ABSTRACT

Title of Dissertation: EFFECT OF ENDURANCE EXERCISE TRAINING ON

FASTING AND POSTPRANDIAL PLASMA ADIPONECTIN

LEVELS

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The purpose of this study was to investigate the postprandial response of plasma

adiponectin (AN) levels to a high-calorie, high-fat meal, in relatively healthy (free of

diabetes, overt heart disease) sedentary 50- to 75-year-old men and women before and

after a six-month endurance exercise training program (approximately 70% of VO_2 max,

three times per week). AN is an adipocyte-released polypeptide ("adipokine") whose

physiological significance in insulin sensitivity and other health risk factors is well

documented.

VO_2 max was significantly increased with training in both men and women (men,

27.0 ± 0.9 vs. 32.2 ± 1.2 mL/kg/min, $p < 0.0001$; women, 23.3 ± 1.0 vs. 27.1 ± 1.4

mL/kg/min, $p = 0.0002$), while % body fat was decreased (men, 29.9 ± 1.2 vs. $26.0 \pm$

1.3 %, $p = 0.0010$; women, 42.3 ± 1.5 vs. 39.5 ± 1.8 %, $p < 0.0001$). Fasting AN levels

were higher in women than in men (gender main effect, $p = 0.0138$), and fasting as well

as postprandial adiponectin levels decreased significantly with training in men ($p =$

0.014) but not in women. No postprandial changes in plasma AN levels were observed in either gender. Stepwise regression analysis showed insulin sensitivity to be the strongest predictor of fasting AN levels. Postprandial AN levels were mainly dependent on fasting AN concentrations.

In conclusion, fasting plasma adiponectin levels decreased with exercise training in men in the present study, whereas they remained unchanged in women. Postprandial adiponectin levels did not change following consumption of a high-fat meal either before or after exercise training.

EFFECT OF ENDURANCE EXERCISE TRAINING ON FASTING AND

POSTPRANDIAL PLASMA ADIPONECTIN LEVELS

By

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ACRP30   Adipocyte complement-related protein 30; syn. Adiponectin

AHA   American Heart Association

AMPK   5'-AMP activated protein kinase

AN   Adiponectin

ANCOVA   Analysis of covariance

ANOVA   Analysis of variance

apm-1   adipocyte most abundant gene product

AT   Adipose tissue

AUC   Area under the curve

BF   Body fat

BMI   Body mass index

BP   blood pressure

BSA   Body surface area

cm   Centimeter

CT   Computed tomography

CV   Coefficient of variation

DEXA   Dual x-ray absorptiometry

EDTA   Ethylenediaminetetraacetic acid

ECG   Electrocardiogram

F   Final

g   Gram

GERS   Gene Exercise Research Study

GBP-28	Gelatin-binding protein 28
GXT	Graded exercise test
HDL	High-density lipoprotein
HR	Heart rate
HRT	Hormone replacement therapy
i.a.	*inter alia* (among others)
i.e.	*id est* (that is)
IRB	Institutional Review Board
ISI	Insulin sensitivity index
kcal	Kilocalorie
kg	Kilogram
l	Liter
LDL	Low-density lipoprotein
m^2	Square meter
min	Minute
mL	Milliliter
μL	Microliter
NCEP	National Cholesterol Education Program
OGTT	Oral glucose tolerance test
PPAR-γ	Peroxisome proliferator-activated receptor γ
PPLT	Postprandial lipemia test
RER	Respiratory exchange ratio
RIA	Radioimmunoassay assay

sec	Second
SMGU	Skeletal muscle glucose uptake
TG	Triglyceride
TNF-α	Tumor necrosis factor-α
TZD	Thiazoledinedione
VO_2	Oxygen consumption
VO_2 max	Maximal oxygen consumption

Chapter 1 – Introduction

Background

Although a possible endocrine function of adipose tissue (AT) was first

suggested in 1953 (36), the preeminent role of adipose tissue in human physiology

has historically been thought to be one of lipid storage (59). Despite detailed

knowledge about the regulation of lipolysis and lipogenesis within AT, the role of AT

as an endocrine organ has remained unknown until recently. The discovery of leptin

in 1994 by Friedman's group (79) effectively started the field of 'adipokine' or

'adipocytokine' research (the latter term due to some sequence homology between

certain adipocyte-released polypeptides and inflammatory cytokines). Since 1994,

over 20 adipokines have been identified. Besides leptin, other adipokines which have

been reported to be biologically significant are, *i.a.*, adiponectin (AN), resistin, tumor

necrosis factor α (TNF-α), and interleukin-6. Undoubtedly, adipose tissue must now

be considered a major endocrine organ (3).

AN was independently discovered and reported in 1995 and 1996 by several

groups. Hence, different terms have been used for this protein. Adipocyte

complement-related protein 30 (ACRP30), gelatin-binding protein 28 (GPB-28) ,

adipose most abundant gene product 1 (apm-1), and adipoQ all refer to the same

polypeptide (4, 29, 43, 50, 61). Human AN consists of 244 amino acids (43). It is

made up of a signaling (most likely, a secretory signal) region at the NH$_2$-terminal, a

highly variable (inter-species) region connecting the globular to a relatively well-

preserved collagen-like region, and a complement factor C1q-like globular domain at

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the COOH-terminal (29, 63). The globular region can be biologically active by itself (30). In human plasma, AN is present in di-, tri-, or multimeric quaternary structures. The biological effects of AN may be affected by the molecule's quaternary state (67, 69).

In humans, fasting plasma AN levels are reported to be ~ 2 to 30 µL/mL plasma (4, 16). This makes AN one of the most abundant plasma proteins (15). Interestingly, plasma AN levels were widely reported to decline with increased body fat mass in animals and humans (4, 15). Thus, initial reports on the regulation of plasma AN levels suggested mainly a negative feedback inhibition by adipocytes. Because there is strong evidence for obesity as a diabetes risk factor, however, it has been suggested that AN may be a mediator of, or regulated by, insulin sensitivity (47). This theory has received support via descriptive as well as *in vivo* and *in vitro* studies in which AN administration improves insulin sensitivity (5), which is thought to occur by both increasing skeletal muscle glucose uptake (SMGU), as well as suppressing hepatic glucose production. Additionally, results from animal studies suggest that AN reduces fat weight in rodents (45), which may have independent beneficial effects on insulin sensitivity.

A study by Kubota et al. showed insulin resistance to be less severe in heterozygous AN-deficient mice compared to AN knockout animals (42). The same group reported ameliorated insulin resistance in AN deficient and previously insulin resistant lipoatrophic mice (74). In humans, AN is the only adipokine known to be negatively correlated with obesity indices (e.g., BMI, body fat mass). Additional evidence of a direct connection between insulin resistance and AN levels comes from

genome-wide linkage studies where the AN gene (*apM1*) locus 3q27 has been identified as a diabetes susceptibility locus (38, 65).

Considerable effort has been put into elucidating pathways of AN expression regulation. Because insulin levels are commonly elevated in insulin resistant individuals, Fasshauer et al. sought to determine the possible regulatory effects of insulin on AN expression levels (17). It was determined that in 3T3-L1 adipocytes, insulin administration suppressed AN gene expression levels by ~ 75 %. Additionally, there is some evidence that adrenergic agonists such as isoproterenol decrease AN gene expression in adipose tissue (14).

In a recent study by Tiikkainen et al., drug-naïve type-2 diabetic patients were either treated with rosiglitazone or metformin, both powerful antidiabetic agents, for 16 weeks (66). Rosiglitazone is a thiazoledinedione (TZD) which acts by activating peroxisome proliferator-activator receptor-γ (PPAR-γ). PPAR-γ is a powerful adipocyte-specific transcription factor which regulates the synthesis of many adipocyte proteins (56). Conversely, metformin has been shown to exert its effects in a PPAR-γ independent manner by activating the AMP-dependent protein kinase (AMPK) cascade (80). Briefly, their results showed that metformin acted mainly on hepatic insulin sensitivity, whereas TZD administration increased AN levels twofold despite unchanged body weight. These findings support the hypothesis that AN synthesis is increased by PPAR-γ agonists (66).

Gender also appears to have an effect on plasma AN levels, as AN levels are found to be higher in women than in men (27, 72). Evidence to support this observation stems from research conducted in mice that showed elevated AN levels in

castrated male mice (52). In this study, plasma AN levels in the castrated animals were reduced by testosterone administration. Conversely, ovariectomy did not change AN levels in female mice. In the same study, plasma AN levels did not differ between pre- and postmenopausal women (52)

Data from several exercise training studies suggests that exercise training by itself without significant concomitant loss in body or fat mass does not increase fasting plasma AN concentration (31, 58, 76). In 2002, Hulver et al. reported that fasting AN levels did not change in overweight individuals (average BMI, 29 kg/m^2) who had performed endurance exercise training for six months and significantly improved insulin sensitivity without changing body or fat mass (31). In the same study, a separate group of morbidly obese individuals (average BMI, 47 kg/m^2) who underwent gastric bypass surgery (average weight loss, 53 kg) more than tripled mean fasting plasma AN concentration to a level roughly twice that of the exercise group. Ryan et al. did not detect AN level decreases in overweight or obese postmenopausal women who lost moderate amounts of fat mass through caloric restriction or a combination of diet and endurance exercise or strength training (58). Yatagai et al. actually reported decreased fasting AN concentration in healthy men undergoing six weeks of endurance exercise training despite improved insulin sensitivity (76).

In conclusion, although initial reports pointed to the amount of fat mass as the main predictor for fasting AN levels, most recent evidence suggests that insulin sensitivity is an important independent factor in determining plasma AN concentration. It is well understood that central fat is highly predictive of insulin resistance (10). Although no tissue studies comparing central and peripheral

adipocytes with regards to AN expression have been conducted, there is limited evidence from cross-sectional studies that central adiposity measurements from CT scans or indices such as the waist-hip ratio are more predictive of plasma AN levels than whole-body adiposity measurements (subcutaneous fat), or indices such as BMI (12, 64).

Humans spend most of their lives in the postprandial state. Merely reporting fasting concentrations of a particular biomolecule is often not sufficiently indicative of a molecule's role in human physiology. For example, fasting plasma insulin concentration is generally viewed a poor index of an individual's ability to maintain plasma glucose homeostasis following a meal (46). Accordingly, it is possible that postprandial changes in plasma AN levels relative to fasting AN hold distinct and relevant predictive value regarding an individual's health status. In this regard, is interesting to note that English et al. have reported differences between obese and lean individuals' postprandial plasma AN levels (16). Specifically, lean individuals displayed increased insulin sensitivity compared to the obese subjects, and showed no significant AN plasma level changes over a three-hour postprandial period following a mixed-composition meal. Conversely, obese subjects had significantly lower fasting AN plasma levels which increased dramatically after meal ingestion and, on average, peaked 60 minutes into the postprandial period.

In the English et al. study, no mention was made of subjects' cardiovascular training status. In accepting the notion that lean individuals on average display superior cardiovascular fitness compared to otherwise matched overweight or obese

individuals, there exists a possibility that this variable may have a confounding influence on postprandial plasma AN levels independent of or in conjunction with the degree of fat mass in these individuals. To our knowledge, no published reports on a possible endurance exercise training effect on postprandial adiponectin response exist.

Specific Aims

Adiponectin is an adipocyte-released polypeptide ("adipokine") whose physiological significance is well documented. There is substantial evidence that AN increases SMGU while suppressing hepatic glucose production, both hallmarks of improved insulin sensitivity. These findings strongly support the contribution of AN to overall health in regards to maintenance of glucose homeostasis and other related processes.

Fasting AN levels have been reported to be inversely proportional to body fat mass as well as insulin sensitivity, and a possible link exists between central fat and both insulin sensitivity and AN levels. Postprandial AN kinetics may differ between lean and obese individuals. That is, lean individuals experience little or no change in plasma AN levels in the postprandial state, whereas obese individuals' plasma AN levels increase substantially after meal ingestion. It is unclear at this time whether postprandial AN kinetics change as a result of aerobic exercise training, especially if a training effect is observed without a concomitant change in body fat mass.

Hence, the purpose of this study was to investigate the postprandial response of plasma AN levels to a high-calorie, high-fat meal, in sedentary 50- to 75-year-old

men and women before and after a six-month endurance exercise training program. This study was conducted based on the following hypotheses:

Hypothesis 1: In sedentary individuals, postprandial changes in AN levels as defined by the % difference between fasting and 60-min postprandial AN levels are predominantly a function of an individual's central fat tissue mass.

Hypothesis 2: Six months of endurance exercise training will decrease 60-min postprandial Adiponectin levels compared to before training, and will do so independent of training-induced changes in central fat mass. Further, we hypothesize that exercise training-induced changes in postprandial AN are a function of changes in insulin sensitivity as assessed by oral glucose tolerance test.

Chapter 2 – Methods

The present study was conducted as part of the Gene Exercise Research Study (GERS) at the University of Maryland, College Park. GERS was initially conceived to describe the effects of endurance exercise training on plasma lipoproteins and blood pressure (BP). Written approval for the use of GERS data for this project was obtained from the University of Maryland Institutional Review Board (IRB) by submitting an addendum to the original GERS IRB application.

Participants

Fifty-four participants completed a baseline PPLT. Of those individuals, 33 performed a PPLT after completing the endurance training program.

Screening

GERS study volunteers were recruited from the Washington, D. C. Metropolitan area. Initial recruitment was done via direct mailings, radio public service announcements, and personal referrals from current participants. The advertisements targeted sedentary individuals of age 50 – 75, free of cardiovascular disease and diabetes, and women who were at least two years postmenopausal. Women were asked not to change their hormone replacement therapy (HRT) status (on/off) during the study. All advertisements encouraged interested individuals to call the GERS study office for further information and to enter the screening process.

Telephone screening

Prospective participants were given a thorough overview of GERS study procedures in regard to qualifications placed on and demands asked of study participants. After callers gave oral consent, GERS staff conducted a thorough interview in which subjects were interrogated on potential exclusionary factors. Main exclusion criteria were: current physical activity (> 20 min > twice/week), history of documented heart or peripheral vascular disease, diabetes, age of < 50 or > 75 years at the time of the telephone interview, females < two years postmenopausal, body mass index (BMI) > 37 kg/m^2, blood pressure outside desired range (systolic: 120 – 159 mm Hg, diastolic: 80 – 99 mm Hg) in combination with normolipemia (i.e., no NCEP lipid abnormality), orthopedic conditions that preclude regular physical exercise and/or maximal exercise testing, hematocrit value of < 35, liver, lung, or chronic obstructive lung disease, and being on > two BP medications. If any of these criteria applied, individuals were informed that they did not qualify for the present study as soon as the exclusion criterion was identified. Potential subjects who were not excluded based on the telephone interview and who agreed to participate further in the study were scheduled for an on-site orientation meeting, and sent an informed consent form, a physical activity questionnaire, and a health history questionnaire.

On-site screening

Orientation meeting: Immediately after arrival, potential subjects were weighed. Height was ascertained to verify self-reported values during the telephone interview, and BMI was calculated [BMI = kg (body weight)/m^2 (height)]. Subjects

with a BMI of over 37 were excluded from the study at this point. The main goal of this appointment was for potential study participants to meet with GERS staff who reiterated the study outline including time commitments, testing procedures, and risks and benefits associated with participation in the study. After receiving a tour of GERS facilities, those individuals still willing to participate signed the informed consent form after all remaining questions had been addressed.

Oral glucose tolerance test (OGTT): During the initial screening visit, a 2-hour OGTT was performed to exclude from the study diabetic individuals who were unaware of their condition. Also, this test was performed to assess individuals' resting seated blood pressure, and basic blood chemistries (*i.a.*, LDL and HDL cholesterol, triglycerides, hematocrit, fasting glucose, complete blood cell count). After completing a phlebotomy questionnaire, approximately 20 mL blood were obtained by venipuncture using standard aseptic laboratory techniques (10-mL K_3 ethylenediaminetetraacetic acid [EDTA], 10-mL SST gel & clot activator tubes). Immediately after obtaining the blood sample, blood glucose was measured using a OneTouch Basic® Glucometer that had been calibrated according to manufacturer's instructions. If estimated plasma glucose was ≥ 126 mg/dL, subjects were informed that they no longer qualified to participate in the study. Moreover, they were strongly encouraged to immediately contact their personal physician for further evaluation. In case of estimated plasma glucose values of < 126 mg/dL, subjects consumed 296 mL of a solution containing 75 g of glucose (Fisher Scientific). Two hours after the glucose beverage has been consumed, another 5-mL blood sample was obtained using techniques mentioned above. Fasting and postprandial samples were analyzed by a

commercial diagnostic laboratory service (Quest Diagnostics, Baltimore, MD), and reviewed by GERS medical staff. If 2-hour plasma glucose levels were $\geq$ 200 mg/dL, subjects were excluded from the study, and referred to their personal physician for further evaluation.

Physical examination/graded exercise test (GXT): Although only individuals free of documented heart disease were included in on-site screening, all prospective participants underwent an exercise test to exclude those with signs of cardiovascular disease before, during, or after an acute exercise bout. After undergoing a general physical examination by GERS medical staff, subjects were prepared for a 12-lead exercise electrocardiogram (ECG). Resting ECG rhythm strips were recorded and monitored by the physician in the supine, seated, and standing positions. Additionally, women also had their ECG recorded in the final 10 seconds (sec) of a 30-sec voluntary hyperventilation period. In case of resting ECG abnormalities, GERS medical staff took the appropriate measures including test cessation and encouraging participants to follow up with their personal physician.

For the exercise test, the Bruce treadmill protocol was used (9). Briefly, during this protocol, subjects undergo a warm-up procedure (2 mph, 0 % grade) for several minutes before the actual test starts. Initially, subjects exercise at 1.7 mph and a 10 % grade. Every three minutes, speed increases alternating by 0.8 and 0.7 mph, and treadmill grade increases by 2 %. Expired air is sampled with an on-line indirect calorimetry system. The system consists of a two-way breathing valve (model 2300, Hans Rudolph, Inc., Kansas City, MO) which is connected to a mixing chamber (Rayfield, Waitsfield, VT) via 1.5-inch ventilatory tubing. Sampling is performed by

a calibrated mass spectrometer (Perkin Elmer 1100; MA Tech, St. Louis, MO), and a ventilation measurement module (VMM-400, Interface Associates, Aliso Viejo, CA). Thirty-second averages for participants' oxygen consumption (VO_2) and respiratory exchange ratio (RER) were determined. Exercise intensity is assessed by monitoring subjects' RER. Tests were stopped due to any of the following: subject indicates fatigue, $RER \geq 1.15$, physician or technician detects ECG abnormalities warranting cessation of exercise, blood pressure > 260 mm Hg systolic or > 115 mm Hg diastolic, or technical problems.

In case of an incomplete test (i.e., peak RER < 1.15 due to technical problems, or subject's inability to perform maximum-intensity exercise), the GXT was repeated on a different day. Exclusion criteria for this test were: elevated BP at rest (> 200 mm Hg systolic or > 110 mm Hg diastolic) or during exercise (> 240 mm Hg systolic or > 120 mm Hg diastolic), significant indication of cardiovascular disease based on resting ECG, and indication of significant myocardial ischemia and/or rhythm abnormalities during the exercise test. Subjects exercised until the attending physician signaled for the cessation of exercise, or until subjects themselves indicated an inability to continue exercise due to fatigue or other discomfort (e.g., tightness or pain in chest, dizziness, nausea, etc.). Subjects were also made aware of an emergency off switch located within their reach on the treadmill which could be used in an emergency.

Dietary stabilization and drug tapering

The main goal of this part of the study was for participants to consume a diet consistent with the American Heart Association (AHA) Dietary Guidelines for the General Population. After the screening process was completed, subjects attended 12 'dietary stabilization' lectures over a period of six weeks. Briefly, this diet mandates a maximum caloric intake from calories derived from fat sources of $\leq 30\%$ ($\leq 10\%$ saturated, 10-15% monounsaturated, and $\leq 10\%$ polyunsaturated; < 300 mg cholesterol/day). Carbohydrates should account for $> 50\%$ of daily caloric intake, with the remainder coming from protein. Compliance throughout the duration of the study was ensured by having participants complete individual 7-day diet records which were analyzed by a registered dietician using a computerized nutrition analysis program (Computrition, Chatsworth, CA).

The six-week dietary stabilization period was also used to taper subjects off of all blood pressure medications. Tapering proceeded only after a medication taper schedule had been approved by the participant's personal physician. Most commonly, tapering occurred over the last four weeks of dietary stabilization. In most cases, subjects will take 100 % of the originally prescribed medication in the first week of tapering. In week two, subjects take 50% of the original dosage, followed by 25% in week three, and no medication at all in week 4. In the case of an individual being on two blood pressure medications, tapering was typically staggered, i.e., subjects stayed on one medication while they tapering off another one, followed by a taper off the remaining medication. During the tapering period, subjects had their blood pressure measured at least twice per week during dietary lectures. If subjects missed a lecture,

they were asked to have their blood pressure checked elsewhere, or to report to the GERS office to have a staff member assess their blood pressure. In subjects where there was reason to assume that blood pressure varied highly or was at the high end of GERS inclusion criteria, an automated blood pressure monitor was provided for the duration of the tapering process and baseline testing. Subjects whose blood pressure exceeded either 159 mm Hg systolic or 99 mm Hg diastolic for three consecutive weeks were excluded from the study and referred to their personal physician for treatment.

Testing procedures

Baseline tests were conducted after the dietary stabilization period, and before the onset of exercise training. All participants who had stopped taking BP medication during the dietary stabilization period were at least 2-weeks medication free before baseline testing began. Testing procedures were identical at baseline and final testing, with the exception that at baseline, subjects were not permitted to have performed strenuous physical exercise (*e.g.*, heavy yard work, etc.) for at least 72 hours prior to testing. In contrast, all final tests were conducted between 24 and 36 hours after the last exercise bout to ensure a true test of training, not acute exercise effects.

Exercise training intervention

The goal of the exercise training intervention was to have subjects exercise four times weekly for 40 minutes at approximately 70% of VO_2 max. Exercise heart rate (HR) as measured by a Polar Beat heart rate monitor (Polar Elektro, Oy, Finland),

in conjunction with the heart rate reserve formula [Karvonen formula; (35)], was used to gauge exercise intensity. Resting and maximum heart rate data (RHR, MHR, respectively) were used to calculate heart rate ranges for a given exercise intensity using the following formula: $HR = RHR + (MHR - RHR) \times$ (intensity %). To allow subjects to adjust to the demands of regular physical exercise, subjects started exercise at ~ 50% of VO_2 max for 20 min in week one. Subjects were provided with a training heart rate range which corresponds to ± 5% of their target exercise intensity. Exercise duration increased by 5 min weekly until, in week 5, subjects exercised for 40 minutes. Starting in week 6, exercise intensity was increased weekly by ~ 5% of VO_2 max until in week 9, subjects exercised for 40 min at approximately 70% of VO_2 max three times weekly. This duration and intensity was maintained throughout the rest of the 24-week training program. In week 10, subjects were asked to perform one 'weekend exercise session' for approximately 40 min in the lower end of each individual's 70% intensity target heart rate range. During all workouts, subjects were instructed to only count minutes spent within the appropriate HR rate range towards their exercise duration goal.

All weekday sessions were held under supervision at the GERS training facility. For the initial 10 weeks of exercise training, subjects reported to GERS staff upon arrival, and had their blood pressure taken daily before, during, and after exercise if their established baseline blood pressure was > 120 mm Hg systolic and/or > 80 mm Hg diastolic. After ten weeks, these individuals had their blood pressure checked once weekly before, during, and after exercise. Subjects considered normotensive (both < 120 mm Hg systolic and < 80 mm Hg diastolic) had their blood

pressure checked before, during, and after exercise once weekly for the first ten weeks of exercise, after which they had their casual BP assessed once weekly, and sporadically during exercise.

Maximum oxygen uptake (VO$_2$ max)

Treadmill VO$_2$ max was assessed using the same equipment that was used for the treadmill GXT (see above). Subjects warmed up on a treadmill until they achieved 70% of the highest recorded heart rate during the GXT by adjusting treadmill speed at a grade of 10 %. Subjects then performed some light stretching exercises. The VO$_2$ max test consisted of incremental two-minute stages. At the end of each stage, treadmill grade was increased by 2 % until the subject reached exhaustion, or any of the other reasons for stopping the exercise test discussed above occurred. A test was considered a 'true' VO$_2$ max test if three of the following four conditions applied: RER $\geq$ 1.15, maximal HR (age-predicted or based on maximal HR data from GXT) was reached, a plateau in the VO$_2$ with an increase in work load (< 250 mL VO$_2$ increase) could be observed, and if the subject indicated physical exhaustion preventing a continuance of exercise.

Postprandial lipemia test (PPLT)

All potential subjects for the PPLT were queried to exclude those with lactose intolerance, gall bladder dysfunction, chronic gastritis, diverticulosis/diverticulitis, ulcers, or other gastrointestinal conditions. Individuals who reported to be intolerant of high-fat meals were also excluded from this test.

For the PPLT, subjects reported to the GERS facility on a morning after a 12- to 16-hour overnight fast. To limit the effects of diurnal variation, PPLTs were started between 07:00 and 09:00 o'clock. Subjects were asked to refrain from alcohol consumption during the 24-hour period before the test, and to adhere to the dietary guidelines discussed above (documented by 1-day food record).

After obtaining the subject's weight, an indwelling 20-gauge or 22-gauge Teflon catheter was inserted into an arm vein at or distal to the antecubital fossa using aseptic techniques. The catheter was secured in place with ½-inch tape, and the puncture site was sealed with a sterile, transparent wound cover. Blood samples were drawn directly into blood collection tubes using a Luer adapter system. Between draws, the catheter line was flushed and maintained using 0.9% sterile saline. A 10x8-inch heating blanket was placed over the catheter for most of the test to maintain circulation.

After the catheter was in place and secured, subjects sat quietly in an armchair, and expired air was collected into 120-l meteorological balloons (Kaysam, Totowa, NJ) for 20 min (4 bags of 5 min each) using the two-way breathing valve described above. Each bag was analyzed for CO_2 and O_2 fractions by the mass spectrometer described above, and for volume in a chain-linked weight-compensated gasometer (W.E. Collins, Boston, MA). Among other variables, ventilation (l/min), VO_2, and RER were calculated, and substrate utilization was estimated (% energy from carbohydrate vs. lipid sources). Ventilatory measurements were repeated ~ 100 min and 220 min into the postprandial phase for 20 min each.

Blood samples were drawn immediately before, and every 30 minutes for four hours following, ingestion of the liquid fat meal for analysis of plasma adiponectin, triglyceride (TG), insulin, and glucose (all 15% EDTA tubes) as well as serum FA (additive-free glass tube) concentrations. The PPLT protocol used in the present study was based on the methods by Patsch et al. (54). A 386-gram (g) dose of liquid fat meal was administered per 2 m^2 of body surface area (BSA) where BSA (m^2) = 0.00949 × [(weight in kg)0.441] × [(height in cm)$^{0.655}$]. Hence, an adult of a height of 185 cm and 82.5 kg body weight would receive a meal consisting of 325 g heavy whipping cream, 39 g chocolate syrup, 14 g granular sugar, and 8 g non-fat powdered milk. Of the 1362 kilocalories (kcal) in the fat meal, ~ 84% are from fat, ~ 3% are from protein, and ~ 14% are from carbohydrate sources. Exactly 240 min after finishing ingestion of the high-fat meal, the catheter was removed, and pressure was applied to the puncture site for ~ 3 min. The subject was given a light snack and instructions on how to prevent phlebitis.

Oral glucose tolerance test

For the three days prior to the OGTT, participants were advised to consume ≥ 250 grams of carbohydrate per day. A 3-day diet record was provided in which all foods consumed were recorded. To account for diurnal variation, all OGTT appointments started in the morning between 6:30 and 9:00 am following a 12- to 14-hour fast. On arrival, participants were questioned to confirm that they complied with preparation instructions, and the diet record was collected and examined by GERS staff for a estimate regarding adequacy of carbohydrate intake.

A 20- or 22-gauge indwelling venous catheter was inserted into an arm vein at, or distal to, the antecubital fossa. The catheter was secured in place with ½-inch tape, and the puncture site was sealed with a sterile, transparent wound cover. Blood samples were drawn before and at 30, 60, 90, and 120 minutes after an oral 75-gram dose of D-glucose in a 296-mL solution. Blood was immediately transferred into tubes containing 15% potassium EDTA, and placed on ice until centrifugation. The draw line was maintained using a 0.9% sodium chloride solution flush after each draw. Aseptic technique was used at all times.

Insulin sensitivity was calculated using the insulin sensitivity index (ISI) devised by Matsuda and DeFronzo (46): ISI = 10,000/(square root of {[(fasting glucose)*(fasting insulin)]*[(mean of 0-, 30-, 60-, 90-, and 120-min glucose values)*(mean of 0-, 30-, 60-, 90-, and 120-min insulin values)]}).

Body composition

Dual x-ray absorptiometry (DEXA) was used to assess total and regional body composition (DPX-L or DPX-IQ; Lunar Corp., Madison, WI) while subjects maintained a relaxed supine position. Central adiposity was assessed via single slice computed tomography (CT). Both procedures took place at the Geriatric Research, Education, and Clinical Center in Baltimore, MD, using procedures previously described (51).

Sample analyses

Plasma adiponectin

Immunoreactive plasma AN concentration was determined through competitive radioimmunoassay (RIA; kit HADP-61HK, Linco Research, St. Charles, MO). Samples were analyzed in duplicate. When duplicate values' coefficient of variation (CV) was > 10%, the sample was re-analyzed in a subsequent assay. In case of one clear outlier, it was eliminated from mean AN concentration calculation. Otherwise, all values within one standard deviation of the mean of the four values were used to calculate sample AN concentration. In addition, between-assay variation was assayed using a control sample which was used in all adiponectin, insulin, triglyceride, and FFA assays. Within-assay CV was < 10% in all assays.

Plasma glucose

Plasma glucose concentration was determined using the glucose oxidase method and a glucose analyzer (YSI 2300 Stat Plus, YSI Inc., Yellow Springs, OH). All samples were measured in duplicate. When duplicate assessments of a sample differed by > 2 mg/dL, the sample was re-analyzed until two values differed by ≤ 2 mg/dL. All values of within 2 mg/dL were averaged to represent sample glucose concentration.

Plasma insulin

Immunoreactive plasma insulin concentration was determined through competitive radioimmunoassay (RIA; kit HI-14K, Linco Research, St. Charles, MO). Samples were analyzed in duplicate. When duplicate values' coefficient of variation (CV) was > 10%, the sample was re-analyzed in a subsequent assay (see "*Plasma adiponectin*", above).

Plasma TG

Plasma TG concentrations were determined using a colorimetric assay (kit 337-B, Sigma Diagnostics Inc., St. Louis, MO), in combination with additional standards (NERL Diagnostics, East Providence, RI) and a plate reader (Emax, Molecular Devices, Sunnyvale, CA). Samples were run in duplicate, and repeated if the CV of the duplicate sample was > 10% (see *"Plasma adiponectin"*, above).

Serum FA

Serum fatty-acid concentrations were assessed using a colorimetric assay kit (kit NEFA C, Wako Chemicals USA, Inc., Richmond, VA), and a plate reader (Emax, Molecular Devices, Sunnyvale, CA). Duplicate measures for each sample were performed. Discrepancies between duplicate samples were handled as described in *"Plasma adiponectin"*, above.

Statistical analyses

All statistical analyses were performed using SAS software (SAS version 9.1, SAS Institute, Cary, NC). Before any statistical analysis, residuals of dependent variables (individual values minus group means) were assessed for homogeneity of variance and normal distribution where applicable. Data transformation using appropriate methods was performed if necessary, and noted when reporting statistical outcomes. Statistical significance was set at $p \leq 0.05$.

Adiponectin total area under the curve (AUC) levels were calculated via the trapezoidal method by using fasting (i.e., 0-min) and 60-min, 120-min, and 240-min postprandial AN levels in the following formula: AN AUC total = fasting AN*30 + 60-min value*60 + 120-min value*90 + 240-min value*60. Insulin, TG, FFA AUC were calculated similarly, although plasma levels from all PPLT time points were used for AUC calculation. For both fasting and total AUC AN levels, a repeated-measures ANOVA (main effects: time point, gender) was conducted. A compound symmetry covariance structure was used in all repeated-measures procedures to adjust for correlation among time point values.

To determine the presence of a change in AN levels between fasting conditions and 60 min into the postprandial phase, paired t-tests between fasting and 60-min AN levels were performed. Finally, in order to determine predictors of both fasting and postprandial AN levels, multiple stepwise regression procedures on fasting and total AUC AN were performed, using central fat tissue amount, % total body fat, insulin sensitivity, TG total area under the curve, and (for postprandial AN levels) fasting AN as independent variables. Because of gender differences in body

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fat and other variables, regression analyses were conducted separately for men and

women. The selection of these independent variables was partly based on previously

published reports (15).

Descriptive data (Table 1)

VO$_2$ max was increased with training (main training effect, p < 0.0001), and

was consistently higher in men (main gender effect, p = 0.016). Men were

significantly heavier than women before and after training (main effect, p = 0.012),

and body weight decreased significantly with training in the combined group (i.e.,

men and women; p < 0.0001). Percent body fat was significantly greater in women

than in men (p < 0.0001), and significantly decreased overall with training (main

effect, p < 0.0001). To the contrary, visceral fat was greater in men than in women (p

= 0.013), and did not change with training in either group (p for combined group =

0.41).

Table 1. Descriptive data

Variable	Men			Women		
	Total Baseline (n=30)	Before Training (n=19)	After Training (n=19)	Total Baseline (n=24)	Before Training (n=14)	After Training (n=14)
Age (yrs)	59.2±1.1	60.6±1.3	-	57.2±1.2	58.0±1.3	-
VO$_2$ max (mL/kg/min)	27.0±0.9	27.9±1.2	32.5±1.2[†]	23.3±1.0	24.0±1.2	27.5±1.5[#]
Weight (kg)	87.9±2.5	85.0±3.3	83.7±3.4[*]	75.0±3.1	72.2±3.6	70.2±3.5[#]
Body fat (%)	29.9±1.2	27.7±1.4	25.8±1.4[#]	42.3±1.5	40.7±1.8	38.7±1.0[†]
Visceral fat (cm^2)	161±15	164±21	163±21	106±7	105±7	102±10

Means ± S.E.M. yrs, years; mL/kg/min, milliliters/kilogram/minute.
[*], p < 0.01; [#], p < 0.001; [†], p < 0.0001

Insulin sensitivity

Insulin sensitivity index (ISI) was calculated using the method of Matsuda and DeFronzo (46). In a repeated-measures compound-symmetry ANOVA, ISI was not significantly different between men and women ($p = 0.39$), and improved in individuals who underwent an OGTT both before and after training (baseline ISI = 3.3 ± 0.30; final ISI = 3.6 ± 0.3; $p = 0.034$; $n = 32$). There was no gender-training interaction effect on ISI ($p = 0.39$).

Fasting and postprandial adiponectin levels

Residuals of raw adiponectin data were found to be non-normally distributed and were log10 transformed. Residuals after transformation were normally distributed. Statistical procedures on AN concentration data were executed using transformed data, and subsequently reverse transformed for presentation. In testing hypothesis 1 (peak postprandial AN levels are a function of central fat tissue mass), adiponectin levels 60 minutes into the postprandial phase did not rise compared to fasting adiponectin levels in either women or men, either before or after training (figure 1). Tests for postprandial variations of plasma AN relative to fasting levels showed that 60-minute AN levels were not significantly different from fasting levels in men ($p = 0.26$; baseline and final tests combined), women ($p = 0.96$; baseline and final tests combined), at baseline ($p = 0.73$; men and women combined) or after exercise training ($p = 0.51$ men and women combined), respectively. In a smaller but representative subset of tests across both genders and time points ($n = 21$), the 30- and 90-min time points were also analyzed for plasma AN concentration. In these samples,

mean log10 plasma AN concentrations were 0.73 ± 0.059 µg/mL (fasting concentration), 0.75 ± 0.057 µg/mL (30 min), 0.74 ± 0.060 µg/mL (60 min), and 0.73 ± 0.053 µg/mL (90 min; mean $\pm$ S.E.M.). When reverse transformed, these values correspond 5.4 µg/mL (fasting), 5.6 µg/mL (30 min), 5.5 µg/mL (60 min), and 5.4 µg/mL (90 min). No significant changes in AN levels compared to fasting levels were detected at those time points (log transformed data were used for statistical analysis). These findings were independent of visceral fat mass. Similarly, the absence of a postprandial AN peak in the sedentary state was unchanged after training.

Figure 1. Fasting and postprandial AN levels
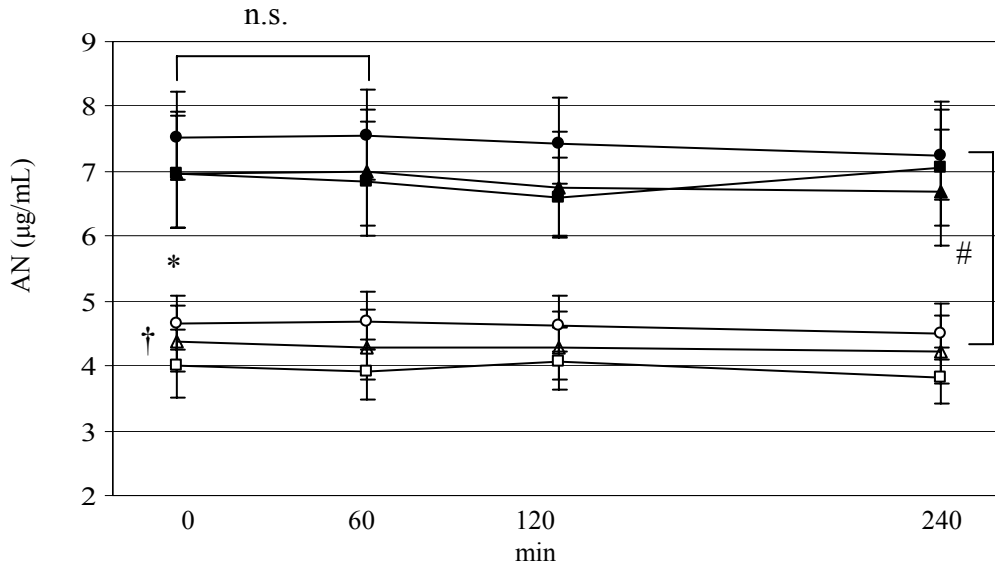
Data are means ± S.E.M.
● Women, total baseline (n = 24); ▲ Women, before training (n = 14); ■ Women, after training (n = 14);
○ Men, total baseline (n = 30); Δ Men, before training (n = 19); □ Men, after training (n = 19).
*, main gender effect on fasting plasma AN levels; p = 0.0074.
†, training effect on fasting plasma AN levels in men; p = 0.0095;
#, main gender effect on plasma AN AUC; p = 0.014. Refer to Figure 2 for detailed analysis of postprandial AN AUC data.
ns, no significant difference between 0-min and 60-min AN sample.

As expected, fasting AN levels were significantly higher in women compared to men (p = 0.0074). There was a tendency for overall fasting AN (p = 0.069) but not AN AUC (p = 0.39) to be decreased after training. The interaction effect between gender and training status approached statistical significance for fasting AN levels (p = 0.096) and was statistically significant (p = 0.048) for AN total AUC levels. Further analysis revealed significantly decreased AN AUC levels after training in men (p = 0.031), while there was no difference in women (p = 0.44; figure 2). Similarly, fasting plasma AN levels were decreased in men but not in women after training (figure 1).

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Elevated plasma AN levels in women compared to men appeared to be consistent throughout the four-hour test, since AN total AUC was also significantly greater in women compared to men (figure 2). After including fasting AN levels as a covariate in the AN AUC statistical analysis, both the training status-gender interaction as well as the gender effect became statistically non-significant, indicating that fasting AN levels are a major determinant for postprandial plasma AN concentration.

Figure 2. Total AUC AN
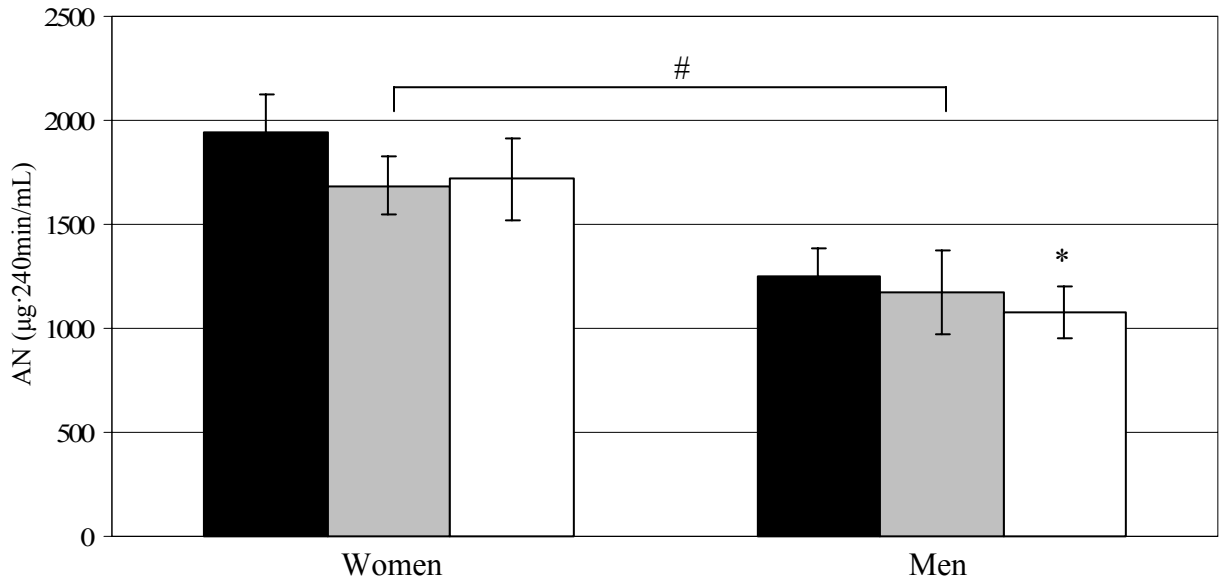
Data are means ± S.E.M.
AN, adiponectin; AUC, area under the curve; μg, microgram.
Black bars, total baseline (n = 24, 30 for women, men); gray bars, before training (n = 14, 19 for women, men); white bars, after training (n = 14, 19 for women, men); #, main gender effect, p = 0.014; *, significantly different from Baseline, p = 0.031.

In either men or women, there was no detectable relationship between changes in visceral adiposity and changes in fasting AN levels before compared to after training (see figure 3). Pearson correlation coefficients between those two variables were 0.081 (p = 0.77) in men and -0.163 (p = 0.59) in women, respectively.

Figure 3. Relationship between changes in visceral fat and fasting adiponectin levels before versus after training
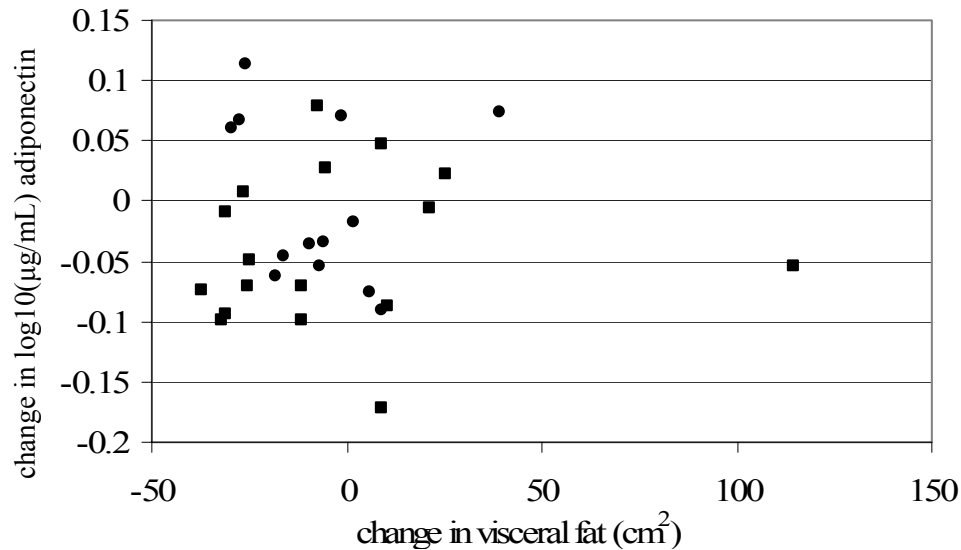
• Women (n = 13); ■ Men (n = 17).

Insulin levels (table 2)

Fasting PPLT plasma insulin levels did not change significantly with exercise training for the combined group (i.e., men and women p = 0.34). Also, fasting PPLT insulin levels did not differ between men and women before or after training. Contrary to PPLT insulin results, however, overall fasting OGTT insulin levels were significantly lower after training (before training, 14.3 ± 1.0 mU/mL, after training, 12.9 ± 1.0 mU/mL, p = 0.050). This difference in results may be due to the standardized carbohydrate ingestion protocol preceding the OGTT. There was no difference between men and women in PPLT insulin total AUC, but overall total insulin AUC decreased significantly with training (p = 0.0009) in the combined group, as well as independently in men and women (table 2, figure 4).

Table 2. Fasting and postprandial insulin levels

	Fasting (µU/mL)			PPLT total AUC (µU·240min/mL)		
	Total Baseline	Before Training	After Training	Total Baseline	Before Training	After Training
Men (n=30,19,19)	13.4 ± 1.1	13.7 ± 1.0	13.1 ± 1.3	8138 ± 739	8224 ± 1114	7411 ± 868[*]
Women (n=24,14,14)	13.0 ± 1.1	14.9 ± 1.9	12.7 ± 1.1	8135 ± 730	8000 ± 978	6682 ± 803[§]

Data are means ± S.E.M. µU/mL, microunits per milliliter.
[*], $p < 0.05$ compared to baseline; [§], $p < 0.01$ compared to baseline.

Figure 4. Fasting and postprandial PPLT plasma insulin levels for all subjects
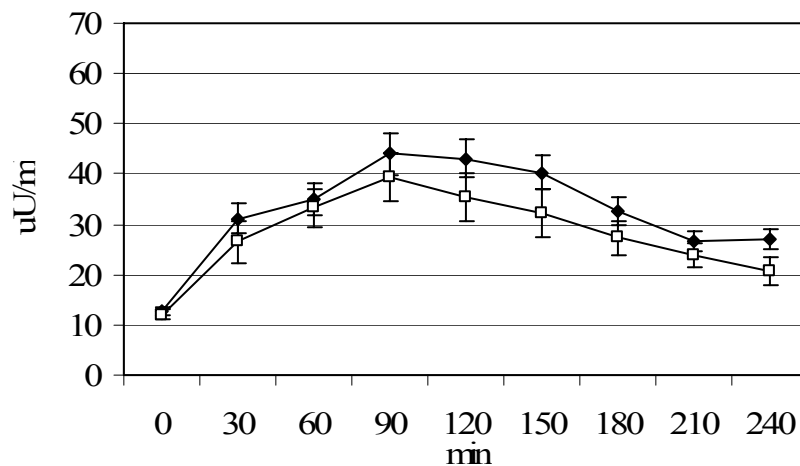
Data are means ± S.E.M. ♦ Baseline; □ Final. µU/mL, microunits per milliliter
Training effect for total AUC, $p = 0.0009$.

Plasma Triglycerides

Overall fasting TG levels did not change with exercise training ($p = 0.21$), and were not significantly different between men and women ($p = 0.84$; table 3). When analyzed by gender, men significantly decreased fasting plasma TG levels with training, but women did not. Similarly, overall PPLT TG total AUC did not change with training ($p = 0.19$; figure 5), although there was a tendency for a training-gender

interaction effect ($p = 0.089$). Closer evaluation showed that PPLT TG total AUC did

not change in women, but did in men (see table 3).

Table 3. Fasting and postprandial triglyceride levels

	Fasting (mg/dL)			PPLT total AUC (mg·240min /dL)		
	Total Baseline	Before Training	After Training	Total Baseline	Before Training	After Training
Men (n=30,19,19)	113 ± 9.1	122 ± 12.7	96 ± 9.5[§]	39652 ± 2902	41734 ± 4092	34806 ± 2586[*]
Women (n=24,14,14)	122 ± 21.6	100 ± 14.7	111 ± 17.1	42336 ± 6131	36782 ± 4713	38974 ± 6133

Data are means ± S.E.M. mg/dL, milligrams per deciliter.
[*], $p < 0.05$ compared to baseline; [§], $p < 0.01$ compared to baseline.

Figure 5. Fasting and PPLT plasma TG levels for all subjects
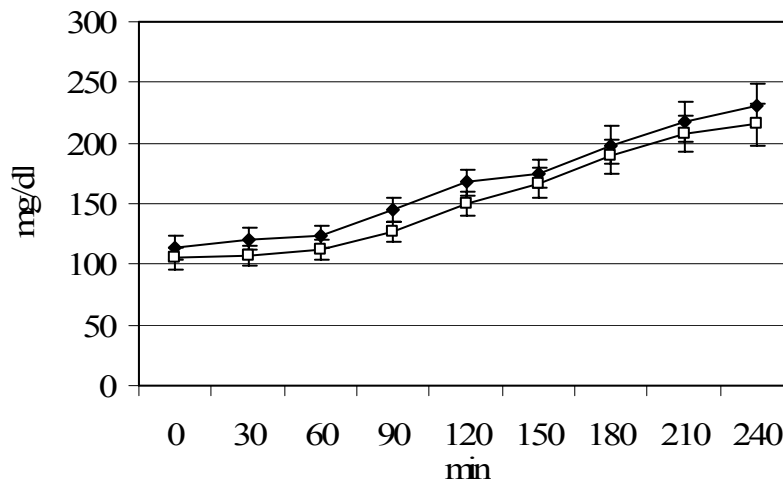
Data are means ± S.E.M. mg/dL, milligrams per deciliter. ♦ Baseline; □ Final.
Training effect for total TG AUC, $p = 0.19$.

Serum Free Fatty Acids

No gender difference was detected for either fasting or PPLT FFA (table 4,

figure 5). In addition, both overall fasting (p = 0.29) and postprandial FFA (p =

0.3661) levels did not change with training (see figure 6).

Table 4. Fasting and postprandial free fatty acid levels

	Fasting (μMol/L)			PPLT total AUC (μMol·240min/L)		
	Total Baseline	Before Training	After Training	Total Baseline	Before Training	After Training
Men (n=30,19,19)	0.37 ± 0.0277	0.364 ± 0.0363	0.313 ± 0.0327	78.2 ± 4.45	73.5 ± 5.90	67.1 ± 5.83
Women (n=24,14,14)	0.413 ± 0.0467	0.334 ± 0.0449	0.35 ± 0.0502	86.9 ± 6.61	77.4 ± 6.63	77.7 ± 7.32

Data are means ± S.E.M. μMol/L, micromoles per liter.

Figure 6. Fasting and postprandial serum FFA levels for all subjects
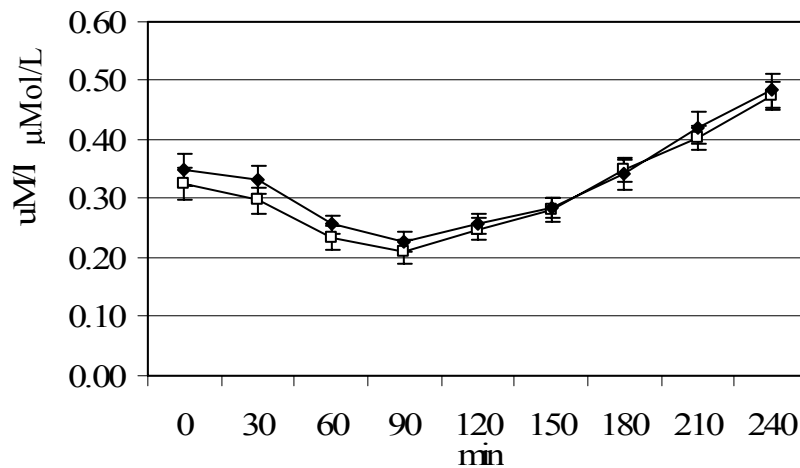
Data are means ± S.E.M. μMol/L, micromoles per liter. ♦ Baseline; □ Final.
Training effect for total AUC, p = 0.37.

Since there were significant gender differences in variables such as visceral fat and % body fat, multiple regression procedures were performed separately for men and women. Stepwise regression analysis in men revealed relationships between fasting plasma AN and ISI ($p = 0.026$) at baseline. Interestingly, ISI was not a significant regression variable after training, but VO_2 max was ($p = 0.011$). Similar to men, in women, ISI was the sole statistically significant predictor of AN levels ($p = 0.01$). After training, ISI ($p = 0.024$), weight ($p = 0.04$) and % body fat ($p = 0.05$) were found to be statistically significant in our stepwise regression model. When stepwise regression analyses procedures were performed on total AUC AN levels, fasting AN was highly significant in both men and women, before and after training ($p < 0.0001$ in all cases). In addition, ISI ($p = 0.0024$), visceral fat ($p = 0.025$), and VO_2 max ($p = 0.026$) were statistically significant in women after training.

Chapter 4 – Discussion

The main finding of the present study was a significant decrease in fasting and postprandial plasma adiponectin levels in men following six months of structured and supervised endurance training at approximately 70% of VO$_2$ max. The decrease in adiponectin levels in men was remarkably consistent between fasting and postprandial measurements. These findings were in contrast to unchanged fasting and postprandial adiponectin levels in women. At first glance, our results may appear somewhat unexpected, since several exercise training studies have reported unchanged plasma adiponectin levels following endurance training alone (7, 31) or in combination with moderate weight loss (58). However, further investigation shows that these studies are mostly consistent with our findings. For example, Ryan et al. (58) did not find decreased fasting adiponectin levels following endurance exercise training in women. Similarly, Hulver et al. studied mostly, but not exclusively, women in their exercise training study, and reported no change in adiponectin levels (31). In addition, our findings are supported by Yatagai et al., who reported decreased plasma adiponectin levels in men following exercise training.

We are confident in stating that postprandial adiponectin levels do not differ markedly from fasting levels. Although the first report on this matter, a study by English et al. (16), showed very interesting results, we are not aware of any published data in support of the English group's findings of a postprandial adiponectin increase relative to fasting levels in obese or lean individuals. Our findings are supported by Karlsson et al. (34), who, in a comment on English's findings, were first to note the

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absence of a postprandial spike in adiponectin levels in obese individuals in their data. In fact, English et al. were subsequently unable to reproduce their original findings (34). Since there was no well-defined or significant postprandial increase of plasma adiponectin in our data, we attempted to identify determinants of fasting and postprandial adiponectin levels in our subjects. Our first hypothesis originally stated that in sedentary individuals, postprandial changes in AN levels as defined by the difference between fasting and 60-min postprandial AN levels are predominantly a function of central fat tissue mass. When we analyzed our data using stepwise regression, we found that the amount of central adipose tissue was not the main predictor of either fasting or postprandial (expressed as total AUC) adiponectin levels in men or women. Rather, insulin sensitivity determined from a 2-hour oral glucose tolerance test, and to a lesser extent, total body fat, body weight, and VO_2 max were independent predictors of fasting adiponectin levels. Somewhat surprisingly, however, central adipose tissue was not a significant predictor in either of these models.

Considering that visceral fat is known to be a metabolically highly active tissue and inherently involved in regulating insulin sensitivity and other metabolic processes, the apparent relationship between total body fat mass, but not visceral fat, and fasting adiponectin in some of our subjects is counterintuitive. Generally, results regarding visceral fat in our subjects were not quite as expected. Specifically, visceral fat was not decreased significantly after training. This was surprising inasmuch as endurance exercise training has been shown to attenuate the increase in visceral fat accumulation in rodents (unpublished personal observations) and humans (53). It must be said, however, that subjects in the current study were strongly encouraged to

maintain body weight as much as possible, and this aspect of our study may have prevented significant changes in visceral fat. Since both VO_2 max as well as insulin sensitivity increased significantly with our exercise training protocol, suggesting an appropriately chosen training stimulus, we are confident that the lack of a change in visceral fat is not simply due to a lack of a training stimulus.

In addition to fasting levels, we also analyzed adiponectin AUC using regression to gain insight into the regulation of postprandial levels of this adipokine. Fasting adiponectin was by far the most important predictor of postprandial adiponectin levels. Interestingly, however, insulin sensitivity, VO2 max, and visceral fat were statistically significant during regression analysis in women after training. To our knowledge, these data on predictors of postprandial adiponectin have not been reported previously.

The decreased fasting and postprandial adiponectin levels in our male subjects present an intriguing finding. As mentioned previously, fasting adiponectin levels have been reported to be decreased (76) or unchanged (7, 31, 58) following various endurance exercise training protocols. We are not aware of any training studies that investigated postprandial adiponectin levels. Several explanations are possible for the decreased adiponectin levels following exercise training (also see Yatagai et al. (76) for a discussion of this topic). In all likelihood, decreased adiponectin synthesis/secretion is regulated within the adipocyte itself, although the original stimulus may well be extracellular. One possibility to explain our findings may be that enhanced adipocyte insulin sensitivity has an effect on adiponectin synthesis. A recent paper by Peres et al. showed that isolated rat adipocytes from trained animals

are more sensitive to insulin than adipocytes from sedentary control animals (55). Fasshauer et al. have demonstrated that a variety of hormones such as TNF-α or insulin can, in fact, downregulate adiponectin synthesis (17). Considering enhanced insulin sensitivity after training, this may be one mechanism by which adiponectin levels were downregulated after training. It must be noted, however, that insulin sensitivity was increased in both men and women in the present study, and that adiponectin levels were only decreased in men. Yatagai et al. also mentioned persistent effects of catecholamine release during and after acute exercise bouts as one possible explanation for decreased adiponectin levels, since β-agonists also have been shown to downregulate adiponectin synthesis (14). In this regard, it is critical to note that Yatagai et al. obtained blood samples 16 hours after an exercise bout (76), whereas in the present study, we attempted to eliminate acute exercise effects by collecting blood samples 24 to 36 hours after the last exercise bout. It is also interesting to note that in men, plasma triglyceride levels decreased in tandem with adiponectin levels. Although it is presently not clear if this reflects altered biochemical conditions in the adipocyte, and if so, if this coincides, precedes, or is followed by altered adiponectin levels. Finally, it is also possible that men underwent an increase in 'adiponectin sensitivity', i.e., similar to the concept of insulin sensitivity, peripheral tissues may become more sensitive to adiponectin following endurance training, and require lower physiological adiponectin concentrations to achieve the desired effect. We are unaware of data in support of such a theory.

Overall triglyceride levels (fasting or postprandial) were not decreased, although men experienced significantly reduced triglyceride levels in the fasted and

postprandial state. In women, both fasting and postprandial triglyceride levels were decreased, although neither reduction achieved statistical significance. This gender-specific decrease is not due to elevated triglyceride levels in men, since both fasting and total AUC plasma triglyceride levels were actually higher (although not significantly so) in women. It is also unlikely that men achieved greater reductions in triglyceride levels due to a difference in training response, since both absolute and relative VO_2 max increases were similar between men and women. Overall, average fasting triglyceride levels before training were relatively low in both men and women.

Fasting insulin levels remained unchanged with training in blood samples obtained as part of the PPLT, but fasting insulin from OGTT studies were lower after compared to before training. While these data may appear to contradict each other at first glance, the preparation leading up to those two tests differed in one important point. Whereas prior to the PPLT, no special dietary modifications except avoidance of alcoholic beverages for 24 hours were necessary, subjects were asked to consume a diet high in carbohydrates for the three days prior to the OGTT. This fairly common procedure was included to ensure that subjects were not carbohydrate depleted at the beginning of the test. A side effect may have been a 'normalization' of insulin levels in the fasted state, which may have reduced some variability in the OGTT insulin samples. It is also interesting to note that mean fasting insulin levels from the OGTT tended to be higher compared to PPLT fasting insulin both before and after training, which is consistent with increased systemic glucose/glycogen levels. Regardless of the unchanged fasting insulin levels in the PPLT, total insulin AUC levels were significantly decreased after training. This most likely reflects

improved peripheral insulin sensitivity secondary to training, and is consistent with increased insulin sensitivity calculated from OGTT insulin and glucose measurements.

Contrary to triglyceride values in men, fasting or postprandial plasma free fatty acid levels were not affected by exercise training in either men or women. Both in baseline and final tests, postprandial free fatty acids exhibited a characteristic drop at 90 – 120 min which coincides with peak insulin levels during this test. These two variables are functionally related, as insulin increases glucose uptake into the adipocyte. The resultant increase in intracellular glucose-3-phosphate increases plasma fatty acid clearance, and decreases fatty acid mobilization (32).

In conclusion, 6 months of exercise training at approximately 70% of VO_2 max significantly increases aerobic capacity and insulin sensitivity in 50 – 75-year-old men and women. Furthermore, we determined that improved fasting and postprandial triglyceride levels in men coincide with decreased adiponectin levels in men. Adiponectin levels in women were not changed in the present study. The molecular mechanisms behind the decrease in adiponectin with training in men are not clear from our data, and further investigation is needed on this topic. Additionally, we have shown conclusively that postprandial adiponectin levels do not change compared to fasting levels as initially assumed. Whereas fasting adiponectin levels were largely determined by insulin sensitivity in the present study, postprandial adiponectin is in turn mostly related to fasting adiponectin levels.

Chapter 5 – Review of Literature

Background, introduction

For over five decades, adipose tissue has been thought to not only be the central site for lipid storage (59), but to also hold endocrine function (36). It was not until 1994, however, that the discovery of leptin (*gr.* leptos = "thin, slender") in 1994 by Friedman's group (79) effectively started the field of 'adipokine' or 'adipocytokine' research. The term 'adipocytokine' pays homage to the fact that some sequence homology exists between certain adipocyte-released polypeptides and inflammatory cytokines (as is the case, e.g., with complement factor C1a and acylation-stimulating protein).

Friedman's discovery received a great amount of attention at the time of its publication not only because of the perceived novelty of considering adipose tissue an endocrine organ, but also because of the leptin knockout animals' inactive and obese phenotype. Since then, over 20 adipokines have been identified. Besides leptin, other adipokines such as adiponectin, resistin, tumor necrosis factor α (TNF-α), visfatin, and interleukin-6 have received much attention in the field of exercise physiology.

Based on these findings, adipose tissue is now generally considered a major endocrine organ (3, 59). The consequences of the paradigm shift of viewing adipose tissue simply as an excess lipid storage site to a metabolically highly active organ are far-reaching, and especially important in diabetes and insulin resistance research. The study of the connection of adipose tissue to these conditions seems especially prudent in light of the fact that Western populations are currently experiencing a significant

obesity problem which is mirrored by rising diabetes rates, and that there is a well-documented connection between obesity and a variety of other 'civilization diseases' such as hypertension, atherosclerosis, chronic inflammation, etc.

Adiponectin

The discovery of adiponectin was reported in 1995 and 1996 by several independent groups. Different terms (i.e., Adipocyte complement-related protein 30 [ACRP30], gelatin-binding protein 28 [GPB-28], adipose most abundant gene product 1 [apm-1], adipoQ) thus refer to the same polypeptide (4, 29, 43, 50, 61). At the present time, the terms most commonly used are adiponectin and ACRP30 (the latter especially in rodent models).

In humans, fasting plasma adiponectin levels are reported to be ~ 2 to 30 µL/mL plasma (4, 16), making it one of the most abundant plasma proteins (15). Interestingly, plasma adiponectin levels have been widely reported to decline with increased body fat mass in animals and humans (4, 15). As indicated above, adiponectin is found in human blood at very high concentrations. Estimates of adiponectin accounting for 0.01% of total plasma protein have been published (4).

Adiponectin levels are generally higher in women than in men (27, 72). In this regard, cross-sectional findings have been supported through findings in castrated mice by Nishizawa et al. who reported higher plasma adiponectin levels than in uncastrated mice (52). In addition, adiponectin levels decreased in the castrated mice following administration of testosterone. Conversely, ovariectomy did not change adiponectin levels in female mice. In the same study, plasma adiponectin levels did

not differ between pre- and postmenopausal women (52). In humans, Bottner et al. (2004) showed that in prepubescent children, adiponectin levels do not differ between boys and girls. Male-female separation of adiponectin levels occurs during puberty, however, and is dependent on serum androgen levels (6).

Physiological effects of adiponectin

On a physiological and molecular level, adiponectin has been given credit for a multitude of effects, many of which concern obesity and/or insulin sensitivity. For instance, Fruebis et al. have shown that administration of the biologically active globular region of adiponectin results in weight loss in mice consuming a high-fat, high-sucrose diet (19). These findings are in agreement with those of Masaki et al. (2003) who showed that adiponectin injection may contribute to a reduction in visceral fat weight in rodents (45), which is thought to be a critical factor in preventing insulin sensitivity.

Regarding insulin sensitivity, Kubota et al. generated full or partial adiponectin knockout mice and showed that $AN^{+/-}$ animals are mildly, and $AN^{-/-}$ animals are moderately insulin resistant, respectively (42). A gene-diet interaction effect may exist, however, since Maeda et al. did not detect impaired insulin sensitivity in mice fed on normal chow, although findings in mice on high-fat chow were similar to those by Kubota et al. (44). Maeda et al. also showed delayed FFA clearance in $AN^{-/-}$ mice. The molecular mechanisms behind the actions of adiponectin are still not fully elucidated, but it seems certain that activity of the 'cellular fuel gauge' 5'-activated protein kinase (AMPK) is at least partly mediated by the

biological effects of adiponectin. Several recent reviews describe how adiponectin can be both an activator of and substrate for AMPK (21, 24).

Because of their pronounced insulin resistance and almost complete lack of body fat, lipoatrophic animals are another fascinating model to study the endocrine effects of adipose tissue on insulin sensitivity. Yamauchi et al. showed that administration of physiological doses of adiponectin and leptin in lipoatrophic mice can recover insulin sensitivity completely, whereas injection of either leptin or adiponectin only partially reverses insulin resistance (74). In the same study, adiponectin administration decreased triglyceride concentrations in muscle and liver in obese animals (74). Analysis of the human genome also supports the role of adiponectin in insulin resistance and diabetes since genome-wide linkage scans have mapped a diabetes-susceptibility locus to chromosome 3q27, the locus of the adiponectin gene *apM11* (65).

Further *in vivo* and *in vitro* studies have provided additional evidence for the significance of adiponectin for insulin sensitivity. Berg et al. demonstrated that i.p. injection of purified adiponectin into normal healthy mice lowers fasting glucose (5). In insulin resistant mouse models (ob/ob, NOD, streptozotocin mice), adiponectin injection consistently decreased hyperglycemia without changing insulin levels. Finally, incubation of isolated hepatocytes with adiponectin increased suppression of hepatocyte glucose production via sub-physiological insulin levels.

Protein structure

Human adiponectin is a 244-amino acid protein which is expressed solely and at great rates in differentiated adipocytes (43, 61). Sequence analysis has predicted a secretory four-domain protein with a signaling sequence at the NH_2 terminal, a non-homologous sequence, a collagen-like region, and a globular C-terminal domain which is in sequence homology with C1q-like proteins (29, 63). Interestingly, the globular region can be biologically active by itself (30). Schaffler et al. (1999) were the first group to determine that the product of the adiponectin gene is a 33-kDa protein that is also detectable in serum (60). An interesting detail about adiponectin's quaternary structure was revealed when Shapiro and Scherer studied the crystal structure of adiponectin homotrimers (63). These trimers are common in human plasma, and were surprisingly similar to TNF-α in structure, folding topology, residue conservations, and other factors. These findings are especially interesting since the function of TNF-α is thought to be mainly as an important regulator of inflammatory pathways, and is thus counter to adiponectin action. A more recent finding in the field of adiponectin research has been the discovery of adiponectin multimers. That is, adiponectin molecules tend to aggregate in trimers, hexamers, or so-called high-molecular weight adiponectin complexes. Recently, Waki et al. have presented evidence that the distribution of adiponectin multimers larger than trimers is dependent on a disulfide bond, and that multimer distribution may play a role in adiponectin's effects on modulating insulin sensitivity (67, 69, 70).

Regulation of adiponectin expression

Fasshauer et al. sought to determine possible regulatory effects of insulin on AN expression levels (17). They determined that in 3T3-L1 adipocytes, insulin administration suppressed AN gene expression levels by ~ 75%. Similarly, TNF-α decreased adiponectin expression in these cells. These findings may provide a mechanistic connection between well-documented increased insulin levels in the insulin resistant state and depressed adiponectin levels in these individuals.

In a study conducted by Tiikkainen et al., drug-naïve type-2 diabetic patients were either treated with rosiglitazone or metformin, both powerful antidiabetic agents, for 16 weeks (66). Rosiglitazone is a thiazoledinedione (TZD), which acts by activating peroxisome proliferator-activator receptor-γ (PPAR-γ). PPAR-γ is a powerful adipocyte-specific transcription factor which regulates the synthesis of many adipocyte proteins (56). Conversely, metformin has been shown to exert its effects in a PPAR-γ independent manner by activating the AMP-dependent protein kinase (AMPK) cascade (80). Results showed that metformin acted mainly on hepatic insulin sensitivity, whereas TZD administration increased AN levels twofold despite unchanged body weight. These findings indicate that AN synthesis is increased by PPAR-γ agonists (66).

Additionally, there is some evidence that adrenergic agonists such as isoproterenol decrease AN gene expression in adipose tissue (14). Interestingly, McGarry and others have hypothesized that a slight but persistent oversecretion of adrenaline may be the first step in the development of insulin resistance and diabetes (47). Finally, as mentioned above, there is some evidence from animal (52) and

human (6, 52) studies that adiponectin synthesis is directly or indirectly affected by androgen, most likely testosterone, levels.

Relationship between adiponectin and obesity/weight loss and insulin sensitivity

There is ample circumstantial evidence suggesting a connection between plasma adiponectin levels and obesity (15). As mentioned previously, adiponectin is the only adipokine known to be negatively correlated with obesity indices (e.g., BMI, body fat mass), in addition to indicators of insulin sensitivity such as fasting insulin levels or calculated insulin sensitivity index (4), or blood lipids (e.g., serum triglycerides) in humans and most animal models (4, 15, 27). Longitudinal studies in rhesus monkeys have shown that decreases in adiponectin mirror the progression of insulin resistance in these animals (28). In murine adiponectin gene knockout models homozygous (i.e., $AN^{-/-}$) mice are significantly more insulin resistant than heterozygous ($AN^{+/-}$) animals (42).

Keeping the apparent correlation of obesity and insulin sensitivity in mind, there is actually little direct evidence that physiological weight loss and the often-observed improvements in insulin sensitivity in insulin resistant individuals with weight loss coincide with increased adiponectin levels. In two studies published in 2004, Abbasi, Reaven, et al. studied the effects of weight loss on adiponectin levels. In the first study, obese women participated in a moderate weight loss program via caloric restriction and administration of an appetite suppressant (2). Subjects were grouped into insulin sensitive and insulin resistant groups. Adiponectin levels were somewhat higher in the insulin sensitive group before intervention ($p = 0.10$).

Although weight loss in both groups was between 8 and 9 kg over the duration of the study and insulin sensitivity improved in the insulin resistant group, adiponectin levels were not affected by treatment.

In another study by the same group, 60 nondiabetic individuals were stratified into four groups according to insulin sensitivity (steady-state plasma glucose concentration following octreotide, glucose, and insulin infusion), and obesity (obese vs. non-obese). Results showed that insulin resistant individuals had significantly lower plasma adiponectin levels independent of the degree of obesity. In addition, adiponectin levels were more varied in the insulin resistant compared to the insulin sensitive individuals (1). Abbasi's conclusion from these studies was that "adiponectin levels are more closely related to differences in insulin-mediated glucose disposal than obesity." (1).

Bariatric surgery studies

Much of the evidence in support of increased adiponectin levels after weight loss comes from studies where individuals lose large amounts of body mass by direct surgical removal of fat (e.g., liposuction, (23)), or indirect fat loss by dramatic restriction of caloric intake or other means (e.g., various forms of bariatric surgery). For instance, Yang et al. published data suggesting weight loss to be a mechanism for increasing adiponectin levels (75). 22 obese individuals (BMI $= 40$ kg/m^2) decreased their BMI by an average of 21%; this dramatic decrease was concomitant with an increase in insulin sensitivity, and increased adiponectin levels (46%). Similarly,

Hulver et al. reported even greater changes in adiponectin levels (average increase, 281%) after subjects decreased BMI from 47 to 28 kg/m^2 (31).

Immediate effects of bariatric surgery

It is interesting to note that following bariatric surgery, insulin sensitivity improves quickly and before large amounts of weight loss are realized. For example, Rubino et al. (2004) reported normalized insulin and glucose levels in type 2 diabetics three weeks after gastric-bypass surgery (57). At that point, BMI had not statistically significantly decreased. In a similar study, Clements et al. (2004) studied the effects of gastric-bypass surgery in 20 morbidly obese type 2 diabetics. Two weeks after surgery, plasma glucose levels had normalized. There was only a tendency for insulin levels, however, to decrease over a twelve-week follow-up period. Plasma levels of the incretin gastric inhibitory polypeptide (incretins are hormones produced by the gastrointestinal tract following nutrient entry) were significantly decreased and may provide an explanation for the apparently improved insulin sensitivity so quickly after surgery (11). Although none of these papers reported adiponectin levels, these findings suggest that weight loss may not be the primary or only factor in changing adiponectin levels after gastric-bypass surgery, and that results from such studies have to thus be interpreted with caution if attempting to draw a connection between weight loss and changes in adiponectin. Similar to bariatric surgery, there are some reports suggesting that liposuction will improve an individual's adiponectin profile. Giugliano et al. reported that removing an average of 2.7 kg of lipid from 30 obese

premenopausal women resulted in increased insulin sensitivity and adiponectin levels six months after surgery (23).

Weight loss by caloric restriction

Xydakis et al. (2004) enrolled 80 individuals of whom 40 had been previously diagnosed with Metabolic Syndrome into a weight loss program to study adipokine and other plasma metabolite levels as a result of weight loss. After approximately four to six weeks of caloric restriction, glucose, leptin, insulin, and triglyceride profiles had improved, although there was no change in either TNF-α or adiponectin levels (71). Monzillo et al. (2003) have reported that when diabetic individuals participated in 'lifestyle modification' programs (moderate exercise and weight loss), they improved their adipokine (adiponectin, leptin, TNF-α) profile. These findings contrasted with non-diabetic individuals whose adiponectin levels did not increase following a similar intervention (49).

The inconsistency in adiponectin response due to weight loss is puzzling, since a clear relationship between adiponectin and body fat mass exists (15). One possible explanation for discrepant findings could be that most cross-sectional studies compare lean to obese individuals. In the first report of the inverse relationship of adiponectin to body fat mass, Arita et al. reported inverse linear relationships in both men and women between adiponectin and BMI (4). Interestingly, correlation coefficients increased slightly when adiponectin levels were log transformed. This would indicate an actual curvilinear, rather than linear, relationship between adiponectin and BMI, and a visual inspection of Arita's graphs

confirms this notion. In their subjects, plasma adiponectin levels seem to drop off relatively steeply from a BMI of 20 to 30. BMI values greater than 30 kg/m^2 did not seem to further affect adiponectin levels (4). If proven correct, this theory would present a unifying explanation why a) adiponectin levels are increased in obese compared to lean individuals, b) moderate weight loss due to caloric restriction often does not result in increased adiponectin levels (due to a failure to reach a 'critical threshold' necessary for improved adiponectin profile around a BMI of 30), and c) adiponectin levels are often increased following gastric bypass surgery (due to drastically decreased fat mass). Unfortunately, we are not aware of any published studies which have further pursued this question based on this observation.

Effects of thiazolidinedione administration on adiponectin levels

Thiazolidinediones (TZD) are a group of commonly prescribed and very powerful anti-diabetic drugs. It is known that TZDs activate the peroxisome proliferator activator receptor γ (PPAR-γ), which is an important factor in gene expression (8). PPAR-γ is highly expressed in adipose tissue. Yamauchi et al. showed that PPAR-γ activation via supraphysiological TZD administration improves insulin sensitivity by reducing lipid concentration in liver and skeletal muscle (73) (73). In addition, Kintscher and Law suggested another role, namely the regulation of secretory protein synthesis, for PPAR-γ (37). In human studies, these findings of TZD administration and concomitant improved insulin sensitivity are supported by consistent reports of elevated adiponectin levels subsequent to PPAR-γ activation in humans. For example, Yu et al. demonstrated that 600 mg/day of the TZD

troglitazone significantly improved adiponectin levels at the same rate in non-diabetic obese and lean, and type-2 diabetic individuals (78). Similarly, data from Ralph DeFronzo's lab support the role of TZD administration in raising adiponectin levels in type-2 diabetic men. In their study, 23 subjects who received pioglitazone for four months significantly increased plasma adiponectin levels despite an increase in total fat mass (48).

Adiponectin and exercise training

Surprisingly few studies have been conducted to assess the effects of endurance exercise training on fasting plasma adiponectin levels. In 2002, Hulver et al. investigated potential adaptations in fasting plasma adiponectin levels following endurance exercise training absent weight loss or significant weight loss following gastric bypass surgery, respectively (31). Subjects were older, mostly insulin resistant or type II diabetic, and mostly female, although some men also participated in the study. Exercising subjects performed approximately 3 hours of exercise per week for 6 months, whereas weight loss subjects lost approximately 57 kg body weight on average. Both groups experienced significant improvements in insulin sensitivity (exercise group, 98%, weight loss group, 432%) following their intervention. Whereas there was no significant change in fasting adiponectin levels following exercise training (6.3 ± 1.5 to 6.6 ± 1.8 μg/mL), gastric bypass surgery resulted in significantly increased plasma adiponectin levels (4.4 ± 0.8 to 13.6 ± 2.2 μg/mL). Hulver et al. suggested that weight loss but not endurance exercise training has the potential to significantly improve fasting plasma adiponectin levels.

In an attempt to determine the weigh loss-independent effects of exercise on adiponectin levels, Yatagai et al. enrolled 12 healthy non-obese men in 6 weeks of endurance exercise training (60 min/d at lactate threshold intensity, 5 d/week) (76). With no change in body or fat mass, insulin sensitivity was improved after exercise training when assessed 16 h after the last exercise bout. Although serum adiponectin levels were significantly and positively correlated with insulin sensitivity at baseline ($r^2 = 0.63$), adiponectin levels unexpectedly decreased slightly but significantly after training (20.9 ± 17.2 μg/mL to 17.2 ± 6.6 μg/mL) despite evidence of enhanced insulin sensitivity after training. Adiponectin levels returned back to baseline levels after one week of inactivity (76). Yatagai et al. suggested that, considering AN's function as an insulin-sensitizing hormone, decreased AN levels may have occurred in response to the enhanced insulin sensitivity after exercise training.

In a study conducted by Ryan et al. (2003), post-menopausal women were placed on a moderate-weight loss diet, and randomized into a resistance training, endurance training, or non-exercise group (58). Although these individuals lost significant amounts of body fat, this did not result in decreased fasting plasma AN levels, even if combined with endurance or resistance exercise training. Conversely, fasting glucose, leptin, and insulin concentrations did decrease in these individuals (58). Ryan et al. concluded that moderate weight loss secondary to caloric restriction and/or regular exercise at moderate intensities is not enough of a stimulus to increase plasma adiponectin.

Boudou et al. (2003) studied the effects of exercise training on adiponectin and leptin levels in diabetic males (7). 16 middle-aged type II diabetic men were

either randomized into a 'sedentary' or 'exercise' group. Exercisers performed a combination of steady-state endurance exercise at 75% of VO_2 max (twice/week), and one session of high-intensity interval training (85% of VO_2 max) once per week for eight weeks. Following exercise training, no change in AN levels was observed in trained compared to sedentary individuals, although the trained group significantly decreased visceral fat (44%) and increased insulin sensitivity (58%) without changing body weight significantly (7). Although there was no significant correlation between change in AN and change in central adiposity, changes in fasting AN levels were significantly correlated to changes in body weight in trained individuals. These findings suggest that although adiponectin may affect insulin sensitivity, exercise training-induced improvements may be independent of adiponectin. Finally, in a letter to the editor published in Diabetes Care, Yokoyama et al. reported no change in AN levels after three weeks of exercise in 50-year-old and older type-2 diabetics despite improved insulin sensitivity. Subjects performed cycle ergometry at approximately 50% of maximal heart rate five days per week for three weeks. Although insulin sensitivity measured via euglycemic-hyperinsulinemic clamp improved significantly as a result of exercise, plasma adiponectin levels did not change in those individuals (77).

Adiponectin and acute exercise

Since there is some evidence that adiponectin may be involved in regulation of what is thought to be a major fuel sensor of the eukaryotic cell, 5'-AMP activated protein kinase, there has been some interest regarding changes in adiponectin levels

during and immediately after exercise. In a study published in 2004, Ferguson et al. described adiponectin levels in men and women before and immediately after a 60-minute cycle ergometry exercise bout at $\sim$ 65% of VO_2 max (18). Although TNF-α levels increased significantly in a gender-independent manner, adiponectin levels did not change following the exercise bout.

Similarly, Kraemer et al. et al. investigated the effect of short-term exercise (running at $\sim$ 79% of VO_2 max, graded running test between 65 and 100% of VO_2 max) (40). In both tests, adiponectin levels immediately after exercise were no different after accounting for exercise-induced shifts in plasma level (1st experiment), or in comparison to a rested control group (2nd experiment), respectively. Contrasting Kraemer's findings, Jurimae et al. presented evidence that adiponectin is, in fact, increased immediately after exercise in some circumstances (33). In their study, highly trained rowers performed rowing ergometry (6000-m time-trial). Adiponectin levels were assessed immediately and 30 minutes after exercise. Results showed that adiponectin levels were significantly decreased immediately after exercise (average decrease from baseline, 11%), and appeared to supercompensate 30 minutes after exercise (average increase from baseline, 20%). Curiously, however, insulin levels did not decrease with the onset of exercise in this study. Decreased insulin levels are commonly observed during acute exercise (20). It is not quite clear how the lack of an insulin decrease, which is known to be at least partly caused by adrenergic inhibition of insulin synthesis of the β-cell, can be explained in light of an apparent adrenergic effect on adiponectin levels.

Kriketos et al. reported an increase in adiponectin levels following two to three exercise sessions in obese males (41). On average, adiponectin increased by 260% (7.0 to 18.2 ug/mL). The authors did not state how long after the last exercise bout blood samples were drawn, however, and could thus have been describing a combination of acute and 'early chronic' effects of exercise.

Postprandial plasma adiponectin levels

In the first study that described postprandial AN increases in obese individuals, English et al. reported differences between obese and lean individuals' postprandial plasma AN levels (16). Specifically, lean individuals displayed increased insulin sensitivity compared to the obese subjects, and showed no significant AN plasma level excursion over a three-hour postprandial period following a mixed-composition meal. Conversely, obese subjects experienced significantly lower fasting AN plasma levels which increased dramatically (on average approximately three-fold) after meal ingestion and, on average, peaked 60 minutes into the postprandial period.

In the English et al. study, no mention was made of subjects' cardiovascular training status. In accepting the notion that lean individuals on average display superior cardiovascular fitness compared to otherwise matched overweight or obese individuals, there exists a possibility that this variable may have a confounding influence on postprandial plasma AN levels independent of or in conjunction with the degree of fat mass in these individuals. To our knowledge, no published reports on a possible endurance exercise training effect on postprandial adiponectin response exist.

It is important to note, however, that the results of the English et al. study have been subsequently questioned by Karlsson et al. (34). Karlsson et al. studied fasting and postprandial adiponectin levels following a standardized 770-kcal mixed meal in very lean (average BMI = 23.5) and morbidly obese (average BMI = 43.9) individuals (5 men, 5 women in each group). Although fasting and postprandial adiponectin levels were higher in morbidly obese compared to lean individuals as previously reported, Karlsson et al. did not observe the postprandial deflection in adiponectin levels in obese individuals that English et al. had reported. In their response to the Karlsson group's letter, English et al. were not able to replicate their initial findings (16).

Effects of acute and chronic exercise on postprandial adiponectin

We currently are not aware of any studies which have investigated the effect of exercise training on postprandial adiponectin levels. Similarly, to our knowledge there are no published data on the issue of changes in postprandial adiponectin levels in the presence or absence of a previous acute bout of exercise.

Postprandial lipemia

Most humans in Western civilizations are in a postprandial state for a majority of their lifetime (54). This fact notwithstanding, data on postprandial fluctuations on most hormones except insulin are scarce. Comparisons of different insulin sensitivity indices that are based on either fasting and/or postprandial insulin and glucose concentrations, however, make it clear that fasting levels of a given biomolecule are

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often insufficient to draw reliable conclusions on, e.g., an individual's insulin sensitivity or other health variables (46). Considering that the typical Western diet is relatively high in fat (62), studies on postprandial lipemia – most commonly measured as deviations in plasma triglyceride levels – are relatively rare. This may partly be due to practical reasons. For instance, peak lipemia following a high-fat meal typically does not occur until approximately four hours after meal ingestion, whereas peak glycemia occurs about 60 minutes after ingestion a glucose beverage. In some conditions, such as hypertension, peak lipemia may be further delayed (39).

Postprandial lipemia after acute and chronic exercise

In 1983, Patsch et al. attempted to establish a connection between HDL_2 and postprandial lipemia (54). Twenty-eight volunteers were recruited and underwent a postprandial lipemia test. It was determined that HDL_2 levels were strongly and negatively correlated ($r = -0.86$) with postprandial triglyceride levels. There was also a weaker, yet still significant, correlation between running mileage (some of the subjects were athletes), and postprandial triglycerides (54). In a sub-section of the study, Patsch et al. were able to monitor one of their subjects' lifestyle changes over the course of several years. Upon baseline screening, the subject (male) was sedentary, and postprandial triglyceridemia was pronounced. Over the course of a little over a year, regular training seemed to contribute to drastically reduced postprandial lipemia in this subject. The extent of postprandial lipemia continued to decrease until minor surgery necessitated cessation of exercise for approximately four weeks. When the subject underwent a lipemia test after this period of relative inactivity, postprandial

triglyceride levels were similar to those in the sedentary state at the beginning of the study. Conversely, approximately 5 months after the subject had resumed training, postprandial lipemia had returned to 'trained' levels (54).

This study was important because it helped define the effects of chronic exercise on an important phenotype. Unfortunately, however, times between the last exercise bout and each lipemia test were not recorded in this study, so it can not be said with certainty whether reduced lipemia in the trained state was due to the acute or chronic effects of exercise, or a combination of both.

To our knowledge, only four studies have been conducted in which the acute and chronic effects of exercise on postprandial lipemia have been compared in the same study. In a recent review, Gill and Hardman discussed the relationship between exercise and lipemia (22). The main points of their synopsis of recent literature were that postprandial lipemia is indeed attenuated by exercise, and that acute effects of exercise are the main cause for decreased lipemia in exercise studies. Additionally, the extent of caloric consumption during exercise seems to be inversely correlated to the severity of lipemia (22).

Gill and Hardman's review is in part based on a thorough study by Tsetsonis et al. where the acute and chronic effects of exercise were compared in trained and untrained women (68). Subjects performed a PPLT either after 3 ½ days of abstinence from structured exercise, or ½ day after a supervised exercise bout (90 minutes of exercise at $60 - 65\%$ of VO_2 max). Postprandial lipemia was lower, but not significantly so, in trained compared to untrained individuals. However, the reduction in postprandial lipemia following the supervised exercise bout was significantly

smaller in trained compared to untrained individuals (68). Hence, Tsetsonis et al. concluded that although acute effects of exercise account for much of the attenuated lipemia ascribed to exercise in general, there is an additive and possible even synergistic effect of chronic and acute exercise.

In 1998, Herd et al. enrolled volunteers in a thirteen-week endurance exercise training program (25). Subjects either participated in exercise training, or maintained a sedentary lifestyle. After training was completed, subjects underwent lipemia tests 15 hours, 60 hours, and nine days after the final exercise session. Sedentary individuals underwent lipemia tests at the same time. Results showed that lipemia significantly increased between the 15-hour and the 60-hour tests in the trained compared to the control individuals, and that lipemia between the 60-hour and the 9-day tests were no different between the sedentary and trained individuals. Herd et al. concluded that this worsening of postprandial triglyceridemia was due to the removal of the acute exercise stimulus in those individuals. However, training adaptations in this study were relatively small, because subjects were already aerobically fit at baseline. Specifically, average VO_2 max values among men was 58.6 ml/kg/min, and for women it was 47.1 ml/kg/min (25). Considering these obvious limitations, these findings must be interpreted with caution, as they may not apply to strictly sedentary individuals.

In a follow-up study to their 1998 paper, Herd et al. published cross-sectional data on both acute and chronic effects of exercise on postprandial lipemia in 2000 (26). In this study, trained and untrained males and females underwent postprandial lipemia tests; trained individuals abstained from structured exercise for approximately

a 60-hour period preceding the test. Consistent with previous findings, it was shown that 60 hours after the last exercise bout, no effect of exercise on postprandial lipemia was detectable in comparison to sedentary individuals. Conversely, postprandial insulin levels were still lower in the trained group at this time point (26).

In an attempt to elucidate the relationship between training status and lipemia in men, Cohen et al. recruited 58 men (29 sedentary, 29 trained) to undergo postprandial lipemia tests (13). The trained and sedentary individuals were matched according to fasting triglyceride levels. Results showed that postprandial triglyceride area under the curve in trained individuals, who underwent the lipemia test 12 hours after the last structured exercise bout, was significantly lower than in untrained individuals. Since there was a possibility that this difference was a residual acute rather than chronic exercise effect, some of the sedentary individuals were also asked to complete a lipemia test 12 hours following a supervised exercise bout. In contrast to the trained group, lipemia in the untrained individuals was not reduced after the exercise bout, and Cohen et al. concluded that the observed diminished lipemia in the trained individuals was due to chronic, rather than acute effects of training (13). It is important to note, however, that there is also an alternative explanation for these results. Namely, it is possible that acute effects of exercise 12 hours after an exercise bout are only present in already trained individuals, similar to what has been reported by Tsetsonis et al. (68).

Overall conclusions

Although initial reports pointed to the amount of fat mass as the main predictor for fasting AN levels, most recent evidence suggests that insulin sensitivity is an important independent factor in determining plasma AN concentration. It is well understood that central fat is highly predictive of insulin resistance (10). Although no tissue studies comparing central and peripheral adipocytes with regards to AN expression have been conducted, there is limited evidence from cross-sectional studies that central adiposity measurements are more predictive of plasma AN levels than whole-body adiposity measurements (subcutaneous fat), or indices such as BMI (12, 64). There are relatively few studies on the effects of acute and chronic exercise on postprandial lipemia. Although much of the reduction in lipemia associated with exercise is likely due to acute exercise effects, there is some evidence suggesting that chronic exercise also plays a role in changing postprandial triglyceride response from before to after training.

Appendix A: Informed Consent Forms

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 De4partment 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 do not have a prior history of ulcers or bleeding disorders. I also understand that I must have somewhat abnormal levels of cholesterol to enter the study.

I understand that I will complete three screening visits. For my first visit, a sample of my blood will be drawn to obtain my DNA. I understand that if I have a version of the gene that is appropriate for this study, 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. A blood sample will also be drawn 2 hours after I drink a sugar solution. I understand that a total of 4 tablespoons of blood will be drawn during these visits. 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, 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 6 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

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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 understand that a blood sample will be drawn just after the end of this test to measure how my immune system responds to exercise. I understand that 2 tablespoons of blood will be drawn during this visit. 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 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 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 7 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 use 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 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 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 warm-up 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.

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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 27 tablespoons of blood will be drawn.

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 a laboratory at the University of Pittsburgh that is part of this study. I understand, however, that my DNA samples sent to the University of Pittsburgh will be identified only by a numeric code and that only investigators at the University of Maryland 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 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

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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 the 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. 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 this research is exploratory and I understand that, if I chose, I will be told of the results of one specific genetic test and any results of other genetics studies that might be useful to me. The one specific genetic test is for a gene that affects my cholesterol levels, known as apolipoprotein E or APO E. The different versions of this gene are called APO E2, APO E3, and APO E4 and, since we all have two strands of DNA, a person could have one of six possible combinations of this gene (2/2, 2/3, 2/4, 3/3, 3/4, 4/4). APO E3 is by far the most frequent and APO E2 the least frequent form of this gene. It has been found that people with at least one APO E4 gene may be at increased risk of developing Alzheimer's disease. This is based on the results of studies with a very large number of research subjects and the relationship is only evident when the results of all of these studies are combined. I understand that it is not possible to predict my own individual risk, or that of any single person, based on the current knowledge about the APO E gene because many persons with the APO E4 gene do not develop Alzheimer's disease and many people with Alzheimer's disease do not have an APO E4 copy of the gene. Thus, I understand that scientists do not generally use APO E testing to determine a person's risk for Alzheimer's disease. I understand that I have a right to know the results of my genetic tests and if I choose to know them, I understand that I need to keep in mind the facts presented above about the importance of these genetic results.

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 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.

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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.

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CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY
Project Title: **ACE genotype, blood pressure, and exercise training in hypertensives**

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 pressure changes with exercise training. This research project will require visits to University of Maryland College Park and the Baltimore VA Medical Center. The specific tests, their requirements, and the time commitments are described below.

I already completed a telephone interview that determined that I am not physically active, am 50 - 75 years of age, not a diabetic, 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. It is also probable that I have a blood pressure that is in the high normal or stage 1 hypertension range (systolic blood pressure: 130-159; diastolic blood pressure: 85 – 99). 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.

The flow chart on the next page indicates the different testing sessions and time required by this study. I understand that if I qualify and complete this study my total involvement will last approximately 9 months. Two of the testing visits are performed at the Baltimore VA Medical Center. I understand that I will also be asked to sign University of Maryland Baltimore consent from for the tests conducted in Baltimore.

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Gene Exercise Research Study

SUBJECT FLOW CHART: Blood Pressure Study

Phase II

Screening Visits (3)
Visit 1: Review and sign informed consent, blood sample for DNA typing. (30 minutes)
Visit 2: Blood chemistries and glucose tolerance test. (2.5 hours)
Visit 3: General physical exam, cardiovascular treadmill test. (1 hour)

Phase I

Recruitment and Preliminary Screening

Phase III

Dietary Stabilization (6 weeks)
Two instructional sessions per week (40-45 minutes each)

Drug Tapering
With approval of personal physician, a gradual stopping of blood pressure medications. Blood pressure monitoring done at Dietary Stabilization sessions.

Phase IV

Baseline Tests (4)
Test 1: Treadmill exercise test (1 hour)
Test 2: Glucose tolerance test and cholesterol sample (3.5 hours)
Test 3: High-fat meal and cholesterol test (5 hours)
Test 4: Body composition scans and cholesterol test; also, if you have elevated cholesterol, post-heparin lipase activity test (PHLA) (Baltimore: 4-5 hours)

Phase V

Exercise Intervention (24 weeks)
Aerobic exercise training, 3 sessions per week, 40-50 minutes per session. At week 10, add one home-exercise session (45-60 minutes) per week and continue through week 24.

Phase VI

Final Tests (5)
Test 1: Treadmill exercise test (1 hour)
Test 2: Glucose tolerance test, cholesterol and blood pressure tests (3.5 hrs)
Test 3: High-fat meal, cholesterol and blood pressure tests (5 hours)
Test 4: Body composition scans and cholesterol test; also, PHLA if done at baseline (Baltimore: 4-5 hours)
Test 5: Blood pressure test (30 minutes)

I understand that I will complete three screening visits. For my first visit, a sample of my blood will be drawn to obtain my DNA and I will have my height, weight, and blood pressure measured; this visit will last about 30 minutes. I understand that if I have a version of the gene that is appropriate for this study, I will report to the laboratory in the morning after an overnight fast and a blood sample will be drawn for blood chemistries. I will also have my blood pressure measured. I understand that I may be excluded from the study if this initial blood sample shows elevated levels of glucose in my blood. A blood sample will also be drawn 2 hours after I drink a sugar solution. This visit will last about 2 ½ hours. I understand that a total of 3 tablespoons of blood will be drawn during these two visits. I understand that I will be excluded from the study at this point if I have low red blood cell count, evidence of kidney or liver disease, evidence of diabetes, or if my blood pressure is too high or too low.

I understand that if I remain qualified to this point, 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. 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 understand that this visit will last about 1 hour and 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 and if I am taking medications to control my blood pressure, I give my permission for my private physician to be contacted to obtain their approval for me to stop taking these medications for the remainder of this study. I understand that I will be slowly withdrawn from these medications according to the plan my physician provides and that my blood pressure will be measured weekly for the remainder of the study. I also understand that if my blood pressure is too high (systolic blood pressure: >159; diastolic blood pressured: >99) for three consecutive weeks at any time during the study, I will be excluded from further participation in the study and referred back to my physician. If I am in the exercise training portion of the study, I understand that if this happens I will complete all Final Testing (see below) and then be referred back to my physician. I also understand that a physician from the University of Maryland School of Medicine is directly involved in this study and that he can be contacted for any medical questions, but on as they concern my involvement in this study.

I understand that if I meet these requirements to enter the study, I will undergo 6 weeks of instruction in the principles of an American Heart Association low-fat and low-salt diet and must follow this diet for the remainder of this study. This program consists of two 40-minute classes each week for the 6-week duration of the program. During the final 3 weeks of this dietary program, I understand that I will have my blood pressure measured weekly for 3 weeks. I understand that my blood pressure must average in the range of 130- 159 for systolic or 85 – 99 for diastolic blood pressure for me to continue in the study.

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After this I will undergo Baseline Testing that includes the following tests that will be completed in 5 testing sessions (4 at the University of Maryland, College Park and one at the Baltimore VA Medical Center). I will have my blood drawn on 2 occasions from a vein in my arm in the morning after an overnight fast to measure my cholesterol levels; these visits will each last about 20 minutes. 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 understand that this visit will last about 1 hour. 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 I will collect my urine for 24 hours in a container that must be refrigerated so that the amount of salt I eat in my diet can be measured. I also understand that I will undergo a 3 hour glucose tolerance test where I will come to the laboratory in the morning after an overnight fast, have a small catheter inserted in an arm vein for blood sampling, and have blood samples drawn before and for every 30 minutes after I drink a glucose solution. Additional samples will be drawn before this test to measure hormone levels in my blood that affect my blood pressure, immunological (disease-fighting), and blood clotting systems. I understand that a maximum of 5 tablespoons of blood will be drawn for this portion of the study. I understand that on another occasion after an overnight fast, I will have blood samples drawn 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 7 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 use 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 the amount of fat I have around my waist will be measure with a CAT can while I lie quietly on a table. Another study will be done to measure my total body fat mass and total body muscle mass while I lie quietly on a table. I also understand that if I have elevated blood cholesterol levels, I will have blood samples drawn before and after a substance that temporarily stops blood from clotting is injected into my arm vein. The blood samples will be used to measure chemicals that affect my blood cholesterol levels. I understand that I will remain in the VA Medical Center for 2 - 3 hours after this test to make sure that all bleeding is stopped. I understand that these tests will be done at the VA Medical Center in Baltimore. This entire visit will require approximately 4 – 5 hours including travel time.

I understand that the maximum total amount of blood that will be drawn during this Screening g and Baseline Testing is 22 tablespoons over 2 – 3 months. This is approximately 75% of the amount of blood that is typically drawn during a single blood donation.

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 warm-up 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 this is not designed as a weight loss program and that if I lose more weight than expected from the amount of exercise that I complete, 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 I may also be asked to collect my urine for 24 hours during the exercise training portion of the study.

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 4 weeks of Final Testing a maximum of 25 tablespoons of blood will be drawn; this is approximately two-thirds of the amount of blood that is typically drawn during a single blood donation.

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 blood pressure 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 blood pressure, cholesterol levels, glucose and insulin levels, bone density, body composition, immunology (disease-fighting), and cardiovascular and 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 a laboratory at the University of Pittsburgh that is part of this study. I understand, however, that my DNA samples sent to the University of Pittsburgh will be identified only by a numeric code and that only investigators at the University of Maryland 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 electrocardiogram and a physical examination prior to 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) There is some risk associated with the elevate blood pressure that I have and some risk associated with stopping the mediations I take to control my blood pressure. However, a 12-month lifestyle change program including diet and exercise is part of the medical recommendations for blood pressure control for individuals with levels of blood pressure similar to mine. In addition, I understand that my blood pressure will be monitored weekly and that this exceeds the blood pressure follow-up guidelines recommended for physicians. I also understand that if my blood pressure I too high for three consecutive weeks anytime during the study, my participation in the study will be discontinued and I will be referred back to my private physician. I also understand that a physician associate with this project is available to deal with concerns related to my participation in this study. (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) There are no risks associated with the 24 hour urine collection. (7) The only risks associated with the measurement of the hormones in my blood that affect my blood pressure are those associated with blood drawing. (8) The risks associated with the oral glucose tolerance test and the high-fat meal test are those associated with the blood drawing, the possibility that my blood sugar may go too low levels at the end of the test, and the possibility of having an upset stomach, primarily a stomach ache, after drinking the glucose or high-fat meal. I understand that I will be given a juice drink and small snack to minimize the chances of my blood glucose levels decreasing too much. (9) The risk of genetic testing is the possibility of finding that a person has genes indicating they may be at risk for developing a disease in the future. However, I understand that the only genes that this study will look at that are related to a specific disease are associated with Alzheimer's disease. This information is discussed in detail in the following paragraph. (10) 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. (11) If I have elevated blood cholesterol levels, I understand that 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 bandage on the intravenous access site after the blood sampling and observing the subject for 2-3 hours.

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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 this research is exploratory and I understand that, if I chose, I will be told of the results of one specific genetic test and any results of other genetics studies that might be useful to me. The one specific genetic test is for a gene that affects my cholesterol levels, known as apolipoprotein E or APO E. The different versions of this gene are called APO E2, APO E3, and APO E4 and, since we all have two strands of DNA, a person could have one of six possible combinations of this gene (2/2, 2/3, 2/4, 3/3, 3/4, 4/4). APO E3 is by far the most frequent and APO E2 the least frequent form of this gene. It has been found that people with at least one APO E4 gene may be at increased risk of developing Alzheimer's disease. This is based on the results of studies with a very large number of research subjects and the relationship is only evident when the results of all of these studies are combined. I understand that it is not possible to predict my own individual risk, or that of any single person, based on the current knowledge about the APO E gene because many persons with the APO E4 gene do not develop Alzheimer's disease and many people with Alzheimer's disease do not have an APO E4 copy of the gene. Thus, I understand that scientists do not generally use APO E testing to determine a person's risk for Alzheimer's disease. I understand that I have a right to know the results of my genetic tests and if I choose to know them, I understand that I need to keep in mind the facts presented above about the importance of these genetic results.

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 after 3 months of exercise training if I complete at least 90% of my exercise training sessions. I also understand that I will earn another $100 after completing 90% of my training sessions for 6 months and all final testing. I understand that the total amount that I earn will be paid to me at the completion of my participation in the study. I understand that if my participation in the study has to be terminated because I change my hormone replacement therapy regimen, I will 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.

Initials _____

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

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Subject's signature   Date

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Witness   Date

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Investigator   Date

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