severe insulin resistance, diabetes, dyslipidemia and atherosclerosis, making it an exceptional positional candidate gene for type 2 diabetes and related phenotypes (7).

The LMNA gene products, lamins A and C, are important elements of the nuclear lamina. The nucleus of higher eukaryotic cells is comprised of three parts: the nuclear membranes, nuclear pore complexes, and the nuclear lamina. The lamina, associated with the inner nuclear membrane, is a structural component and also functions in DNA replication and chromatin organization (27). The lamina consists of proteins (called lamins) arranged in a fibrous network. There are two classes of nuclear lamins in human cells identified as lamin A or B (27). The LMNA gene encodes proteins that produce the two major A-type lamins; lamin A and lamin C (27). Human lamins A and C are identical in the initial 566 amino acids at which point alternative splicing in the LMNA gene results in two similar but functionally different isoforms(23) .

As already stated nuclear lamins A and C function as structural components of the nuclear membrane and also play a role in DNA replication. Mutations in the LMNA A/C gene are collectively referred to as laminopathies. Mutations in the genes that code for A type lamins result in these inherited laminopathies (27). Tissues that are affected by mutations in lamins A and C include peripheral nerves, striated muscle and adipose tissue. Mutant lamins expressed in cultured cells have been shown to disrupt the lamina structure which, in turn, impairs DNA synthesis (26; 33) and inhibits RNA polymerase II activity (33). Specifically, mutations in lamin A/C have been related to Emery-Dreifuss muscular dystrophy (5), Limb girdle muscular dystrophy type 1B (28), dilated cardiomyopathy (11), Charcot-Marie-Tooth neuropathy type 2 B1 (9) and Dunnigan's familial partial lipodystrophy (7).

Dunnigan's familial partial lipodystrophy (FPLD), a rare autosomal-dominant disease, is characterized by complete or partial absence of adipose tissue. Patients with

FPLD have normal fat distribution until they reach puberty, at which point adipocytes in their trunk and extremities degenerate (6; 13; 21) . Another characteristic of FPLD is insulin resistance, which is associated with dyslipidemia and often precedes type 2 diabetes (16).

A genetic variation in lamin A/C has also been reported to be associated with obesity-related anthropometric and biochemical traits in aboriginal Canadians and Inuit (17; 18), higher fasting triglyceride, lower HDL-cholesterol concentrations and the metabolic syndrome in an Amish population (34) and reduced subcutaneous abdominal adipocyte size in Pima Indians (39). Specifically, the mutation is a common single nucleotide polymorphism (SNP) in exon 10 of LMNA. This silent C→T substitution occurs at nucleotide 1908 and affects the third base within codon 566, which is also the last codon shared between lamins A and C before alternative splicing produces one of these proteins (17). Exercise training has been shown to have a positive effect on all of the above mentioned factors which are a result of the C1908T SNP and independently contribute to type 2 diabetes and cardiovascular disease. The interactive effect of exercise training and this polymorphism has not yet been investigated.

STATEMENT OF THE PROBLEM

The purpose of this project was to determine the effect that the C1908T polymorphism has on body composition, plasma lipoprotein profile and insulin sensitivity in sedentary subjects before, and their changes with, 24-weeks of exercise training.

EXPERIMENTAL HYPOTHESES

1. Subjects carrying the C-allele will have significantlybetter body composition, plasma lipoprotein profile and insulin sensitivity at baseline.
2. After 24 weeks of exercise training subjects carrying the C-allele will have significantly less improvement in body composition, their plasma lipoprotein profile and insulin sensitivity.

CHAPTER 2: METHODS AND PROCEDURES

SUBJECTS AND SAMPLING

The subjects for this study were participants in the Gene Exercise Research Study at the University of Maryland, College Park. These subjects were volunteers from the Washington, D.C. metropolitan area who responded to media advertisements (newspaper and magazine advertisements, radio public service announcements, flyers mailed to citizens in the metropolitan area). Subjects initially took part in a telephone screening interview to confirm that they met the following criteria: sedentary, nondiabetic, nonsmoking, no history of CVD or lung disease, normotensive or hypertensive with blood pressure <160/90 controlled with non-lipid and non-glucose altering medication, aged 50 to 75 years, no history of liver or kidney disease, no history of ulcers or other bleeding disorders, a hematocrit greater than 35, a body mass index (BMI) less than 37 kg/m^2, and no physical or orthopedic conditions that would preclude exercise. In addition, women were required to be postmenopausal and agreed to maintain their hormone replacement therapy (HRT) status, either receiving or not receiving HRT. It was necessary that all of the subjects meet these criteria to ensure that subjects had no known conditions limiting participation in any of the testing procedures and to aid in the control of extraneous variables that could affect body composition, plasma lipoprotein-lipid levels and insulin sensitivity. In addition, individuals aged 50-75 were chosen as the subject population since they are a high risk group for CVD and preventive measures could make an impact on their health.

EXPERIMENTAL DESIGN AND VARIABLES

This study was a retrospective analysis of data from the subjects of the Gene Exercise Research Study at the University of Maryland, College Park. This study examined the differences in body composition, plasma lipoprotein-lipid levels and insulin sensitivity in 50-75 year old individuals, grouped by genotype for a single nucleotide polymorphism in exon 10 of the Lamin A/C gene. In addition, gender, age, HRT status, ethnicity, body composition and baseline values were accounted for as covariates in this study.

INSTRUMENTATION

Since, the purpose of this study was to determine genotypic effects associated with differences in body composition, plasma lipoprotein-lipid levels and insulin sensitivity, the independent variable was a gene having associations with body composition measures, plasma lipoprotein-lipid levels and insulin sensitivity. Furthermore, the gene examined has a common genetic polymorphism, which served as levels of the independent variable. The independent variable was the C1908T polymorphism. Genotyping took place in Dr. Robert Ferrell's laboratory at the University of Pittsburgh, Pittsburgh, PA. To ensure validity, test samples were genotyped by direct comparison to controls of known genotype on the same gel. Additionally, two independent observers scored the gels to ensure that the correct genotype was determined.

The dependent variables of the study were initial percent body fat, intra-abdominal fat, subcutaneous fat, trunk fat, plasma lipoprotein-lipid levels, fasting plasma glucose, fasting plasma insulin and insulin sensitivity index, changes in percent body fat, intra-abdominal fat, subcutaneous fat, trunk fat, plasma lipoprotein-lipid levels, fasting

plasma glucose, fasting plasma insulin and insulin sensitivity index, thatoccurred after 24 weeks of exercise training.

DATA COLLECTION PROCEDURES

Screening

This study took place following approval by the University of Maryland, College Park Institutional Review Board. Initially, written informed consent was obtained from all individuals interested in participating in the Gene Exercise Research Study, which had been approved by the Institutional Review Board at the University of Maryland, College Park. The following procedures took place during each potential subject's first laboratory visit. Medical history was examined and height and weight were measured to calculate BMI. Following an overnight fast of 12 hours, a blood sample was drawn for cholesterol, genotype, and glucose measurements. Standard blood chemistry tests were used to assess liver, kidney, and blood disorders and a 2-hour, 75 gram oral glucose tolerance test (OGTT) was performed to assess diabetes status. As inclusion criteria, subjects must have had a fasting glucose of less than 126 milligrams per deciliter (mg/dL), a 2-hour glucose less than 200 mg/dL, and at least one National Cholesterol Education Program lipid abnormality (cholesterol greater than 200 mg/dL, HDL less than 35 mg/dL, triglyceride greater then 200 but less than 400 mg/dL, or LDL greater than 130 mg/dL). Individuals who met inclusion criteria after the first laboratory visit underwent a second screening visit. The second screening visit consisted of a general physical examination and a maximal graded treadmill exercise test, using the Bruce protocol, to screen for CVD. Blood pressure, electrocardiogram, and heart rate were recorded before the test, at the end of every stage in the test, and every other minute for 6 minutes during

active recovery from the exercise test. During this screening visit a potential subject was excluded from the study if his or her blood pressure response to exercise was abnormal, the test was terminated due to cardiovascular symptoms, or the subject had ST segment depression greater than 2 mV (1).

Dietary Stabilization

After successfully completing the screening process, subjects attended dietary lessons on the principles of the AHA Step 1 diet, twice a week for six weeks. The emphasis of the AHA Step 1 diet includes consuming 55-60% of dietary calories from carbohydrate sources, less than 30% of dietary calories from fat sources, less than three grams of salt per day and consuming alcoholic beverages in moderation (2). Subjects were required to maintain the AHA Step 1 diet throughout their participation in the study. Periodically diet recalls and food frequency questionnaires were completed by subjects and reviewed by a registered dietician to ensure compliance. Additionally, subjects were instructed to maintain caloric intake throughout the study, since weight loss can influence many health and fitness variables. Subjects were weighed weekly and those losing more weight than expected or gaining weight were advised in properly increasing or decreasing their daily calories.

Baseline Testing

After completing the dietary stabilization program, subjects underwent two body composition scans. Total body composition was measured via dual energy x-ray absorptiometry (DPX-L, Lunar Corporation, Madison, WI) using standard procedures (19; 25). Subjects were measured in the fasted state using either the slow or medium scan mode (depending on the subject's size). Intra-abdominal fat was quantified midway

between L4 and L5 using computerized tomography as described previously (29). Visceral and subcutaneous adipose tissue areas were delineated by encircling the abdominal muscular wall. Both adipose tissue areas were calculated from the pixel distribution with attenuation values -190 and -30 Hounsfield units.

Following a 12-hour overnight fast, plasma samples were drawn and plasma lipoprotein-lipid profiles were determined. Plasma samples were drawn on two separate occasions and the values were averaged. If the values differed by more than 10%, a third separate measurement was included in the average. A CDC-standardized Hitachi 717 autoanalyzer was used to enzymatically measure plasma triglyceride and total cholesterol levels. LDL-C was calculated using the Friedewald equation (12) and HDL-C was measured after precipitation with dextran sulfate (38). A second precipitation using dextran sulfate was used to separate HDL_2 and HDL_3 (14). HDL_2-C was calculated as the difference between total HDL-C and HDL_3-C.

A 2-hour OGTT was performed following a 12-hour overnight fast. Subjects were asked to refrain from using anti-inflammatory medications for 24-hours before the test and to consume at least 250 grams of carbohydrate per day for the 3 days prior to the OGTT. Subjects recorded all food consumed in the 3 days prior to the OGTT and the records were collected and examined to ensure adequate carbohydrate intake. A 20- or 22-gauge indwelling venous catheter was placed in the antecubital vein, and blood sampling occurred before and every 30 minutes for 2-hours following the ingestion of a 75 gram glucose solution. The blood samples were collected in tubes containing potassium ethylenediaminetetraacetic acid (EDTA) and centrifuged at 4 degrees Celsius.

Plasma samples were then separated and stored at -80 degrees Celsius until assayed for glucose and insulin.

Plasma glucose levels were analyzed with a glucose analyzer (2300 STAT Plus, YSI, Inc., Yellow Springs, OH) using the glucose oxidase method. Plasma insulin levels were determined by radioimmunoassay (HI-14K kit, Linco Research, Inc., St. Charles, MO). Insulin sensitivity index was calculated using an equation developed by Matsuda and DeFronzo. (24).

Genotyping

The blood sample for genotyping was drawn during the screening process. All genotyping took place in the laboratories of Dr. Robert Ferrell at the University of Pittsburgh, Graduate School of Public Health, using standard techniques. Briefly, DNA was isolated from peripheral lymphocytes and genotyping for LMNA was carried out by amplification of the target sequence using polymerase chain reaction. The amplified product was digested with the restriction enzyme *NlaIII*, separated on polyacrylamide gels, and then stained and visualized under ultraviolet light (17).

Exercise Training Intervention

Subjects completed 24-weeks of exercise training by attending three supervised exercise sessions per week. During exercise sessions heart rate monitors were worn to control exercise intensity and blood pressure was assessed before, during and after exercise. Exercise modes included treadmill, stair climber, elliptical, rower, cycling and skiing. Initially, exercise sessions consisted of 20-minutes at 50% VO_{2max}. Exercise duration increased five-minutes each week until week five; at this time subjects were completing 40 minutes of 50% VO_{2max} exercise each session. Exercise intensity was then

increased by 5% each week until an intensity of 70% VO_{2max} was reached. When subjects reached week ten an additional 45-60 minute exercise session at less then 70% VO_{2max} was added. Exercise heart rate, mode and duration were recorded after each session in a logbook.

Final Testing

Following completion of the exercise training, subjects submitted a final seven-day diet record to ensure their adherence to the AHA Step 1 diet prior to reassessment of the plasma lipoprotein-lipid profile, body composition, insulin sensitivity and VO_2 max. Subjects continued with the exercise training until all final tests were completed; additionally, blood samples for plasma lipoprotein-lipid profile and the 2-hour OGTT were drawn 24-36 hours after an exercise session.

STATISTICAL ANALYSES

All comparisons were made between C-allele carriers and TT homozygotes. Chi square tests were used to determine if genotype frequencies were in Hardy-Weinberg equilibrium and to compare potential differences in gender and female HRT status between groups. Analysis of variance (ANOVA) mean comparison procedures were used to examine differences in subject characteristics and plasma lipoprotein-lipid levels, body composition and insulin sensitivity at baseline and their changes with training. Analysis of covariance (ANCOVA) was used to account for other variables influencing body composition, plasma lipoprotein-lipid levels and insulin sensitivity index. Any differences found in baseline variables using ANOVA were re-analyzed with age, gender, HRT status, ethnicity and body composition as covariates. Any differences found in change with training variables using ANOVA were re-analyzed with age, gender, HRT

status ethnicity, body composition and baseline level of that variable as covariates. All statistical analyses were performed using SAS. Statistical significance was set at $p \leq 0.05$ level.

CHAPTER 3: RESULTS

Baseline Population Comparisons

The sample size for hypothesis #1, which compared percent body fat, plasma lipoprotein-lipid profile and insulin sensitivity across LMNA genotypes at baseline, ranged from 33-46 for the C allele carriers and 58-98 for the TT homozygotes. There were 56 male subjects and 88 female subjects. Of the 88 female subjects, 43 were using HRT. The ethnicity of the subject population was 75% Caucasian (n = 108), 17% African-American (n = 25), 5% Asian (n = 7), 2% Hispanic (n = 3) and 1% other (n = 1).

Baseline population allele and genotype frequencies for the LMNA C1908T polymorphism are displayed in Table 1. The T and C allele frequencies were 0.68 and 0.32, respectively. Of the 144 subjects, 68% (n = 98) were TT homozygotes, 29 % (n = 41) were CT heterozygotes, and 3% (n = 5) were CC homozygotes. The genotype frequencies did not differ from those predicted by Hardy-Weinberg equilibrium (χ^2 = 0.08, P >0.05). However, the genotypes were regrouped for statistical analysis as C allele carriers versus TT homozygotes as there were only 5 CC homozygotes. Subject characteristics for the genotype groups are shown in Table 2. General linear ANCOVA techniques, with age, gender, ethnicity and baseline percent body fat as covariates, were used to analyze all data for hypothesis #1.

There were no significant differences between the LMNA genotype groups with regard to body composition variables at baseline as shown in Table 3, although the TT homozygotes tended to have a slightly more favorable body composition than the C allele carriers, in terms of intra-abdominal fat, subcutaneous fat area, trunk fat, and total body fat; however, none of these differences were statistically significant.

As shown in Table 4, there were no significant differences among the LMNA genotype groups relative to baseline plasma lipoprotein-lipid variables. The TT homozygotes tended to have a less favorable lipid profile with higher total cholesterol, triglycerides and LDL and lower HDL and HDL_2, however, these differences were not significant. While the baseline values for HLD_2 are approximately 60% higher in the C allele group, this difference was not significant.

There were no significant differences between the C allele carriers and TT homozygotes with regard to OGTT variables at baseline as shown in Table 5. The insulin sensitivity and fasting plasma glucose values were almost identical between the genotype groups. There appears to be no trend toward differences between the genotype groups with respect to the OGTT variables.

Table 1. Allele and Genotype Frequencies for LMNA C1908T Polymorphism – Baseline Comparison Population

Allele Frequency	**Genotype Frequency**
T 0.68 (n = 98) C 0.32 (n = 46)	TT 0.68 (n = 98) CT 0.29 (n = 41) CC 0.03 (n = 5)

Table 2. Subject Characteristics as a Function of LMNA C1908T Polymorphism Genotype Groups – Baseline Comparison Population

	LMNA Genotype Group	
Characteristics	**TT (n = 98)**	**CT/CC (n = 46)**
Men/Women	41/57	15/31
Women on/not on HRT	27/30	16/15
White/Non-White	71/27	37/9
Age (years)	57.3±0.5	58.6±0.9
Height (cm)	169.3±1.0	166.0±1.3
Weight (kg)	81.5±1.5	80.1±2.0
Body Fat (%)	36.6±1.0	39.0±1.4

Note: Values are expressed as counts or least-squares means ± SEM. Probabilities calculated from $\chi^{2,}$ Fishers Exact tests or ANOVA. All differences between genotype groups were not significant.

Table 3. Body Composition Variables as a Function of LMNA C1908T Polymorphism – Baseline Comparison Population

Characteristics	LMNA Genotype Group	
	TT	**CT/CC**
CTIA (cm^2)	124.9±4.7	123.7±5.1
CTSC (cm^2)	350.6±14.5	361.4±18.2
TRUNK FAT (%)	39.0±0.9	40.3±1.2
TOTAL FAT (%)	38.9±1.0	40.1±1.4

Note: Values are expressed least-squares means ± SEM. Probabilities calculated from ANCOVA. All differences between genotype groups were not significant. Number of subjects for TT genotype group ranged from 89 – 98. Number of subjects for CT/CC genotype group ranged from 42 – 46.

Table 4. Plasma Lipoprotein-Lipid Variables as a Function of LMNA C1908T PolymorphismGenotype Groups – Baseline Comparison Population

	LMNA Genotype Groups	
Characteristics	**TT**	**CT/CC**
Total Cholesterol (mg/dL)	213.4±3.3	209.0±5.6
LDL (mg/dL)	133.1±2.8	126.2±4.7
HDL (mg/dL)	49.1±1.4	52.1±2.8
HDL_2 (mg/dL)	4.9±0.7	8.0±1.7
HDL_3 (mg/dL)	44.1±1.0	43.9±1.4
Triglycerides (mg/dL)	159.8±8.8	149.0±11.8

Note: Values are expressed least-squares means ± SEM. Probabilities calculated from ANCOVA. All differences between genotype groups were not significant. Number of subjects for TT genotype group ranged from 83 – 85. Number of subjects for CT/CC genotype group was 38.

Table 5. OGTT Variables as a Function of LMNA C1908T Polymorphism Genotype Groups– Baseline Comparison Population

	LMNA Genotype Groups	
Characteristics	**TT**	**CT/CC**
Fasting Plasma Glucose (mg/dL)	92.3±1.3	92.7±1.9
Fasting Plasma Insulin (pmol/L)	84.5±3.9	80.0±4.1
Insulin Sensitivity Index	1.6±0.7	1.6±0.1

Note: Values are expressed as least-squares means ± SEM. Probabilities calculated from ANCOVA. All differences between genotype groups were not significant. Number of subjects for TT genotype group ranged from 58 – 74. Number of subjects for CT/CC genotype group ranged from 33 – 37.

Comparison of Training Adaptations

The sample size for hypothesis #2, which compared changes with exercise training in percent body fat, plasma lipoprotein-lipid profile and insulin sensitivity across LMNA genotypes, ranged from 23-33 for the C allele carriers and 49-71 for the TT homozygotes. There were 45 male subjects and 59 female subjects. Of the 59 female subjects, 29 were using HRT. The ethnicity of the population was 79% Caucasian (n = 82), 14% African-American (n = 15), 5% Asian (n = 5), 1% Hispanic (n = 1) and 1% other (n = 1).

Allele and genotype frequencies for the exercise training population are presented in Table 6. The T and C allele frequencies were 0.68 and 0.32, respectively. Of the 104 subjects, 68% (n = 71) were TT homozygotes, 28% (n = 29) were CT heterozygotes, and 4% (n = 4) were CC homozygotes. The genotype frequencies did not differ from those predicted by Hardy-Weinberg equilibrium ($\chi^2 = 0.23$, $P > 0.05$). The genotypes were regrouped for statistical analysis as C allele carriers versus TT homozygotes as there

were only 4 CC homozygotes. Subject characteristics for these genotype groups are shown in Table 7. General linear ANCOVA techniques, with age, gender, ethnicity, final percent body fat and the baseline value of each variable as covariates, were used to analyze all data for hypothesis #2.

Among the exercise training population there were no significant differences between the genotype groups with regard to the change in body composition variables with exercise training as shown in Table 8. However, a significant difference was found for changes in intra-abdominal, trunk fat and total fat measurements with exercise training within the total population and within each of the genotype groups. Although both genotype groups decreased subcutaneous fat, there was not a significant difference between or within the LMNA genotype groups, with respect to changes in CTSC measurements with training as shown in Table 8.

As shown in Table 9, there was no significant difference within either of the genotype groups or the total population with regard to the change in total cholesterol or LDL cholesterol levels with exercise training. However, within the total exercise training population and each of the genotype groups, there was a significant change with exercise training in HDL-C, HDL_2, HDL_3 and TG. Triglyceride values were significantly decreased in both LMNA genotype groups and the total exercisepopulation with exercise training and HDL-C, HDL_2, and HDL_3 values were significantly increased with exercise training.

There were significant differences with exercise training in some OGTT variables among both LMNA genotype groups and the total exercise training population as displayed in Table 10. Insulin sensitivity index improved with exercise training in both

LMNA genotype groups and the total exercise training population, as did fasting plasma insulin values. Fasting plasma glucose values, however, were not significantly improved with exercise training in any of the groups.

Table 6. Allele and Genotype Frequencies for LMNA C1908T Polymorphism – Exercise Training Population

Allele Frequency	**Genotype Frequency**
T 0.68 (n = 71) C 0.32 (n = 33)	TT 0.68 (n =71) CT 0.28 (n = 29) CC 0.04 (n = 4)

Table 7. Subject Characteristics as a Function of LMNA C1908T Polymorphism Genotype Groups – Exercise Training Population

	LMNA Genotype Groups	
Characteristics	TT (n = 71)	CT/CC (n = 33)
Men/Women	31/40	14/19
Women on/not on HRT	16/24	13/6
White/Non-White	55/16	27/6
Age (years)	57.5±0.6	58.8±1.0
Height (cm)	169.8±1.1	167.2±1.6
Weight (kg)	79.3±1.7	78.5±2.4
Body Fat (%)	35.8±1.2	36.9±1.6

Note: Values are expressed as counts or least-squares means ± SEM. Probabilities calculated from $\chi^{2,}$ Fishers Exact test, or ANOVA. All differences between genotype groups were not significant.

Table 8. Exercise Training Induced Changes in Body Composition Variables as a Function of LMNA C1908T Polymorphism Genotype Groups – Exercise Training Population

Characteristics	Total Population	LMNA Genotype Groups TT Homozygotes	CT/CC
CTIA (cm^2)			
Baseline	121.9±3.8	122.2±5.0	121.4±5.8
Final	111.7±3.4	112.0±4.3	111.2±5.5
Change	-10.6±2.3*	-10.9±2.8*	-10.2±4.5*
CTSC (cm^2)			
Baseline	316.9±12.5	317.4±16.4	316.0±18.1
Final	310.3±11.6	309.0±14.6	312.8±19.3
Change	-5.9±3.8	-9.2±4.7	-4.6±6.3
TRUNK FAT (%)			
Baseline	37.4±0.8	37.2±1.0	37.8±1.3
Final	35.5±0.8	35.4±1.0	35.7±1.3
Change	-1.7±0.3*	-1.6±0.3*	-1.8±0.5*
TOTAL FAT (%)			
Baseline	36.4±1.0	36.1±1.2	36.9±1.6
Final	34.7±0.9	34.6±1.2	35.0±1.6
Change	-1.4±0.2*	-1.3±0.2*	-1.5±0.3*

Note: Values are expressed as least-squares means ± SEM. Probabilities calculated from ANCOVA. * indicates significant change with training within that group at $p \leq 0.05$. The number of subjects for the total population, TT homozygotes and C allele carriers ranged from 98-102, 66-69 and 32-33, respectively.

Table 9. Exercise Training Induced Changes in Plasma Lipoprotein-Lipid Variables as a Function of LMNA C1908T Polymorphism Genotype Groups – Exercise Training Population

		LMNA Genotype Groups	
Characteristics	**Total Population**	**TT Homozygotes**	**CT/CC**
Total Cholesterol(mg/dL)			
Baseline	207.8±3.2	212.0±3.6	198.8±6.2
Final	206.6±3.1	211.1±3.8	197.0±5.2
Change	-1.2±2.0	-0.9±2.4	-1.9±3.7
LDL (mg/dL)			
Baseline	128.0±2.8	131.9±3.2	119.8±5.2
Final	127.6±2.6	131.7±3.2	118.8±4.4
Change	-0.2±1.9	-0.1±2.4	-0.9±3.4
HDL (mg/dL)			
Baseline	48.0±1.4	47.2±1.6	49.8±2.9
Final	51.1±1.5	50.3±1.7	52.6±3.2
Change	3.4±0.5*	3.6±0.6*	2.8±0.8*
HDL_2 (mg/dL)			
Baseline	4.8±0.7	4.0±0.7	6.3±1.7
Final	6.4±0.9	5.7±0.9	7.8±2.0
Change	1.5±0.4*	1.4±0.6*	1.5±0.6*
HDL_3 (mg/dL)			
Baseline	43.1±0.9	43.0±1.1	43.2±1.5
Final	44.9±0.9	44.8±1.1	45.0±1.5
Change	1.9±0.5*	2.0±0.7*	1.8±0.8*
Triglycerides (mg/dL)			
Baseline	157.5±7.9	164.5±10.0	142.5±12.5
Final	141.8±6.6	146.8±8.1	131.2±10.9
Change	-16.1±4.8*	-18.4±5.9*	-11.2±8.5*

Note: Values are expressed as least-squares means ± SEM. Probabilities calculated from ANCOVA. * indicates significant change with training within that group at p≤0.05. The number of subjects for the total population, TT homozygotes and C allele carriers ranged from 101-104, 68-71 and 33, respectively.

Table 10. Exercise Training Induced Changes in OGTT Variables as a Function of LMNA C1908T Polymorphism Genotype Groups – Exercise Training Population
.

Characteristics	Total Population	LMNA Genotype Groups TT Homozygotes	CT/CC
Fasting Plasma Glucose (mg/dL)			
Baseline	91.4±1.1	90.7±1.2	93.0±2.2
Final	92.8±1.2	92.2±1.2	94.0±1.8
Change	1.3±1.2	1.5±1.3	1.0±2.0
Fasting Plasma Insulin (pmol/L)			
Baseline	81.2±3.5	83.0±4.6	77.3±5.0
Final	70.1±2.5	72.0±3.2	66.0±4.1
Change	-11.1±2.4*	-10.9±3.1*	-11.3±3.5*
Insulin Sensitivity Index			
Baseline	1.5±0.1	1.5±0.1	1.4±0.1
Final	1.7±0.1	1.7±0.1	1.7±0.1
Change	0.2±0.1*	0.2±0.1*	0.2±0.1*

Note: Values are expressed as least-squares means ± SEM. Probabilities calculated from ANCOVA techniques. * indicates significant change with training within that group at $p \leq 0.05$. The number of subjects for the total population, TT homozygotes and C allele carriers was 72, 49 and 23, respectively.

CHAPTER 4: DISCUSSION

Studies have associated the LMNA C1908T gene polymorphism, specifically the T-allele, with increased occurrence of the metabolic syndrome, increased fasting TG, increased indices of obesity, and decreased HDL-cholesterol levels (34; 16; 18). Contrastingly, a study investigating this polymorphism in the Pima Indians found the T allele to be significantly associated with increased insulin sensitivity (39). In addition to genetic influences, exercise training has been shown to affect the metabolic syndrome and its components, therefore this study sought to examine differences in body composition, plasma lipoprotein-lipid profile, and insulin sensitivity index at baseline and the change in these variables with exercise training, between C allele carriers and TT homozygotes of the C1908T polymorphism. The results of this study indicated that there were no significant differences between the genotype groups at baseline or their change with training, regarding body composition, plasma lipoprotein-lipid profile, or insulin sensitivity index.

Hegele and colleagues reported that among a population of Oji-Cree Aboriginal Canadians, TT homozygotes had significantly higher indices of obesity (BMI, wasit:hip ratio) than the C allele carriers. The TT homozygotes also had a higher body fat percentage but this was not significantly different from the C allele carriers (17). Hegele and colleagues also reported on the association between anthropometric traits and the LMNA C1908T polymorphism among the Inuit Indians of Canada (18). They reported that subjects with the T allele had significantly higher body weight, BMI, waist circumference, waist-to-hip ratio, subscapular skinfold thickness and subscapular to triceps skinfold thickness ratio. These finding are consistent with their previous findings

among the Oji-Cree Aboriginal Canadians (17). Weyer and colleagues investigated an association between the LMNA C1908T polymorphism and various physical and metabolic characteristics among a population of Pima Indians (39). Their results show that among this population, the SNP is not associated with weight, percentage of body fat, or waist-to-thigh ratio. They did, however, find that TT homozygotes had a 10% smaller mean subcutaneous abdominal adipocyte size (39).

In contrast to the findings of the previously mentioned studies, the TT homozygotes in our baseline population tended to have a slightly more favorable body composition, however these differences were not significant. Among the Pima Indian population, TT homozygotes had a significantly smaller mean subcutaneous adipocyte size, while adipocyte size was not measured in our subjects. Among the exercise training population from our study, the C allele carriers tended to show a greater improvement in body composition with exercise training, although again this improvement was not significantly different from that in the TT homozygotes. There was a significant improvement with exercise training within both genotype groups regarding intra-abdominal fat, trunk fat and total body fat. Subcutaneous fat was lower in both groups following the exercise training, but these reductions were not significant.

Steinle and colleagues reported that the LMNA C1908T polymorphism was associated with the metabolic syndrome as well as higher fasting TG and lower HDL-C among a population of Old Order Amish subjects (34). Amish subjects carrying the T allele had less favorable lipid profiles and higher prevalence of the metabolic syndrome (34).

In our study, there was no significant difference between the C allele carriers and TT homozygotes regarding TG or HDL-C values at baseline, and although the TT homozygotes tended to have a less favorable lipid profile with higher TC, TG and LDL and lower HDL, these differences were not significant. After 24-weeks of exercise training TG levels were significantly lower and HDL-C was significantly higher within both genotype groups, although there was not a significant difference in the changes with exercise training between the two groups. While TC levels were also lowered in both genotype groups with exercise training, there was not a significant difference in these changes with training either between or within the two LMNA genotypegroups or the total exercise training population.

In their investigation of the LMNA C1908T polymorphism in the Oji-Cree Aboriginal Canadians, Hegele and colleagues measured fasting plasma insulin (FPI), which was found to be somewhat lower in TT homozygotes; however, this difference was not significant. While Hegele et. al. reported taking fasting glucose samples, these results were not presented (17). Weyer and colleagues investigated an association between the LMNA C1908T polymorphism and metabolic characteristics among a population of Pima Indians (39). Their results show that in this population, the SNP is not associated with fasting insulin and glucose values. Additionally, Weyer et. al. reported that among the Pima Indians, the T allele was significantly associated with increased insulin sensitivity (39).

In our baseline population, the TT homozygotes and C allele carriers had almost identical FPI, FPG and insulin sensitivity index (ISI) values, and there were no significant differences between the genotype groups. Among the exercise training

population in our study, ISI and FPI improved significantly in both genotype groups following exercise training, but there was not a significant difference between the genotype groups. Exercise training did not significantly improve FPG within the genotype groups or the exercise training population.

The purpose of our study was to determine the effect of the C1908T polymorphism on body composition, plasma lipoprotein-lipid profile and insulin sensitivity. Our first hypothesis proposed that subjects in our baseline population carrying the C allele would have significantly lower percent body fat, a more favorable plasma lipoprotein-lipid profile and higher insulin sensitivity. This hypothesis was based upon the results of the previously discussed studies. Our results show that while there were some slight differences between the genotype groups in the baseline population, none of these differences were significant regarding any of the variables.

Our second hypothesis proposed that after 24 weeks of exercise training subjects carrying the C allele would have significantly less improvement in percent body fat, plasma lipoprotein-lipid profile and insulin sensitivity. There have been no previous exercise training studies conducted concerning the LMNA C1908T polymorphism, therefore, this hypothesis was based upon the idea that if, in fact, the TT homozygotes had a less favorable profile relative to the components of the metabolic syndrome at baseline, they would have more room for improvement than the C allele carriers. Our results show that although both genotype groups improved with exercise training in relation to many of the variables, the TT homozygotes did not improve significantly more for any of the variables than the C allele carriers.

There are a few differences between our study and those previously mentioned, in addition to the results. A large difference is the homogeneity of the populations. Each of the previously mentioned studies investigated a very ethnically homogeneous group, often times consisting of family members. In addition to the ethnic homogeneity, these other populations were geographically and/or culturally isolated for many generations which resulted in very similar eating and activity behaviors. The subjects in our study were not related and had a varied ethnicity. While a majority of the baseline population was Caucasian (75%), there were also African-American, Asian and Hispanic subjects. Statistical analysis for differences between ethnic groups relative to our variables was not conducted. Our protocol attempted to diminish the variability among subjects with regard to diet and exercise. Our subjects attended dietary classes to instruct them in the principles of the AHA Step 1 diet and submitted diet recalls at various stages of the study, however; all dietary information was self-reported. Additionally, subjects participated in structured and supervised exercise sessions three times a week for 24 weeks. The structured dietary classes and exercise sessions were used to increase homogeneity among the subjects.

Another difference between our investigation and those previously mentioned involves the sample size and genotype and allele frequencies which are shown in Table 11. Our investigation had the fewest number of subjects at 144, while the number of subjects in the other studies ranged from 186 to 908. Because of the small percentage of CC homozygotes (n = 5), our statistical analysis was conducted on C allele carriers versus TT homozygotes. As shown in Table 11, the only previous study to make

comparisons between all three genotype groups was Weyer et. al in their study on the Pima Indians (39).

Table 11. Allele and Genotype Frequencies

Subjects	Allele Frequencies	Genotype Frequencies
Old Order Amish n = 908 T allele carriers vs. CC homozygotes (34)	C = 65% T = 35%	CC = 42% CT = 45% TT = 12%
Oji-CreeAboriginal Canadians n = 306 C allele carriers vs. TT homozygotes (17)	C = 23% T = 77%	CC = 5% CT = 36% TT = 59%
Inuit Canadians n = 186 T allele carriers vs. CC homozygotes (18)	C = 52% T = 48%	CC = 23% CT = 58% TT = 19%
Pima Indians n = 255 compared all three genotype groups (39)	C = 55% T = 45%	CC = 29% CT = 51% TT = 20%
GERS – Baseline n = 144 C allele carriers vs. TT homozygotes	C = 32% T = 68%	CC = 3% CT = 29% TT = 68%
GERS – Exercise Training n = 104 C allele carriers vs. TT homozygotes	C = 32% T = 68%	CC = 4% CT = 28% TT = 68%

Another difference between our study and the previous studies was the inclusion of an exercise training intervention. Exercise training has been shown to improve the components of the metabolic syndrome that we investigated and among our subjects various body composition, plasma lipoprotein-lipid and OGTT variables were improved to a substantial extent with exercise training. However, the extent of improvement or change in these variables with exercise training did not differ significantly between the genotype groups.

It is not yet clear how mutations in the lamin A and C proteins, which are expressed in nearly all differentiated somatic cells, cause different diseases. The current hypotheses which have been proposed to address this question are the mechanical stress or structural hypothesis and the gene-expression hypothesis.

The structural hypothesis suggests that mutations in lamins causing abnormalities in nuclear structure lead to increased susceptibility of cellular damage due to physical stress. This physical stress is likely to occur in skeletal and cardiac muscles where contractile forces are present. Lammering and colleagues studied LMNA deficient mice and found that the lack of A-type lamins compromised the structural integrity of the nuclear envelope. These cells were susceptible to stress-induced damage which led to loss of cell viability (22). Further support for this hypothesis is presented by Stewart et al., (36) who also studied LMNA knockout mice. These mice developed cardiac myopathies and skeletal abnormalities which resembled EDMD and also showed changes in peripheral nerve axons, but did not show signs of lipodystrophy. Despite the evidence supporting this hypothesis there are some limitations to it. For example, weakening of the nucleus is less likely to account for defects in adipose tissue because there is a lack of mechanical stress in this type of tissue (27).

The gene-expression hypothesis proposes that mutations in A-type lamins, which are important regulators of gene-expression, will alter their interaction with various regulatory proteins and ultimately promote disease in different tissues (42). Lamins interact with many nuclear factors, help regulate transcription and influence the organization of heterochromatin. Changes in chromatin organization that have been observed in LMNA knockout mice and AD-EDMD patients could lead to altered tissue

specific gene-expression (30). Stierle et al. showed that there are two peptide regions of lamins A and C that take part in DNA-binding and alterations in this interaction may cause some laminopathies (35).

The gene-expression hypothesis may be useful in explaining lipodystrophy disorders where the mechanical stress hypothesis had its inadequacies. While LMNA deficient mice have been shown to develop cardiomyopathy and muscular dystrophy they do not exhibit lipodystrophy or insulin resistance, suggesting that lipodystrophy is not caused by loss of lamin A or C function. Fibroblasts from lipodystrophy affected individuals display alterations in chromatin structure suggesting the mutations causing lipodystrophies obstruct the regulation of adipocyte-specific gene expression (4).

Some limitations in this study should be noted. The sample size for our study was smaller than other studies investigating this polymorphism and we were not able to compare all three genotype groups. The lack of influence of the C1908T polymorphism may be due to small sample size and the grouping of subjects into two genotype groups. Analyzing each genotype group separately, with adequate sample sizes, may enable detection of significant differences.

CHAPTER 5: CONCLUSION

To conclude, the LMNA C1908T polymorphism does not appear to be associated with the baseline or change with training values of body composition, plasma lipoprotein-lipid and OGTT variables. However, more research is needed, particularly with a larger sample size of rare allele carriers. Future studies should focus on obtaining a more balanced study population across genotype groups and examine other prospective candidate genes for interactions. Finally, the effect of genes on body composition, plasma lipoprotein profile and insulin sensitivity merits further study.

APPENDIX A: PROPOSAL INFORMATION

Statement of the problem
Experimental hypotheses
Delimitations
Limitations
Operational definitions

APPENDIX A

PROPOSAL INFORMATION

Statement of the Problem

The purpose of this project was to determine the effect of the C1908T polymorphism on body composition, plasma lipoprotein profile and insulin sensitivity in sedentary subjects before, and their changes with, 24-weeks of exercise training.

Experimental Hypothesis

1. Subjects carrying the C-allele will have significantly more favorable body composition, plasma lipoprotein profile and higher insulin sensitivity at baseline
2. After 24 weeks of exercise training subjects carrying the C-allele will have significantly less improvement in body composition, their plasma lipoprotein profile and insulin sensitivity.

Delimitations

1. Subjects for the study ranged in age from 50 to 75 years and have taken part in the Gene Exercise Research Study at the University of Maryland, College Park.
2. As members of the Gene Exercise Research Study, the subjects have been recruited from the University of Maryland and Washington D.C. metropolitan areas.
3. As participants in the Gene Exercise Research Study, the subjects were nonsmoking, nondiabetic, sedentary, healthy with the exception of having high cholesterol levels, calculated as having a body mass index less than 37 kilograms per meter squared (kg/m^2), not taking medications known to affect glucose levels, and postmenopausal, if female.

Limitations

1. The subjects recruited as part of the Gene Exercise Research Study were from the University of Maryland, College Park and Washington D.C. metropolitan areas and may not be representative of the entire population.
2. The frequencies of genotypes were not controlled, so rare allele carriers were grouped together in the analyses because sample sizes were inadequate.
3. This study is retrospective in nature.

Operational Definitions

1. *Glucose* – A monosaccharide containing six-carbon atoms that is used as a major energy substrate in the body.
2. *Insulin* – A pancreatic 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.
3. *Oral Glucose Tolerance Test* – Diagnostic assessment measuring the ability of the body to maintain euglycemia in response to a glycemic challenge.
4. *Genetic Polymorphism* – Common variation in genes at the DNA level.
5. *Allele* – Alternative form of a DNA sequence at a given locus.

APPENDIX B: LITERATURE REVIEW

The Metabolic Syndrome
Heritability of the Metabolic Syndrome and its Components
Effects of Exercise Training
Heritability of Exercise Training Adaptations
Nuclear Lamina
Lamins A & C
The C1908T Polymorphism
Summary
References

APPENDIX B

LITERATURE REVIEW

Previous studies have suggested that the C1908T polymorphism of the Lamin A/C gene (LMNA) may be associated with obesity, unfavorable plasma lipoprotein lipid profiles and the metabolic syndrome in certain populations. Exercise training has also been shown to affect these cardiovascular disease (CVD) risk factors. Accordingly, the role of the C1908T polymorphism and exercise training in the development and treatment of the previously mentioned CVD risk factors will be the focus of this review.

The Metabolic Syndrome

Insulin resistance and obesity, in conjunction with dyslipidemia, is referred to as the metabolic syndrome. The metabolic syndrome affects approximately 25% of adults over the age of 20 and up to 45% of adults over 50. The components of this syndrome, which serve as risk factors for type II diabetes mellitus and CVD, in addition to having environmental propensity, may be the result of genetic susceptibility (4; 42).

Insulin resistance and Obesity

Insulin resistance, to which obesity and physical inactivity contribute, is caused by a combination of insulin resistant cells and insufficient insulin secretion by the pancreas. The normal response to an increase in blood glucose levels after a meal involves release of insulin from the pancreas. Insulin facilitates the diffusion of glucose into a cell by binding with receptors. Once insulin is bound to the cell, glucose can enter, where it is either used for energy or stored for later use. By facilitating the diffusion of glucose into a cell, insulin aids in the maintenance of normal blood glucose levels. When an individual is insulin resistant, or has decreased insulin sensitivity, the normal amount

of insulin secreted does not assist the diffusion of glucose into the cell. The pancreas overproduces insulin in response to increased blood glucose levels, which is referred to as hyperinsulinemia. Despite the overproduction of insulin a high blood glucose level is maintained and often leads to type 2 diabetes. Obesity contributes to insulin resistance because as adipocytes increase in size insulin receptor density decreases (14).

Dyslipidemia

Dyslipidemia is characterized by increased levels of triglyceride, total cholesterol and LDL in conjunction with decreased HDL concentrations. Lipoproteins, formed in the liver from the union of triglycerides, phospholipids or cholesterol with protein, are the main form for lipid transport in the blood. These compounds are divided into various classes based on their density: chylomicrons, high-density, low-density and very-low density.

Very-low-density lipoprotein (VLDL), which contains the highest percentage of lipid and therefore has the lowest density, transports triglycerides to adipose and muscle tissue. VLDL is degraded by lipoprotein lipase into low-density lipoprotein (LDL), which contains fewer lipids. LDL conveys cholesterol to the linings of the blood vessels to which it adheres, forming plaque. High levels of VLDL and LDL are associated with CVD (36).

High-density lipoprotein (HDL-C) is produced by the liver and small intestine, and contains the largest percentage of protein and the lowest percentage of cholesterol and lipids. High-density lipoproteins protect against CVD by conveying cholesterol away from blood vessels and to the liver for eventual excretion (36). Numerous studies have

established an inverse relationship between the concentration of HDL-C and cardiovascular disease (18).

Type II diabetes mellitus, the metabolic syndrome and their shared risk factors for CVD can, in many cases, be managed or altered markedly by aerobic exercise training.

Heritability of the Metabolic Syndrome and its Components

The HERITAGE Family Study (HEalth, RIsk factors, exercise Training And GEnetics) has undertaken the task of studying the role of genetics in cardiovascular, metabolic, and hormonal responses to aerobic exercise training. Their subjects consist of 90 Caucasian families and 40 African-American families. The families, consisting of both parents and at least three biological adult offspring, participated in the same 20-week exercise training program (5).

Results from the HERITAGE Family Study show that the metabolic syndrome significantly accumulates within families (33). Additional reports from the HERITAGE Family Study show that genetic influences play a role in insulin levels, abdominal visceral fat and plasma lipoprotein lipid profile. (25; 13).

Heritability of Dyslipidemia

Pollin et al., investigated the heritability of plasma lipoprotein-lipid levels among 28 Old Order Amish families. The specific variables studied included total serum cholesterol, high density lipoprotein cholesterol, triglycerides and low-density lipoprotein cholesterol. Linkage analyses indicated that lipid levels were substantially heritable among the Old Order Amish (39). Austin et al., also established the heritability of plasma lipoprotein-lipid levels among Japanese Americans. Results reveal that in this population

52% of the variation in plasma lipoprotein-lipid levels is attributable to genetic factors (3).

Heritability of Obesity

The role of genetics in determining body composition has also been investigated. Hong et. al. focused on the heritability of abdominal visceral fat (AVF) as it has been reported that AVF is a predictor of diabetes and correlates more strongly to insulin resistance than other indices of obesity in many studies. The heritability for AVF was found to be 42% among the 98 families investigated (23). Hsu et al. also examined the role of genetics in body composition. This study concentrated on percentage of fat mass (%FM), whole body fat mass (FM) and lean mass (LM) as measured by DEXA, in subjects from 244 families. Study outcomes show that %FM, FM and LM are 48%, 69% and 49% heritable, respectively (25).

Effects of Exercise Training

Body Composition and Lipids

Exercise training has been shown to have a favorable impact on phenotypes associated with diabetes and the metabolic syndrome. Halverstadt et al., recently showed that body composition and plasma lipoprotein lipid profile improve significantly in men and women following 24-weeks of aerobic exercise training. The exercise training consisted of three sessions a week for 24-weeks. Training began with 20 minutes of exercise at 50% of VO_{2max} and progressed to 40 minutes at 70% VO_{2max} for the remaining 14 weeks. At week 12 an additional exercise session lasting 45-60 minutes at a lower intensity was added. Among healthy, sedentary men and women, body weight, percent body fat and visceral adipose tissue decreased significantly following the exercise

training. Similarly, triglycerides decreased, while HDL-C levels increased (20). Review articles by Durstine et. al. and Poirier et. al. show that results similar to these have been found in numerous other laboratories as well (11; 38).

Insulin

A study by McKenzie et al., utilizing the same previously mentioned aerobic exercise training protocol, showed a significant increase in insulin sensitivity index as well as a decrease in fasting insulin with aerobic exercise training (34). Ryan et. al., indicates that these responses have also been found by other investigators (41).

Heritability of Exercise Training Adaptations

Heritability of Changes in Insulin Values with Exercise Training

Adaptations to exercise training have also been shown to be heritable. Lakka et al. found strong evidence for linkage in fasting insulin response to aerobic exercise training. Using data from the HERITAGE Family Study, involving 99 white families and 105 black families, this group measured fasting plasma glucose and insulin before and following 20-weeks of aerobic exercise training. Single and multipoint linkage analyses were performed on the phenotype data for baseline and final measures. No significant training effects were found for the glucose measures and subsequently this data was eliminated from the linkage analysis, however, insulin was found to decrease significantly following the aerobic exercise training. Changes in insulin levels with aerobic exercise training were shown to be heritable among both black and white families (31).

Heritability of Changes in Plasma Lipoprotein-Lipid Values with Exercise Training

Another investigation within the HERITAGE Family Study explored the heritability of plasma lipoprotein-lipid responses to aerobic exercise training. The data for this study involved 99 white families and 115 black families whose plasma lipoprotein-lipid profiles were assessed at baseline and following 20-weeks of training. Total cholesterol (TC), triglycerides (TG), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL-C) and high-density lipoproteins (HDL-C, HDL_2-C, HDL_3-C) were measured. There was significant family resemblance in the changes with training with approximately 30% of the phenotypic variance accounted for by familial factors (40).

Heritability of Changes in Body Composition Values with Exercise Training

The heritability of changes in body composition with aerobic exercise training was examined among 99 white families participating in the HERITAGE Family Study. The outcome of this multipoint linkage analysis investigation showed that lean body mass, body fat content, and BMI changes with aerobic training were heritable. LOD scores between 2.0 and 3.0 indicate suggestive results and the LOD scores for change in percent body fat and fat mass were both 2.2 for a locus on chromosome 1. BMI presented an LOD score of 2.4 for a locus on chromosome 5 and fat-free mass showed an LOD score of 2.3 for a locus on chromosome 12 (9).

Nuclear Lamina

The nuclear lamina, the innermost layer of the nuclear envelope (NE), is a fibrous meshwork composed of intermediate filament proteins called lamins. There are two classes of lamins; A-type and B-type. B-type lamins, B1 and B2, are encoded by the genes LMNB1 and LMNA2 respectively, while A-type, lamins A and C, arise by

alternative splicing of RNA encoded by a single gene, LMNA. The structure and function of A-type lamins will be discussed in detail in the following section.

While perhaps the most significant role of the nuclear lamina is that of maintaining the integrity of the NE (6), it also determines the size and shape of the nucleus (27). Another function of the lamina is to attach to the annular subunits of the nuclear pore complexes controlling their arrangement in the NE (19). Lamins have been found not only lining the inner nuclear membrane but also further inside of the nucleus, suggesting that they also form a structural framework deeper inside the nucleus (19). The close proximity of the nuclear lamina to chromatin has led some to propose that it also has a role in anchoring and organizing chromatin (2). It has also been hypothesized that lamins play a role in DNA replication, specifically the elongation phase (2).

Lamins A & C

A-type lamins, which include lamin A and lamin C, are encoded by a gene (LMNA) located on chromosome 1q21.2. Alternative splicing of this gene produces either lamin A or lamin C. These proteins are identical for the first 566 amino acids and diverge at their carboxyl termini. Pre-lamin A has 98 distinctive carboxyl terminal amino acids, of which the last 18 amino acids are removed to form lamin A, while lamin C has 6 carboxyl terminal amino acids (22). The last four amino acids of lamin A form a CaaX (cysteine-aliphatic-aliphaticany amino acid) sequence. Post-translational modifications involving farnesylation and proteolytic cleavage are necessary for lamin A's insertion into the inner nuclear membrane (INM). Lamin C does not contain this CaaX sequence, therefore it's insertion into the INM is dependent upon lamin A. An additional structural

aspect in which lamins A and C differ is that lamin C contains six amino acids in the middle of exon 10 that lamin A does not (30).

An investigation by Lin et al., determined the structure of the LMNA gene. This gene contains 12 exons spread over approximately 24 kilobases. All 11 introns contain the nucleotides GT at their 5' end, and a pyrimidine-rich region followed by AG at the 3' end. Exon 1 codes for the globular amino-terminal head in the first part of the central ά helical rod domain of both lamins A and C. The remainder of the central ά helical rod domain for both proteins is encoded by exons 2-6. Exons 7-9 code for the carboxyl-terminal tail domains, that are common among lamins A and C. The splice site for generating either lamin A or C is contained within exon 10. Finally, located in exons 11-12 are lamin A specific sequences coding for the CaaX box. Exons 11 and 12 also comprise the 3' region of lamin A (32).

As previously stated, alternative splicing of the LMNA gene in exon 10 gives rise to either pre-lamin A or lamin C mRNA. Amino acid 566 is the last 3' amino acid common to the two proteins. Following amino acid 566 are six additional lamin C specific amino acids and a TGA termination codon. Located 77 nucleotides 3' to the TGA termination codon is the polyadenylation signal of lamin C. Pre-lamin A is formed by the splicing of 743 nucleotides to the 3' end of nucleotide 566. The termination codon, TAA, is located in exon 12 and 952 nucleotides 3' to this codon is the polyadenylation sequence. Pre-lamin A is polyadenlyated 16 nucleotides 3' to this sequence (32).

Lamins A and C are expressed in most terminally differentiated mammalian cells; however, in early mammalian embryos and some undifferentiated cells these proteins are

absent. Lamins A and C seem to be expressed in equal amounts or not at all in cells; therefore, it is unlikely that alternative splicing favors one over the other (32).

It has been hypothesized that lamins A and C function in DNA replication and transcription. Moir et al showed that disruption of lamin organization blocks the elongation phase of DNA replication (38). Furthermore, Spann et al showed that disruption of the lamin organization inhibits RNA polymerase II, which ultimately affects transcription (16).

Sometimes diseases resulting from mutations in two or more different genes will exhibit similar phenotypes; however, rarely do mutations in just one gene result in numerous diseases. In recent years, mutations in one gene, lamin A/C, have been found to be associated with several seemingly different and unrelated diseases. Diseases caused by mutations in lamin A/C are collectively referred to as laminopathies. The laminopathies can be grouped according to the tissue they affect; striated muscle, adipose and bone tissues, peripheral nervous tissue and finally an ailment affecting many tissues and causing premature aging (45).

Laminopathies associated with muscular diseases differ in the specific muscle tissue affected, either cardiac or skeletal, and also in onset of the condition. Emery-Dreifuss muscular dystrophy (EDMD) is distinguished by three clusters of ailments: (a) early onset contractures at the neck, ankles and elbows, (b) slow and progressive muscle wasting, and (c) cardiac conduction defects. Heart problems, beginning with conduction defects, do not usually develop until the second decade. Most EDMD patients develop cardiac arrthymias, leading to complete heart block and sudden heart failure. Muscles in the shoulders, upper arm and lower leg exhibit the most severe muscle wasting. The joint

contractures are the least understood of the symptoms; it is yet to be determined if they are a result of muscle shortening or due to shortening of the tendons (2).

There are two forms of EDMD, the X-linked and the autosomal dominant varieties (AD-EDMD). X-linked EDMD is inherited by a mutation in the emerin gene which encodes the nuclear membrane protein emerin (2). Emerin is closely related in sequence and structure to the other nuclear envelope proteins, lamins A and C which arise from the lamin A/C gene (24). A missense mutation in the lamin A/C gene produces AD-EDMD (4). The similarity in phenotypes of the two forms of EDMD suggests a close functional relationship between the emerin protein and the lamin A/C proteins. Cao et. al showed emerin and lamin A/C are indeed linked (8). Furthermore, lamin A/C has been found to direct normal emerin localization at the inner nuclear membrane (43).

Another disease associated with lamin A/C that affects striated muscle tissue is Limb Girdle Muscular Dystrophy type 1B (LGMD1B). LGMD1B has been mapped to the same chromosomal locus as AD-EDMD and is inherited as a slowly progressive autosomal dominant trait. Symptoms include cardiac conduction disturbances, dilated cardiomyopathy and the absence of early contractures (43). Three mutations in the lamin A/C gene of affected patients that were not present in unaffected control subjects have been identified. There are genetic and non-genetic factors which cause differences in phenotypes between AD-EDMD and LGMD1B; however, both disorders are the result of a disease-causing mutation in the same gene (8).

Dilated cardiomyopathy (DCM), characterized by enlargement of the heart's chambers and impaired systolic contraction, is a principal cause of congestive heart failure (10). DCM can be genetically transmitted and DCM associated with conduction -

system disease was mapped to the same region of chromosome 1 as AD-EDMD (29). As stated previously, heart conduction defects are one of the symptoms of AD-EDMD (2). Given the location of lamin A/C on chromosome 1 and the cardiovascular phenotype associated with AD-EDMD, it was hypothesized that a mutation in the lamin A/C gene caused DCM. Evidence for this hypothesis was established by Fatkin et. al (12).

Dunnigan-type familial partial lipodystrophy (FPLD) is a rare autosomal-dominant disease characterized by loss of adipose tissue. Individuals with FPLD have normal fat distribution at birth, but during puberty they lose subcutaneous fat from the extremities, trunk and gluteal region. While excess fat may be deposited on the face, neck and back, there are normal stores of inter-muscular, intra-abdominal, intrathoracic and bone marrow fat. Additionally, those with FPLD often exhibit insulin resistance with hyperinsulinemia and develop diabetes later in life (17; 7).

The FPLD gene was mapped to the same region of chromosome 1 as lamin A/C by several groups (26; 28; 2). Using previous knowledge of the role of mutations in lamin A/C associated with autosomal-dominant Emery-Dreifuss muscular dystrophy (AD-EDMD), researchers hypothesized that mutations in lamin A/C may also be responsible for the progressive degeneration of adipocytes occurring in FPLD. Subsequently, the LMNA R482Q mutation was identified as underlying the site-specific adipocyte degeneration observed in FPLD (8).

An additional adipose tissue affecting disease, Mandibuloacral Dysplasia (MAD), is caused by a mutation in lamin A/C. MAD is a rare autosomal recessive disorder with onset during midchildhood. This disease is characterized by mandibular and clavicular hypoplasia, delayed closure of the cranial suture, joint contractures, hyperpigmentation,

lipodystrophy and several characteristics of the metabolic syndrome (15). Novelli et. al. studied the adipose tissue distribution among individuals diagnosed with MAD and accordingly described two separate patterns. Type A pattern restricted fat loss to the extremities while type B pattern showed a more generalized subcutaneous fat loss (37).

The metabolic symptoms and partial lipodystrophy characteristics of MAD are similar to FPLD. The similarities in the two conditions prompted investigators to screen MAD patients for mutations in the lamin A/C gene. It was found that a missense mutation, R527H, resulted in MAD (37).

The C1908T Polymorphism

A genetic variation in lamin A/C has been reported to be associated with obesity-related anthropometric and biochemical traits in aboriginal Canadians and Inuit (21; 8), higher fasting triglyceride, lower HDL-cholesterol concentrations and the metabolic syndrome in an Amish population (2) and reduced subcutaneous abdominal adipocyte size in Pima Indians (44). Specifically, the mutation is a common single nucleotide polymorphism (SNP) in exon 10 of LMNA. This silent C→T substitution occurs at nucleotide 1908 and affects the third base within codon 566, which is also the last codon shared between lamins A and C before alternative splicing produces one of these proteins (21).

Hegele et. al. investigated the C1908T polymorphism among Oji-Cree aboriginal Canadians and also among the Canadian Inuit. It was determined that in the Oji-Cree population the frequencies for the C and T alleles were 23% and 77%, respectively, and the genotype frequencies were as follows, 5% CC homozygous, 36% heterozygous and 59% TT homozygotes. Comparisons made between C-allele carriers and the TT

homozygotes showed that the TT homozygous had significantly higher indices of obesity (21). Among the Inuit population, frequencies for the C and T alleles were 52% and 48% respectively, and the genotype frequencies were as follows, 23% CC homozygous, 58% heterozygous and 19% TT homozygous. During this study, comparisons were made between T allele carriers and CC homozygotes, revealing that those with the T allele had increased indices of obesity (8).

Among another population, the Old Order Amish, allele and genotype frequencies were similar to those in the Inuit population. The frequencies for the C and T alleles were 65% and 35% respectively, and the genotype frequencies were as follows, 42% CC homozygous, 45% heterozygous and 12% TT homozygous. Researchers also made comparisons between T allele carriers and CC homozygotes, finding that the T allele was associated with increased occurrence of the metabolic syndrome, increased fasting triglycerides and decreased HDL levels (2).

Weyer et al. reported that allele frequencies among the Pima Indians were 55% for the C allele and 45% for the T allele. The genotype frequencies among this population were 29% CC homozygous, 51% heterozygous and 20% TT homozygous. Weyer et al. made comparisons between all three genotype groups and found that the T allele was associated with decreased subcutaneous abdominal adipocyte size and also increased insulin sensitivity (44).

Exercise training has been shown to have a positive effect on all of the above mentioned factors which are a result of the C1908T SNP and independently contribute to type 2 diabetes and cardiovascular disease. The interactive effect of exercise training and this polymorphism has not yet been investigated.

Summary

This review has discussed the prevalence of and risks associated with the metabolic syndrome and its components, in addition to their heritability. This review has also addressed the effects of aerobic exercise training on the metabolic syndrome and the heritability of exercise training. A genetic variation in the Lamin A/C gene has been reported in the literature to contribute to the prevalence of the metabolic syndrome and its components which are risk factors for type 2 diabetes and cardiovascular disease. It appears that those individuals with the C allele of the Lamin A/C gene are at a greater risk for developing the metabolic syndrome and its components. Also, those with the C allele may receive a greater benefit from aerobic exercise training concerning the metabolic syndrome and its components. These benefits may include a decrease in percent body fat, improved lipoprotein-lipid profile and increased insulin sensitivity.

References - Literature Review

1. **American Heart Association**. *2002 Heart and Stroke Statistical Update*. Dallas, Texas, American Heart Association. 2001.

2. **Anderson JL, Khan M, David WS, Mahdavi Z, Nuttall FQ, Krech E, West SG, Vance JM, Pericak-Vance MA and Nance MA**. Confirmation of linkage of hereditary partial lipodystrophy to chromosome 1q21-22. *Am J Med Genet* 82: 161-165, 1999.

3. **Austin MA, Edwards KL, McNeely MJ, Chandler WL, Leonetti DL, Talmud PJ, Humphries SE and Fujimoto WY**. Heritability of multivariate factors of the metabolic syndrome in nondiabetic Japanese americans. *Diabetes* 53: 1166-1169, 2004.

4. **Bonne G, Di Barletta MR, Varnous S, Becane HM, Hammouda EH, Merlini L, Muntoni F, Greenberg CR, Gary F, Urtizberea JA, Duboc D, Fardeau M, Toniolo D and Schwartz K**. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat Genet* 21: 285-288, 1999.

5. **Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH and Gagnon J**. The HERITAGE family study. Aims, design, and measurement protocol. *Med Sci Sports Exerc* 27: 721-729, 1995.

6. **Burke B and Stewart CL**. Life at the edge: the nuclear envelope and human disease. *Nat Rev Mol Cell Biol* 3: 575-585, 2002.

7. **Burn J and Baraitser M**. Partial Lipoatrophy with Insulin Resistant Diabetes and Hyperlipemia (Dunnigan Syndrome). *Journal of Medical Genetics* 23: 128-130, 1986.

8. **Cao H and Hegele RA**. Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy. *Human Molecular Genetics* 9: 109-112, 2000.

9. **Chagnon YC, Rice T, Perusse L, Borecki IB, Ho-Kim MA, Lacaille M, Pare C, Bouchard L, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC and Bouchard C.** Genomic scan for genes affecting body composition before and after training in Caucasians from HERITAGE. *J Appl Physiol* 90; 1777-1787, 2001.

10. **Dec GW and Fuster V**. Idiopathic dilated cardiomyopathy. *N Engl JMed* 331: 1564-1575, 1994.

11. **Durstine JL, Grandjean PW, Cox CA and Thompson PD**. Lipids, lipoproteins, and exercise. *J Cardiopulm Rehabil* 22: 385-398, 2002.

12. **Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, Atherton J, Vidaillet HJ, Spudich S, De Girolami U, Seidman JG, Seidman CE, Muntoni F, Muehle G, Johnson W and McDonough B**. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *New England Journal of Medicine* 341: 1715-1724, 1999.

13. **Feitosa MF, Rice T, Rankinen T, Almasy L, Leon AS, Skinner JS, Wilmore JH, Bouchard C and Rao DC**. Common Genetic and Environmental Effects on Lipid Phenotypes: The HERITAGE Family Study. *Hum Hered* 59: 34-40, 2005.

14. **Foster DW**. Insulin resistance--a secret killer? *N Engl J Med* 320: 733-734, 1989.

15. **Freidenberg GR, Cutler DL, Jones MC, Hall B, Mier RJ, Culler F, Jones KL, Lozzio C and Kaufmann S**. Severe insulin resistance and diabetes mellitus in mandibuloacral dysplasia. *Am J Dis Child* 146: 93-99, 1992.

16. **Garcia CA**. A clinical review of Charcot-Marie-Tooth. *Ann N Y Acad Sci* 883: 69-76, 1999.

17. **Garg A, Peshock RM and Fleckenstein JL**. Adipose tissue distribution pattern in patients with familial partial lipodystrophy (Dunnigan variety). *Journal of Clinical Endocrinology and Metabolism* 84: 170-174, 1999.

18. **Genest J, Jr., Marcil M, Denis M and Yu L**. High density lipoproteins in health and in disease. *J Investig Med* 47: 31-42, 1999.

19. **Goldberg MW and Allen TD**. The nuclear pore complex and lamina: three-dimensional structures and interactions determined by field emission in-lens scanning electron microscopy. *J Mol Biol* 257: 848-865, 1996.

20. **Halverstadt A, Phares DA, Ferrell RE, Wilund KR, Goldberg AP and Hagberg JM**. High density lipoprotein-cholesterol, its subfractions, and responses to exercise training are dependent on endothelial lipase genotype. *Metabolism* 52: 1505-1511, 2003.

21. **Hegele RA, Cao HN, Harris SB, Zinman B, Hanley AJ and Anderson CM**. Genetic variation in LMNA modulates plasma leptin and indices of obesity in aboriginal Canadians. *Physiological Genomics* 3: 39-44, 2000.

22. **Holtz D, Tanaka RA, Hartwig J and McKeon F**. The CaaX motif of lamin A functions in conjunction with the nuclear localization signal to target assembly to the nuclear envelope. *Cell* 59: 969-977, 1989.

23. **Hong YL, Rice T, Gagnon J, Despres JP, Nadeau A, Perusse L, Bouchard C, Leon AS, Skinner JS, Wilmore JH and Rao DC**. Familial clustering of insulin and abdominal visceral fat: The HERITAGE family study. *Journal of Clinical Endocrinology and Metabolism* 83: 4239-4245, 1998.

24. **Hozak P, Sasseville AM, Raymond Y and Cook PR**. Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. *J Cell Sci* 108 (Pt 2): 635-644, 1995.

25. **Hsu FC, Lenchik L, Nicklas BJ, Lohman K, Register TC, Mychaleckyj J, Langefeld CD, Freedman BI, Bowden DW and Carr JJ**. Heritability of body composition measured by DXA in the diabetes heart study. *Obes Res* 13: 312-319, 2005.

26. **Hsueh WC, St Jean PL, Mitchell BD, Pollin TI, Knowler WC, Ehm MG, Bell CJ, Sakul H, Wagner MJ, Burns DK and Shuldiner AR**. Genome-wide and fine-mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish - Evidence for a new diabetes locus on chromosome 14q11 and confirmation of a locus on chromosome 1q21-q24. *Diabetes* 52: 550-557, 2003.

27. **Hutchison CJ**. Lamins: building blocks or regulators of gene expression? *Nat Rev Mol Cell Biol* 3: 848-858, 2002.

28. **Jackson SN, Pinkney J, Bargiotta A, Veal CD, Howlett TA, McNally PG, Corral R, Johnson A and Trembath RC**. A defect in the regional deposition of adipose tissue (partial lipodystrophy) is encoded by a gene at chromosome 1q. *Am J Hum Genet* 63: 534-540, 1998.

29. **Kass S, MacRae C, Graber HL, Sparks EA, McNamara D, Boudoulas H, Basson CT, Baker PB, III, Cody RJ, Fishman MC.** A gene defect that causes conduction system disease and dilated cardiomyopathy maps to chromosome 1p1-1q1. *Nat Genet* 7: 546-551, 1994.

30. **Kitten GT and Nigg EA**. The CaaX motif is required for isoprenylation, carboxyl methylation, and nuclear membrane association of lamin B2. *J Cell Biol* 113: 13-23, 1991.

31. **Lakka TA, Rankinen T, Weisnagel SJ, Chagnon YC, Rice T, Leon AS, Skinner JS, Wilmore JH, Rao DC and Bouchard C**. A quantitative trait locus on 7q31 for the changes in plasma insulin in response to exercise training - The HERITAGE Family Study. *Diabetes* 52: 1583-1587, 2003.

32. **Lin F and Worman H.J.** Structural Organization of the Human Gene Encoding Nuclear Lamin-A and Nuclear Lamin-C. *J Biol Chem* 22; 16321-16326, 1993.

33. **Loos RJF, Katzmarzyk PT, Rao DC, Rice T, Leon AS, Skinner JS, Wilmore JH, Rankinen T and Bouchard C**. Genome-wide linkage scan for the metabolic syndrome in the HERITAGE Family Study. *Journal of Clinical Endocrinology and Metabolism* 88: 5935-5943, 2003.

34. **McKenzie JA, Weiss EP, Ghiu IA, Kulaputana O, Phares DA, Ferrell RE and Hagberg JM**. Influence of the interleukin-6 -174 G/C gene polymorphism on exercise training-induced changes in glucose tolerance indexes. *J Appl Physiol* 97: 1338-1342, 2004.

35. **Moir RD, Spann TP, Herrmann H and Goldman RD**. Disruption of nuclear lamin organization blocks the elongation phase of DNA replication. *Journal of Cell Biology* 149: 1179-1191, 2000.

36. **Musliner TA and Krauss RM**. Lipoprotein subspecies and risk of coronary disease. *Clin Chem* 34: B78-B83, 1988.

37. **Novelli G, Muchir A, Sangiuolo F, Helbling-Leclerc A, D'Apice MR, Massart C, Capon F, Sbraccia P, Federici M, Lauro R, Tudisco C, Pallotta R, Scarano G, Dallapiccola B, Merlini L and Bonne G**. Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. *Am J Hum Genet* 71: 426-431, 2002.

38. **Poirier P and Despres JP**. Exercise in weight management of obesity. *Cardiol Clin* 19: 459-470, 2001.

39. **Pollin TI, Hsueh WC, Steinle NI, Snitker S, Shuldiner AR and Mitchell BD**. A genome-wide scan of serum lipid levels in the Old Order Amish. *Atherosclerosis* 173: 89-96, 2004.

40. **Rice T, Despres JP, Perusse L, Hong Y, Province MA, Bergeron J, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Bouchard C and Rao DC**. Familial aggregation of blood lipid response to exercise training in the health, risk factors, exercise training, and genetics (HERITAGE) Family Study. *Circulation* 105: 1904-1908, 2002.

41. **Ryan AS**. Insulin resistance with aging: effects of diet and exercise. *Sports Med* 30: 327-346, 2000.

42. **Shoulders CC, Jones EL and Naoumova RP**. Genetics of familial combined hyperlipidemia and risk of coronary heart disease. *Hum Mol Genet* 13 Spec No 1: R149-R160, 2004.

43. **Sullivan T, Escalante-Alcalde D, Bhatt H, Anver M, Bhat N, Nagashima K, Stewart CL and Burke B**. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J Cell Biol* 147: 913-920, 1999.

44. **Weyer C, Wolford JK, Hanson RL, Foley JE, Tataranni PA, Bogardus C and Pratley RE**. Subcutaneous abdominal adipocyte size, a predictor of type 2 diabetes, is linked to chromosome 1q21-q23 and is associated with a common polymorphism in LMNA in Pima Indians. *Molecular Genetics and Metabolism* 72: 231-238, 2001.

45. **Worman HJ and Courvalin JC**. How do mutations in lamins A and C cause disease? *J Clin Invest* 113: 349-351, 2004.

APPENDIX C: FORMS

Informed consent
APO E genotype and HDL Changes with Exercise Training

APPENDIX C
INFORMED CONSENT

CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY

Project Title: **APO E genotype and HDL Changes with Exercise Training**

I state that I am over 18 years of age and wish to participate in a program of research being conducted by Dr. James Hagberg in the Department of Kinesiology, University of Maryland.

The purpose of this study is to determine the role that genetics may play in determining how my blood cholesterol levels change with exercise training.

I already completed a telephone interview that determined that I am not physically active, am 50 - 75 years of age, not a diabetic or have controlled diabetes, not taking cholesterol-lowering medications, have normal blood pressure or high blood pressure controlled on medications not affecting my cholesterol levels, have no evidence of lung disease, have an appropriate body weight for my height, and have no other medical problems that would keep me from exercising vigorously. Furthermore, if I am a woman, I must be postmenopausal, defined as no menstrual cycles for at least the last 2 years. I understand that if I am a woman and change my hormone replacement therapy regimen during the study, my participation in the study will be terminated. I also understand that if I have a prior history of ulcers or bleeding disorders, I will be excluded from one test that is part of this study. I also understand that I must have somewhat abnormal levels of cholesterol to enter the study.

I understand that I will complete one orientation and two screening visits. The orientation session will present all aspects of the study and my written informed consent will be provided after all of my questions have been answered. For my first Screening visit, I will report to the laboratory in the morning after an overnight fast and a blood sample will be drawn for blood chemistries and blood cholesterol levels. I understand that I may be excluded from the study if this initial blood sample shows elevated levels of glucose in my blood. I understand that a part of this blood sample will be used to obtain my DNA. A blood sample will also be drawn 2 hours after I drink a sugar solution. I understand that a total of 3 tablespoons of blood will be drawn during this visit. I understand that I will be excluded from the study at this point if I have low cholesterol levels, high triglyceride levels, a low red blood cell count, evidence of kidney or liver disease, or evidence of diabetes. I understand that if I remain qualified to this point, on my second Screening Visit I will undergo a treadmill exercise test to determine if I have heart disease. A physical examination will precede the exercise test. I will then complete a test on an exercise treadmill where the treadmill speed and grade will increase every 3 minutes until I cannot continue or symptoms of heart disease develop. Blood pressure, heart rate, and ECG will be recorded before, during, and after the test. I understand that I will be excluded from the study at this point if I have evidence of heart disease.

I understand that if I meet all of these requirements to enter the study, I will undergo 6-8 weeks of instruction in the principles of an American Heart Association low-fat diet and must follow this diet for the remainder of this study. After this I will undergo

Baseline Testing that includes the following tests that will be completed in 5

testing sessions. I will have my blood drawn on 2 or 3 occasions from a vein in my arm in the morning after an overnight fast to measure my cholesterol levels and to assess my immune system. I understand that a maximum of 2 tablespoons of blood will be drawn during these visits. I understand that I will also undergo a second exercise test on a treadmill to measure my cardiovascular fitness. This test will start at 70% of the highest heart rate achieved on my first exercise test and the treadmill grade will increase by 2% every 2 minutes. Blood pressure, heart rate, and ECG will be monitored before, during, and after the test. The test will be stopped when I can no longer continue. During this test I will have a noseclip on my nose and I will breathe through a mouthpiece so that the air that I breathe out can be analyzed. I also understand that my dietary habits will be measured by having me record for 7 days all of the food items that I eat. I understand that on another morning after an overnight fast I will have blood samples drawn before and for 3 hours after I drink a glucose solution to assess my risk of developing diabetes; I will also have additional blood drawn prior to this test that will be frozen for future studies that relate directly to the goals of the present study. I understand that 5 tablespoons of blood will be drawn during this visit. I understand that on another occasion after an overnight fast, I will have blood samples drawn from a line (catheter) in my arm before and for 4 hours after drinking approximately 1 – 2 cups of a high-fat liquid meal. The high-fat meal is made of heavy whipping cream with small amounts of chocolate, sugar, and powdered milk and tastes similar to a rich chocolate shake. I understand that 10 tablespoons of blood will be drawn during this test and will be used to measure how my body absorbs and uses fat from a meal and how my blood clotting, and substances that affect hunger are affected by a fat meal. Before and after I drink the high-fat meal, I understand that I will breath through a mouthpiece while my nose is closed-off with a nose clip and the air that I breath out will be collected and used to determine how much fat I use for energy while sitting at rest. I understand that these tests will be done at the University of Maryland College Park.

I understand that in the morning after an overnight fast I will have blood samples drawn to assess my cholesterol levels and blood clotting system. I will then have a substance that temporarily stops blood from clotting injected into my arm vein. Blood samples are drawn 10 minutes later for measurement of chemicals that affect blood cholesterol levels. I understand that if I have a prior history of ulcers or bleeding disorders I will not undergo this test. I understand that I will remain in the laboratory for 2 - 3 hours after this test with pressure on the site where blood samples were drawn to make sure that all bleeding is stopped. I understand that 4 tablespoons of blood will be drawn during this visit. I understand that how much fat and muscle I have will be measured using x-rays while I lie quietly on a table for 15 to 30 minutes. I understand that the amount of fat I have around my waist will be measured with a CAT scan while I lie quietly on a table. I also understand that these last 3 tests will be done at the VA Medical Center in Baltimore.

I understand that during this Baseline Testing a total of 21 tablespoons of blood will be drawn, which is about two-thirds of the amount of blood given when donating blood.

I understand that after completing this testing, for 6 months I will complete 3 exercise sessions each week supervised by study personnel. I understand that I will be instructed on appropriate warmup and stretching exercises to perform prior to each exercise training session. I will be taught to measure my heart rate and to use heart rate monitors to control how hard I am exercising. The first training sessions will consist of 20 minutes of light exercise. The amount of exercise and how hard I exercise will increase gradually until I am completing 40 minutes of moderate intensity exercise every session. Exercise modes include walk/jogging, stairstepping, and cycle, cross-country ski, and rowing ergometry. I will be asked to add a 45-60 minute walk to my exercise program on weekends after the first 10 weeks of the exercise program. I understand that some of the supervised exercise sessions may be done outside of the exercise facility, but still under the direct supervision of study personnel. I understand that if I lose more weight than expected from the exercise, I will be counseled by a dietitian against restricting how much food I eat. I will also be asked to complete food records during the exercise training program and if major dietary changes have occurred, I will also be counseled by a dietitian to resume my original dietary habits.

I understand that after completing 6 months of exercise training, I will have everything reevaluated that was measured before I began the exercise program. I understand that during this testing a maximum of 21 tablespoons of blood will be drawn, which is about two-thirds of the amount of blood given when donating blood.

I understand that if I qualify for this study that my DNA will be isolated from my blood and analyzed at a number of sites for differences in DNA that may affect how my cholesterol levels change with exercise training. I understand that some of my DNA will also be frozen for future studies. However, these studies can only analyze my DNA at sites that might affect how my cholesterol levels, glucose and insulin levels, bone density, body composition, immunology (disease-fighting), and cardiovascular blood clotting systems change with exercise training.

All information collected in this study is confidential, and my name will not be identified at any time. I understand that my DNA (genetic material) will be sent to laboratories at the University of Pittsburgh and the University of Maryland at Baltimore School of Medicine that are part of this study. I understand, however, that my DNA samples sent to the University of Pittsburgh and the University of Maryland at Baltimore will be identified only by a numeric code. I understand that my coded blood samples are also sent to the University of Florida and to a company in North Carolina to measure compounds in my blood that relate to blood cholesterol levels and cardiovascular disease risk. I understand that only investigators at the University of Maryland College Park will know whose name is associated with each coded number. I further understand that the list of names and codes will be retained at the University of Maryland for up to 25 years.

I understand the following risks are associated with my participation in this study. (1) The risk of maximal exercise testing is approximately 1 nonfatal event in 10,000 tests and 1 fatal cardiac event in 70,000 tests. Risks will be minimized by having the test administered by a physician and personnel trained in such tests and emergency procedures. I will be screened with a resting ECG and a physical examination prior to

Initials _______________

this test. An emergency cart with the necessary drugs and a cardiac defibrillator will be present at all testing sessions. (2) There is minimal risk of bruising and infection associated with blood drawing. These risks will be minimized by using sterile techniques and by having experienced personnel draw all blood samples. (3) The risk of the body composition testing is the exposure to X-rays. The amount of x-ray exposure for this test is the same as that occurring during 30 minutes of any activity outside in the sun. (4) The risk associated with the test requiring the injection into an arm vein of a substance that temporarily stops blood clotting is bleeding. This risk will be minimized by excluding persons with bleeding disorders, peptic ulcers, or other blood disorders from the study. The risk is further minimized by placing a pressure bandage on the intravenous access site after the blood sampling and observing the subject for 2-3 hours after the injection. (5) The risk associated with the CAT scan to measure abdominal fat is the exposure to x-rays. The x-ray exposure is less than the maximum radiation dose individuals are permitted to be exposed to each year in their occupation. (6) The risks associated with the blood clotting and immune system studies are those related to blood drawing as listed above. (7) The risks associated with the oral glucose tolerance test and the high-fat meal test are those associated with blood drawing, the possibility of having low blood sugar levels at the end of the test, and the possibility of having an upset stomach, primarily a stomach ache, after drinking the glucose and/or high-fat meals. The risk of low blood sugar levels at the end of the test will be minimized by providing you with a drink and small snack. (8) The risk of exercise training is the possibility of a heart attack or other cardiovascular event. A large physical activity center reported that 1 nonfatal cardiovascular event occurred in 1.7 million walk/jogging miles. These risks will be minimized because I will undergo a cardiovascular evaluation before beginning exercise training. Exercise sessions will be supervised by experienced personnel trained in emergency procedures. An emergency cart with the necessary drugs and a cardiac defibrillator will be present at all supervised exercise training sessions. Two study personnel will supervise the outside exercise sessions done at the University of Maryland, College Park though no emergency equipment will be directly available during these sessions. (9) There are no risks associated with the genetic testing because no results of these tests will be given to the participants. This has to be the case because the genetic results are not from clinically-approved laboratories.

I understand that this study is not designed to help me personally, but may help the investigators to determine who exercise might benefit the most. I understand that I will be provided with my study results and they can be sent to my physician if I request this in writing. I understand that these results are not to be used for clinical diagnostic purposes and that I will not receive the results of my genetic testing. I understand that I am free to ask questions or to withdraw from participation at any time without penalty. I understand that I will be paid $50 for completing Baseline Testing after the dietary stabilization period. I also understand that I will be paid another $50 for completing 3 months of exercise training and another $100, for a total of $200, for completing 6 months of exercise training and all final testing, if I complete at least 90% of my exercise training and testing sessions. I understand that if my participation in the study has to be terminated because I change my hormone replacement therapy regimen, I will

 Initials _______________

only be paid for the portion of the study that I have already completed, that is, which of the stages above that I have completed.

In the event of a physical injury resulting from participation in this study, I understand that immediate medical attention is available at the Washington Adventist Hospital or the Baltimore VA Medical Center. However, I understand that the University of Maryland does not provide any medical or hospitalization coverage for participants in this research study nor will the University of Maryland provide any compensation for any injury sustained as a result of participation in this research study except as required by law.

Principal Investigator: James Hagberg, PhD. Department of Kinesiology. HLHP Building. University of Maryland, College Park, MD 20742-2611, telephone 301-405-2487.

___________________	___________________
Subject's signature	Date
___________________	___________________
Witness	Date
___________________	___________________
Investigator	Date

References

1. **American College of Sports Medicine**. ACSM's Guidelines for Exercise Testing and Prescription. 2000. Philadelphia, Lea and Febiger.

2. **American Heart Association**. Dietary Guidelines for Healthy American Adults. *Circulation* 77: 721-724, 1988.

3. **American Heart Association**. *2002 Heart and Stroke Statistical Update*. Dallas, Texas, American Heart Association. 2001.

4. **Bamburg JR and Wiggan OP**. ADF/cofilin and actin dynamics in disease. *Trends Cell Biol* 12: 598-605, 2002.

5. **Bonne G, Di Barletta MR, Varnous S, Becane HM, Hammouda EH, Merlini L, Muntoni F, Greenberg CR, Gary F, Urtizberea JA, Duboc D, Fardeau M, Toniolo D and Schwartz K**. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat Genet* 21: 285-288, 1999.

6. **Burn J and Baraitser M**. Partial Lipoatrophy with Insulin Resistant Diabetes and Hyperlipemia (Dunnigan Syndrome). *Journal of Medical Genetics* 23: 128-130, 1986.

7. **Cao H and Hegele RA**. Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy. *Human Molecular Genetics* 9: 109-112, 2000.

8. **Chagnon YC, Rankinen T, Snyder EE, Weisnagel SJ, Perusse L and Bouchard C**. The human obesity gene map: the 2002 update. *Obes Res* 11: 313-367, 2003.

9. **De S**. Homozygous defects in LMNA, encoding lamin A/C nuclear-envelope proteins, cause autosomal recessive axonal neuropathy in human (Charcot-Marie-Tooth disorder type 2) and mouse. *American Journal of Human Genetics* 70: 1075, 2002.

10. **Elbein SC, Hoffman MD, Teng K, Leppert MF and Hasstedt SJ**. A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes* 48: 1175-1182, 1999.

11. **Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, Atherton J, Vidaillet HJ, Spudich S, De Girolami U, Seidman JG, Seidman CE, Muntoni F, Muehle G, Johnson W and McDonough B**. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *New England Journal of Medicine* 341: 1715-1724, 1999.

12. **Friedewa.Wt, Fredrick.Ds and Levy RI**. Estimation of Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of Preparative Ultracentrifuge. *Clinical Chemistry* 18: 499-&, 1972.

13. **Garg A, Peshock RM and Fleckenstein JL**. Adipose tissue distribution pattern in patients with familial partial lipodystrophy (Dunnigan variety). *Journal of Clinical Endocrinology and Metabolism* 84: 170-174, 1999.

14. **Gidez LI, Miller GJ, Burstein M, Slagle S and Eder HA**. Separation and Quantitation of Subclasses of Human-Plasma High-Density Lipoproteins by A Simple Precipitation Procedure. *Journal of Lipid Research* 23: 1206-1223, 1982.

15. **Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, Mitch W, Smith SC and Sowers JR**. Diabetes and cardiovascular disease - A statement for healthcare professionals from the American Heart Association. *Circulation* 100: 1134-1146, 1999.

16. **Hegele RA, Anderson CM, Wang J, Jones DC and Cao H**. Association between nuclear lamin A/C R482Q mutation and partial lipodystrophy with hyperinsulinemia, dyslipidemia, hypertension, and diabetes. *Genome Res* 10: 652-658, 2000.

17. **Hegele RA, Cao HN, Harris SB, Zinman B, Hanley AJ and Anderson CM**. Genetic variation in LMNA modulates plasma leptin and indices of obesity in aboriginal Canadians. *Physiological Genomics* 3: 39-44, 2000.

18. **Hegele RA, Huff MW and Young TK**. Common genomic variation in LMNA modulates indexes of obesity in Inuit. *Journal of Clinical Endocrinology and Metabolism* 86: 2747-2751, 2001.

19. **Heymsfield SB, Wang J, Heshka S, Kehayias JJ and Pierson RN**. Dual-Photon Absorptiometry - Comparison of Bone-Mineral and Soft-Tissue Mass Measurements Invivo with Established Methods. *American Journal of Clinical Nutrition* 49: 1283-1289, 1989.

20. **Hsueh WC, St Jean PL, Mitchell BD, Pollin TI, Knowler WC, Ehm MG, Bell CJ, Sakul H, Wagner MJ, Burns DK and Shuldiner AR**. Genome-wide and fine-mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish - Evidence for a new diabetes locus on chromosome 14q11 and confirmation of a locus on chromosome 1q21-q24. *Diabetes* 52: 550-557, 2003.

21. **Kobberling J and Dunnigan MG**. Familial Partial Lipodystrophy- 2 Types of An X-Linked Dominant Syndrome, Lethal in the Hemizygotic State. *Journal of Medical Genetics* 23: 120-127, 1986.

22. **Lammerding J, Schulze PC, Takahashi T, Kozlov S, Sullivan T, Kamm RD, Stewart CL and Lee RT**. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *J Clin Invest* 113: 370-378, 2004.

23. **Lin F and Worman HJ**. Structural Organization of the Human Gene Encoding Nuclear Lamin-A and Nuclear Lamin-C. *Journal of Biological Chemistry* 268: 16321-16326, 1993.

24. **Matsuda M and DeFronzo RA**. Insulin sensitivity indices obtained from oral glucose tolerance testing - Comparison with the euglycemic insulin clamp. *Diabetes Care* 22: 1462-1470, 1999.

25. **Mazess RB, Barden HS, Bisek JP and Hanson J**. Dual-Energy X-Ray Absorptiometry for Total-Body and Regional Bone-Mineral and Soft-Tissue Composition. *American Journal of Clinical Nutrition* 51: 1106-1112, 1990.

26. **Moir RD, Spann TP, Herrmann H and Goldman RD**. Disruption of nuclear lamin organization blocks the elongation phase of DNA replication. *Journal of Cell Biology* 149: 1179-1191, 2000.

27. **Mounkes L, Kozlov S, Burke B and Stewart CL**. The laminopathies: nuclear structure meets disease. *Current Opinion in Genetics & Development* 13: 223-230, 2003.

28. **Muchir A, Bonne G, van der Kooi AJ, van Meegen M, Baas F, Bolhuis PA, de Visser M and Schwartz K**. Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). *Hum Mol Genet* 9: 1453-1459, 2000.

29. **Nicklas BJ, Rogus EM, Colman EG and Goldberg AP**. Visceral adiposity, increased adipocyte lipolysis, and metabolic dysfunction in obese postmenopausal women. *American Journal of Physiology-Endocrinology and Metabolism* 33: E72-E78, 1996.

30. **Novelli G and D'Apice MR**. The strange case of the "lumper" lamin A/C gene and human premature ageing. *Trends Mol Med* 9: 370-375, 2003.

31. **Shoulders CC, Jones EL and Naoumova RP**. Genetics of familial combined hyperlipidemia and risk of coronary heart disease. *Hum Mol Genet* 13 Spec No 1: R149-R160, 2004.

32. **Spann TP, Goldman AE, Wang C, Huang S and Goldman RD**. Alteration of nuclear lamin organization inhibits RNA polymerase II-dependent transcription. *Journal of Cell Biology* 156: 603-608, 2002.

33. **Spann TP, Moir RD, Goldman AE, Stick R and Goldman RD**. Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. *Journal of Cell Biology* 136: 1201-1212, 1997.

34. **Steinle NI, Kazlauskaite R, Imumorin IG, Hsueh WC, Pollin TI, O'Connell JR, Mitchell BD and Shuldiner AR**. Variation in the lamin A/C gene: associations with metabolic syndrome. *Arterioscler Thromb Vasc Biol* 24: 1708-1713, 2004.

35. **Stierle V, Couprie J, Ostlund C, Krimm I, Zinn-Justin S, Hossenlopp P, Worman HJ, Courvalin JC and Duband-Goulet I**. The carboxyl-terminal region common to lamins A and C contains a DNA binding domain. *Biochemistry* 42: 4819-4828, 2003.

36. **Sullivan T, Escalante-Alcalde D, Bhatt H, Anver M, Bhat N, Nagashima K, Stewart CL and Burke B**. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J Cell Biol* 147: 913-920, 1999.

37. **Vionnet N, Hani E, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C and Froguel P**. Genomewide search for type 2 diabetes-susceptibility genes in French whites: Evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *American Journal of Human Genetics* 67: 1470-1480, 2000.

38. **Warnick GR, Benderson J and Albers JJ**. Dextran Sulfate-Mg-2+ Precipitation Procedure for Quantitation of High-Density-Lipoprotein Cholesterol. *Clinical Chemistry* 28: 1379-1388, 1982.

39. **Weyer C, Wolford JK, Hanson RL, Foley JE, Tataranni PA, Bogardus C and Pratley RE**. Subcutaneous abdominal adipocyte size, a predictor of type 2 diabetes, is linked to chromosome 1q21-q23 and is associated with a common polymorphism in LMNA in Pima Indians. *Molecular Genetics and Metabolism* 72: 231-238, 2001.

40. **Wiltshire, Bottazzo GF, Gesu PB and Foxon R**. A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (The Diabetes UK Warren 2 Repository): Analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q (vol 69, pg 553, 2001). *American Journal of Human Genetics* 69: 922, 2001.

41. **Wolford JK, Hanson RL, Bogardus C and Prochazka M**. Analysis of the Lamin A/C gene as a candidate for Type II diabetes susceptibility in Pima Indians. *Diabetologia* 44: 779-782, 2001.

42. **Worman HJ and Courvalin JC**. The nuclear lamina and inherited disease. *Trends Cell Biol* 12: 591-598, 2002.

43. **Xiang KS, Wang YQ, Zheng TS, Jia WP, Li J, Chen L, Shen KX, Wu SH, Lin X, Zhang GD, Wang CR, Wang SJ, Lu HJ, Fang QC, Shi Y, Zhang R, Xu J and Weng Q**. Genome-wide search for type 2 diabetes/impaired glucose homeostasis susceptibility genes in the Chinese - Significant linkage to chromosome 6q2l-q23 and chromosome 1q21-q24. *Diabetes* 53: 228-234, 2004.