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Influence of Gender and Aerobic Training Background on Exercise-Induced Increase in Adiponectin

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Influence of Gender and Aerobic Training Background on Exercise-Induced Increase in Adiponectin

by

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Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

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ABBREVIATIONS

mo	month
ADPN	adiponectin
FPI	fasting plasma insulin
FPG	fasting plasma glucose
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
m	male
f	female
T	trained/experienced runners
U	Untrained/novice beginners
HT	height
WT	body weight
BMI	body mass index
BF	body fat
WST	waist circumference
HIP	hip circumference
WHR	waist-to-hip ratio
HRT	hormone replacement therapy

ABSTRACT OF THE DISSERTATION

Influence of Gender and Training Background on Effect of Exercise on Adiponectin

by

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Adipose tissue secretes the adipokine, adiponectin (ADPN), which is insulin-sensitizing, anti-inflammatory, and anti-atherogenic. Exercise training improves insulin sensitivity and lowers the risk of cardiovascular complications. As some of the metabolic effects of exercise training and ADPN overlap, exercise training has been proposed to increase ADPN. However, most single bout exercise, or short-term (≤3 months) and constant-effort (fixed session duration, fixed number of sessions/week, and fixed intensity) exercise protocols do not produce increases in ADPN in untrained and trained cohorts. Furthermore, most exercise studies were conducted on male-female mixed gender cohorts or male/female single gender cohorts. As a result, no direct comparison of male and female subjects pertaining to the effect of exercise on ADPN levels has been reported. Our **governing hypothesis** is that long-term aerobic exercise increases ADPN, and the increase in ADPN is influenced by gender and exercise training background. We tested two specific hypotheses using different cohorts of human volunteers. **Hypothesis 1** states that ADPN levels will increase significantly in previously untrained, middle-aged males and females in response to a long-term, progressive aerobic training protocol. **Hypothesis 2** states that ADPN levels in multi-year trained female marathoners will increase significantly in response to a long-term, progressive aerobic training, but not in

comparably trained males. We compared ADPN levels in trained marathoner males (n=10) and females (n=8) subjects and untrained males (n=9) and females (n=11) subjects in a 6-mo aerobic training intervention study. Fasting plasma samples were collected at the beginning and end of the 6-mo training period and analyzed. ADPN levels increased significantly in both trained and untrained females and untrained males, but non-significantly in trained males. Ours is the first study to compare changes in ADPN in mean age and BMI-matched male and female groups with two non-overlapping exercise training backgrounds in response to the same long-term, progressive aerobic training. The insight provided by the results of the two studies will help in understanding gender differences in ADPN.

CHAPTER ONE

INTRODUCTION

Obesity… The Much Talked about Issue!

Obesity has currently become a worldwide public health issue in many developed and developing countries among people of all age groups. Increased caloric intake coupled with decreased physical exercise results in obesity. Obesity greatly increases the possibility of developing metabolic complications such as hypertension, atherosclerosis, dyslipidemia, and insulin resistance (IR) that later lead to type II diabetes (DM2). An estimated 24 million children and adult Americans have DM2; another 60 million people have the pre-diabetic condition of high blood sugar [1], increasing their risk for developing the disease. The American Diabetes Association estimates the cost of DM2 to be at least $174 billion a year as of 2007 [1].

What is Adiponectin and Why Should We Care for It?

Adipose tissue, in addition to its function as a major energy store, is now known to function as an active endocrine tissue that secretes several hormone-like proteins termed adipokines. New adipokines are being discovered, but the current list of adipokines includes chemerin, interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), retinol binding protein 4, tumor necrosis factor-alpha (TNF□), visfatin, leptin, and adiponectin. Adipokines regulate satiety, lipid and carbohydrate metabolism, and

insulin sensitivity [2, 3]. Decreased insulin sensitivity [or insulin resistance (IR)] involves excessive insulin secretion in response to elevated blood glucose [3].

Mature adipocytes from visceral fat release an anti-inflammatory, insulin-sensitizing adipokine, adiponectin (ADPN) [4]. ADPN antagonizes the inflammatory effects of pro-inflammatory adipokines, IL-6 and TNF-α and the levels of IL-6 and TNF- are inversely related to ADPN levels [5]. ADPN stimulates free fatty acid (FFA) oxidation in skeletal muscle [6] and ADPN levels positively correlate with circulating FFA concentration [7]. ADPN levels are significantly lower in overweight, obese, insulin-resistant, and diabetic individuals compared to lean or normal-weight individuals [6]. Females have higher basal levels of ADPN than males [8]. Non-Hispanic whites (Caucasians) have higher ADPN levels compared to other ethnic groups, such as non-Hispanic blacks, Asians, and Hispanics [9]. The prevalence of DM2 is higher in all populations other than non-Hispanic whites [10].

Aerobic and Resistance Exercise and Adiponectin, and The Possible Mechanism of Interaction

Aerobic exercise helps maintain healthy levels of body fat, decreases IR, improves DM2, and lowers the risk of developing cardiovascular complications [11]. Aerobic exercise has been at the forefront of life-style efforts to manage pre-diabetes and diabetes. The mechanism by which aerobic exercise brings about favorable changes in blood insulin and glucose levels is not yet known, but studies have noted the "anti-inflammatory nature" of exercise [11].

Metabolic effects of aerobic exercise include reduction in body weight and body fat, improved insulin sensitivity, improved cardiovascular health by improving plasma

lipoprotein profile, reducing blood pressure, and decreasing incidences or preventing the development of DM2 [11]. The reported metabolic effects of ADPN are: reduction of IR; increase FFA oxidation; decrease hepatic glucose production by inhibiting enzymes of gluconeogenesis; produce nitric oxide (NO) and improve endothelium-dependent vasodilation; and reduce in thrombus formation and platelet aggregation [12]. Because some of the metabolic effects of exercise training are similar to the effects of ADPN, aerobic exercise training has been proposed to increase ADPN [11]. Since the discovery of ADPN, studies have been conducted in human and rodent models to understand the connection between aerobic exercise training and ADPN levels [13-15]. Several human studies report that aerobic exercise increases [16-19] or does not increase [20-29] ADPN levels. Differences in study outcomes are mainly due to differences in –

1. Cohort characteristics –
    (i) Metabolic profile of the cohort (*e.g.* trained subjects/athletes [24, 30-32], untrained normal-weight [22], untrained overweight [20, 26], untrained obese [16, 27-29, 33], untrained insulin-resistant, DM2 subjects [16-19, 21])
    (ii) Gender profile of the cohort (*e.g.* male-female combined [18, 19, 22, 23, 26, 27, 33, 34] or single gender male [24, 25, 29, 35] or female [17, 20] cohort)
2. Exercise intervention characteristics –
    (i) Duration of exercise training intervention (*e.g.* short-term (*i.e.* ≤3 mos) [20, 21, 25, 27-29] or long-term (*i.e.* >3 mos) exercise studies [18, 19, 22-24, 26])
    (ii) Nature of exercise training protocol (*e.g.* constant effort [22, 23, 26, 27, 33, 34] or periodically increasing [progressive] protocol [18, 19, 35])

Resistance/Strength training also has been shown to be helpful in managing of DM2 by maintaining muscle mass [36]. Intervention studies employing resistance training are fewer than aerobic exercise intervention studies and show increases [37-39] and no changes [40-42] in ADPN. Similar to aerobic exercise interventions the differences in the outcome of resistance training intervention also is due to

1. Cohort characteristics –
    (i) Cohort metabolic profiles – studies were conducted on overweight, obese, or DM2 subjects.
    (ii) Gender profile of the cohort – studies were conducted on male only or female only cohorts
2. Exercise intervention characteristics –
    (i) Duration of exercise training intervention [*e.g.* short-term (*i.e.* ≤3 mos) [40-42] or long-term (*i.e.* >3 mos) [37-39]].
    (ii) Intensity of exercise training protocol [*e.g.* low-medium intensity (*i.e.* ≤80% repetition maximum) [38, 40-42] or high intensity (*i.e.* >80% repetition maximum [37, 38]].

Anticipated Mechanism and Gaps in The Literature

Some groups studying the effect of a long-term aerobic exercise on ADPN have proposed that increases in ADPN levels might be due to the indirect reduction in total body weight, mainly by reduction in body fat, or insulin resistance and not the direct effect of the exercise intervention. It has been suggested that weight losses of more than 10% are necessary to detect significant increases in circulating ADPN [33]. Some

researchers made similar observation where the obese females lost more than 10% body weight through exercise intervention and showed a significant increase in ADPN [16, 17]. However, Ring-Dimitriou *et al.* found an increase in ADPN with less than 10% decrease in body weight [18]. Some researchers found no increase in ADPN despite decreases in body weight [26-28]. In a cross-sectional study, ADPN levels were higher in physically active young adult females than in BMI- and age-matched sedentary females [43]. Reduction in total body weight may not be necessarily associated with an increase in ADPN.

In general, an inverse correlation exists between ADPN levels and body fat [6]. A mean weight loss of 3% achieved by liposuction alone increased ADPN significantly in obese women [44], thus reduction in body fat may increase ADPN levels. However, the extent of body fat loss necessary to induce an increase in ADPN is not yet established. During a 2-year exercise intervention, changes in levels of ADPN and body fat were not inversely correlated [18] and a large (~44%) decrease in abdominal fat was not associated with an increase in ADPN [21]. Reduction in body fat may not be necessarily associated with an increase in ADPN.

The possibility of a dose-response effect of exercise on ADPN was raised by studies where the intensity of aerobic exercise was varied [26]. Increasing levels of resistance training were found to increase ADPN levels [38]. Increased intensity, as well as increased duration of exercise increased ADPN in obese adolescent males [35].

Gender Differences in Adiponectin Levels

Females, in general, exhibit higher ADPN levels than males [8]. This is primarily due to a significantly higher concentration of the high molecular weight isoform in females [8, 45]. Most long-term (>3 mos) exercise protocols studying the effect on ADPN levels were conducted on combined cohorts of untrained males and females [18, 22, 23, 26, 34, 46] and only a few were conducted separately on male only [24, 35] or female only cohorts [16, 17]. Independent of the duration of the intervention and metabolic profile of the cohort, most aerobic exercise studies conducted solely with males failed to show significant increases in ADPN levels [24, 25, 29], whereas those with overweight and obese females showed increases in ADPN levels [16, 17, 19]. In a two-year, moderate exercise training intervention on obese males and females Ring-Dimitriou *et al.* [18] reported that the females showed twice the mean net increase in ADPN compared to males at the end of 2-yr aerobic training. Long-term (>3 mos) resistance training studies have also observed greater increases in ADPN levels of females than males [37, 39]. Some researchers have raised a possibility that ADPN may change differently in the two sexes due to exercise alone [17] or due to differences in changes in body fat [46].

Significance of the Study

Exercise is at the forefront of DM2 treatment. Metabolic effects of exercise include reduction in body weight and body fat, improved insulin sensitivity, and improved cardiovascular health. Adiponectin (ADPN) is anti-inflammatory, insulin-sensitizing, and anti-atherogenic. Because metabolic effects of exercise and ADPN

overlap, exercise is proposed to increase ADPN. Higher levels of ADPN are noted in athletes and centinarians. Females, in general, exhibit higher ADPN levels than males, but the effect of aerobic training on ADPN levels in males and females has not been compared. We studied the effect of long-term exercise training on ADPN in both untrained and multi-year trained, weight-stable subjects to understand if ADPN increases independently of body weight loss, exercise training conditioning, and gender. Our study findings will help fill the gaps in gender-based effectiveness of exercise with reference to ADPN levels.

Novelty of the Study Design

Females, in general, exhibit higher ADPN levels than males [8], but the effect of aerobic training on ADPN levels in males and females has not been compared. Multi-year trained male athletes have higher baseline ADPN levels than untrained controls [24, 31], but the effect of aerobic training on ADPN levels of trained and untrained individuals has not been compared. Several years of aerobic training reduces and stabilizes body weight, body fat, and resting plasma insulin levels [31]. ADPN levels are significantly and inversely correlated with body fat and resting plasma insulin levels [6]. Significant increases in ADPN in presence [16, 17] and absence [26-28] of significant weight loss have been reported, but changes in ADPN have not been compared in groups with and without weight loss through the same exercise intervention. Most exercise intervention studies did not control for changes in anthropometric measurements before interpreting the changes in ADPN as a result of exercise intervention [14]. Hence no relationship between weight reduction and changes in ADPN can be established. Our comprehensive

hypothesis connects the established findings and assists in filling in the gaps left from previous studies.

This dissertation endeavors to answer following questions to fill in the present gaps in the literature -

1. Does long-term aerobic training increase ADPN in healthy subjects?

Most of the studies in the literature have been conducted on overweight, obese, insulin-resistant, DM2 subjects. Even though these studies showed an increase in ADPN, studies conducted on healthy subjects showed no changes in ADPN.

2. Is weight loss necessary for an increase in ADPN?

The general opinion in the field is that a significant and more than 10% weight loss, brought about by caloric restriction or aerobic exercise intervention or liposuction-type weight reduction surgery procedures is essential for increases in ADPN levels. On the other hand, some studies employing progressive training protocol showed increased ADPN levels without significant weight loss. Many studies did not control for reductions in weight, body fat, waist circumference to correctly interpret if intervention-induced, specifically exercise intervention-induced increases in ADPN levels were due to intervention alone or due to reductions in anthropometric measurements.

3. Are there gender differences in aerobic exercise-induced increases in ADPN?

Most studies were conducted on mixed gender male/female cohorts or male only female only cohorts. Studies employing female only cohorts reported increases in ADPN while most studies employing male only cohorts reported no changes. Ring-Dimitriou *et al.* reported twice higher increase in ADPN in females compared to males [18]. No study compared males and females through same exercise intervention.

4. Are there training background-induced differences in increases in ADPN due to aerobic exercise training?

Most studies were conducted on untrained subjects and only a few were conducted on trained subjects. But no study compared changes in ADPN levels in trained and untrained subjects undergoing same exercise training protocol. Do untrained subjects show higher increases in ADPN levels as they are not conditioned to exercise training? Do trained subjects show lesser increases in ADPN levels because they are conditioned to exercise training? Our comprehensive study strives to answer these questions.

Hypothesis

We hypothesize that long-term aerobic exercise increases total circulating adiponectin and that gender and exercise training background influence the exercise-induced increase in adiponectin. Our governing hypothesis is based on several observations reported in the literature. It also makes several predictions that, to our knowledge, have not yet been tested. Our proposed study is designed to test the specific aspects of our working hypothesis. We will test two specific hypotheses using different cohorts of human volunteers.

Hypothesis 1

We hypothesize that ADPN levels will increase significantly in previously untrained males and females in response to long-term, progressive aerobic training.

Specific Aim 1

To compare ADPN levels before and at the end of a long-term, progressive aerobic training regimen in middle-aged, untrained males and females. We will analyze ADPN levels in both males (M) and females (F) from the untrained group that have never trained for or participated in a full-distance (26.2 mile) marathon before. We will measure markers of insulin-resistance and associated anthropometric parameters in addition to ADPN levels.

Hypothesis 2

We hypothesize that ADPN levels in multi-year aerobic trained females will increase significantly in response to long-term, progressive aerobic training, but not in comparably trained males.

Specific Aim 2

To compare ADPN levels before and at the end of a long-term, progressive aerobic training protocol in middle-aged, multi-year aerobic trained males and females. We will analyze ADPN levels in both males (M) and females (F) from the trained marathoner group that has been training for and completing at least one full-distance (26.2 mile) marathon each year for five consecutive years. We will measure markers of insulin-resistance and associated anthropometric parameters in addition to ADPN levels.

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CHAPTER TWO

LONG-TERM, PROGRESSIVE, AEROBIC TRAINING INCREASES ADIPONECTIN INMIDDLE-AGED, OVERWEIGHT, UNTRAINED MALES AND FEMALES

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Abstract

Adipose tissue secretes the adipokine, adiponectin (ADPN), which increases insulin sensitivity. Because some of the metabolic effects of exercise and ADPN are similar, exercise has been proposed to increase ADPN. However, most short-term (≤3 mos) and constant-effort exercise protocols have not produced increases in ADPN. Furthermore, no direct comparisons of male and female subjects on the effect of exercise on ADPN levels have been reported. We hypothesized that long-term (6 mos), progressive training would increase ADPN levels in both males and females. We recruited middle-aged, untrained males and females to participate in an interventional study employing a marathon training regimen progressing from 9.7 to 88.5 km (6 to 55 miles) per week over 6 mos. At baseline, we matched the mean ages of the male and female groups. We collected and stored fasting plasma samples and recorded body measurements at 0 (baseline) and 6 mos. Stored samples were analysed for insulin, glucose, and ADPN. ADPN increased significantly among both males (from 5.89 ± 2.46 (mean ± SD) to 7.65 ± 3.18 μg/ml; $p < 0.05$) and females (from 8.48 ± 3.22 to 10.56 ± 4.05 μg/ml; $p < 0.05$). The extent of the increase in ADPN was similar in the male (40.7 ± 50%; median, 12.1%) and female (27.0 ± 31.1%; median, 22.3%) groups. However, there was no significant reduction in insulin resistance as measured by the HOMA-IR scores in either group. We conclude that long-term, progressive aerobic training increases circulating ADPN levels in middle-aged, untrained males and females.

Key Words

Exercise, intervention studies, human, adiponectin, sex differences

Introduction

Adipose tissue, once thought to be exclusively a depot of fat, secretes several hormone-like proteins termed adipokines. Adipokines regulate satiety, lipid and carbohydrate metabolism, and insulin sensitivity. Decreased insulin sensitivity in the form of insulin resistance involves excessive insulin secretion in response to elevated blood glucose. Central obesity is on the rise in middle-aged men and women [1, 2] and increased adipose mass decreases the production of adiponectin (ADPN), an adipokine positively correlated with insulin sensitivity [3]. Mature adipocytes express and secrete ADPN (aka gbp28, Acrp30, and apM1), which occurs in human plasma at concentrations from 3 – 30 μg/ml. ADPN knockout mice on a high fat diet develop insulin resistance and coronary artery disease [4] while ADPN over-expression improves insulin sensitivity [5]. ADPN may preserve insulin sensitivity in middle-aged men and women [1, 2] since ADPN levels negatively correlate with obesity, hypertension, type II diabetes, hyperlipidemia, and metabolic syndrome [6].

Aerobic exercise improves insulin sensitivity, blood pressure, lipoprotein profile, and metabolic and cardiovascular health [7]. Because some of these effects are similar to some of the effects of ADPN, exercise has been proposed to increase ADPN [7]. Several studies report that exercise increases [8 – 11] or does not increase [12 – 21] ADPN levels. In general, studies employing short-term (i.e. ≤ 3 mos) exercise protocols showed non-significant changes in ADPN [12, 13, 17, 19 – 21]. Long-term (i.e.≤3 mos) exercise studies on overweight, obese, or insulin-resistant subjects increased ADPN [8 – 11], but not in normal-weight subjects [14,16]. In particular, long-term exercise studies employing progressive protocols showed significant increases in ADPN [10, 11].

However, most long-term protocols used combined male/female cohorts [10, 11, 14, 15, 18], but none have directly compared the effect of exercise in males and females using a long-term, progressive aerobic protocol. We hypothesized that untrained, overweight, middle-aged males and females would exhibit significant increases in ADPN in response to a long-term, progressive aerobic protocol. To test our hypothesis, we compared the effects of a progressive marathon-training program of 6 mos duration on ADPN levels in untrained, age-matched males and females. We found that ADPN levels increased significantly and to similar extents in both untrained males and females. This increase was accompanied by a significant reduction in waist circumference in the male group and by a significant reduction in body weight and hip circumference in the female group.

Methods

Subjects

We recruited subjects from a large ($n \approx 600$) local running club to participate in a 6-mo, progressive, marathon running/walking program. The Institutional Review Board of Loma Linda University approved the research protocol. At the planning stage of our study, the local running club organizers informed us that in previous marathon training programs conducted by the club, the female attrition rate was about twice that of males. For this reason, we recruited more females than males. We presented the objectives and procedures of the study to the club members. We gave interested members an informed consent document explaining the procedures and risks and afforded opportunities to ask questions. Potential subjects signed the informed consent document before completing a screening questionnaire. Inclusion criteria were adult males and females who had never

participated in or trained for a marathon. Exclusion criteria were known cardiovascular, renal, hepatic, pulmonary, adrenal, pancreatic, thyroid, or pituitary disorder and smoking.

Demographic Characteristics

We separated the subjects into two groups: untrained males and untrained females. At baseline, we age-matched the male and female groups by excluding ten outlying female enrollees. The baseline cohort of males and females were numerically similar in mean ages and BMI values (Table 2.1.).

During the 6 months of progressive training, our two groups exhibited different rates of attrition over which we had little control. Males with the highest BMI values (i.e. obese) dropped out to a greater extent than males of lower BMI categories, while females of the next two lower BMI categories dropped out to a greater extent than obese females. These opposing attrition trends caused the mean BMI values and ages of the two groups to diverge slightly (compare Tables 2.1. and 2.2.).

The completing groups were not restricted to a particular ethnic group, but the proportion of Caucasians to non-Caucasians in the study was similar between the male and female groups with 36.4% and 22.2%, being Caucasians, respectively. We included postmenopausal females both on (n = 1) and not on (n = 2) hormone replacement therapy (HRT) because ADPN levels are reported to not differ between pre and postmenopausal women nor between postmenopausal women on HRT and those not on HRT [22]. We also included premenopausal females both on (n = 2) and not on (n = 6) oral contraceptive pills because ADPN levels in females are reported to be stable throughout the menstrual cycle [23], which we verified (data not showed). Female participants on oral contraceptives or HRT continued to use these throughout the study.

Table 2.1. Physical characteristics in male and female groups at the baseline (0 mo).

	Untrained Male (n=14)			Untrained Female (n=38)			
Variable	Mean	±	SD	Mean	±	SD	*p**
age	47.0	±	13.8	44.6	±	11.0	NS
HT	1.71	±	0.03	1.63	±	0.07	<0.0001
WT	81.8	±	13.8	77.9	±	15.6	NS
BMI	28.1	±	4.7	29.4	±	6.0	NS
BF	26.5	±	9.0	37.7	±	6.9	<0.0005
WST	98.4	±	10.1	92.6	±	13.1	NS
HIP	103.5	±	12.7	108.9	±	12.6	NS
WHR	0.96	±	0.08	0.85	±	0.07	<0.0005

Abbreviation key: WT, total body weight (kg); HT, height (m); BMI, body mass index (kg/m^2); WST, waist circumference (cm); HIP, hip circumference (cm); WHR, waist-to-hip ratio; BF, total body fat (%); NS, not significant; *p**, significance between Untrained Male and Female groups by independent t-test.

Table 2.2. Physical characteristics and blood analytes in male and female groups at the baseline (0 mo) and end (6 mos).
Abbreviation key: ADPN, plasma total adiponectin (μg/ml); FPI, fasting plasma insulin (μU/ml); FPG, fasting plasma glucose (mg/dl); HOMAIR, HOmeostasis Model Assessment of Insulin Resistance; WT, total body weight (kg); HT, height (m); BMI, body mass index (kg/m2); WST, waist circumference (cm); HIP, hip circumference (cm); WHR, waist-to-hip ratio; BF, total body fat (%); NS, not significant; ^{LT}, tests were conducted on log transformed variable; *p*, significance within Untrained Male and Female groups by paired t-test; †, effect of exercise training on ADPN levels between Untrained Male and Female groups by repeated-measures ANOVA after controlling for significant confounders; *p**, significance between Untrained Male and Female groups by independent t-test.

Variable	Time	Untrained Male (n=9) Mean ± SD	Untrained Female (n=11) Mean ± SD	*p**
age	0 mo	47.1 ± 12.2	43.2 ± 12.1	NS
HT	0 mo	1.72 ± 0.03	1.62 ± 0.07	<0.005
WT	0 mo	79.5 ± 9.2	79.7 ± 17.0	NS
	6 mo	78.2 ± 7.5	73.8 ± 13.4	NS
	p	NS	<0.05	
BMI	0 mo	26.8 ± 2.5	30.5 ± 7.2	NS
	6 mo	26.4 ± 2.1	28.3 ± 6.0	NS
	p	NS	<0.05	
BF	0 mo	23.3 ± 4.8	38.1 ± 7.4	<0.001
	6 mo	23.8 ± 5.7	36.5 ± 6.9	<0.001
	p	NS	NS	
WST	0 mo	96.7 ± 7.6	93.1 ± 13.0	NS
	6 mo	91.8 ± 7.4	88.0 ± 10.3	NS
	p	<0.05	NS	
HIP	0 mo	100.8 ± 6.6	110.3 ± 11.8	<0.05
	6 mo	99.0 ± 4.2	103.7 ± 8.4	NS
	p	NS	<0.05	
WHR	0 mo	0.96 ± 0.03	0.84 ± 0.08	<0.005
	6 mo	0.93 ± 0.06	0.85 ± 0.06	<0.05
	p	NS	NS	
$ADPN^{LT}$	0 mo	5.89 ± 2.46	8.48 ± 3.22	NS
	6 mo	7.65 ± 3.18	10.56 ± 4.05	NS
	p	$<0.05^{\dagger}$	$<0.05^{\dagger}$	
FPI	0 mo	6.27 ± 3.48	8.97 ± 3.98	NS
	6 mo	5.47 ± 1.66	8.46 ± 4.57	NS
	p	NS	NS	
FPG	0 mo	93.6 ± 10.6	98.6 ± 5.4	NS
	6 mo	97.4 ± 14.3	96.0 ± 9.1	NS
	p	NS	NS	
HOMA-IR	0 mo	1.48 ± 0.88	2.21 ± 1.04	NS
	6 mo	1.32 ± 0.44	2.03 ± 1.12	NS
	p	NS	NS	

Training Protocol

All subjects followed the same progressive training protocol for 6 mos (Figure 2.1.). The subjects met each Sunday at 7 a.m. with a pace group of their preferred speed to complete the assigned weekly long-distance run (Figure 2.1.). Subjects also completed additional progressive distances assigned for weekdays, but at times, days, and places of their choosing. The Sunday distance increased linearly at an average rate of 1.16 km/week (km/week) from 3.2 to 32.2 km (2 to 20 miles). Weekday distance increased non-linearly during the first 8 weeks (from 6.4 to 32.2 km) and thereafter increased linearly at an average rate of 1.13 km/week (from 37 to 56.3 km) until the end of the study period of 24 weeks (i.e. 6 mos). Total weekly distance increased non-linearly during the first 8 weeks (from 9.7 to 45.1 km) and thereafter increased approximately linearly at an average rate of 2.08 km/week (from 53.1 to 88.5 km) until the end of the study (Figure 2.1.). We monitored adherence to the prescribed training protocol using self-reported daily running logs, which were collected each Sunday. Subjects remained in the same pace group throughout the study. We instructed subjects to maintain their pre-intervention dietary habits during the course of the study, but dietary intakes were not monitored.

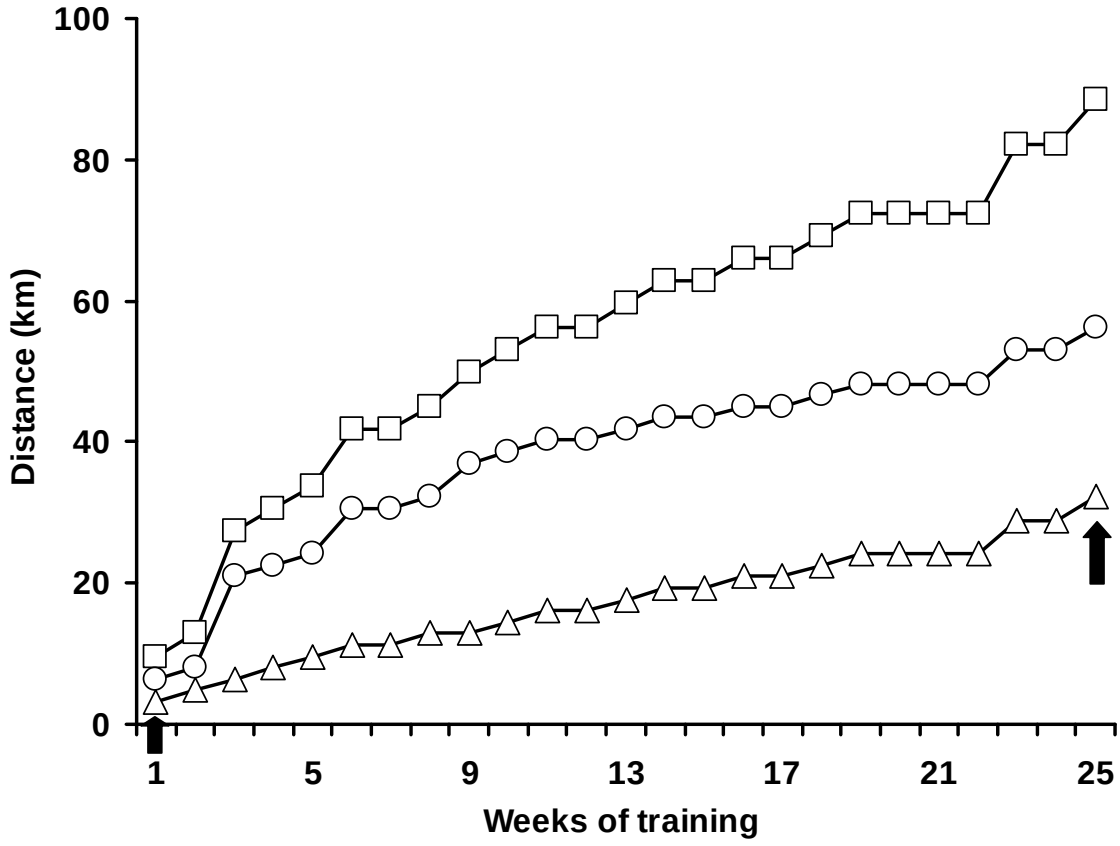

Figure 2.1. Time course of long-term, progressive training protocol. Total weekly distance (□) is the sum of weekday distance (○) and Sunday distance (Δ). Anthropometric data and blood samples were collected at baseline (0 mo) and 6 mos as indicated by arrows.

Biochemical Measurements

We collected fasting blood samples on Sundays, immediately before the start of the 3.2- and 32.2-km (2- and 20-mile), long-run distances corresponding to 0 and 6 mos, respectively. We asked the subjects to avoid strenuous exercise 2 days before the blood draws to avoid possible interfering pro-inflammatory cytokines [18]. We reminded the subjects of the 12-h fast requirement by telephone contact the evening before the morning blood draws. The 0-mo samples provided the baseline measurements, which also served as each subject's control. We collected blood samples between 6 and 7 a.m. to minimize diurnal variation in ADPN levels [24]. A licensed phlebotomist drew venous blood from the median antecubital vein into a 10-ml glass vacuum tube (0268384; BD, Franklin Lakes, NJ) containing liquid K_3 EDTA and protease inhibitor (250 KIU aprotinin /ml of blood; Calbiochem, San Diego, CA) and immediately placed on ice. We collected plasma within 1 h of blood collection by centrifuging at 1,900 x *g* in swinging buckets for 10 min at 4°C, aliquoted plasma samples, and immediately stored them in cryogenic vials at 80°C until analysed. Before drawing the blood, we queried each subject about complying with the fast and omitted those not found to comply. Subjects subsequently found to have both fasting plasma insulin higher than 10 μU/ml and fasting plasma glucose higher than 110 mg/ml would have been determined to have broken the fast and would have been excluded from the study. No subject exceeded the above-mentioned limits.

We used sandwich ELISA-based assays to measure fasting total plasma ADPN (mg/ml; EZHADPN- 61K, LINCO Research, St. Charles, MO) and fasting plasma insulin (FPI; μU/ml; EZHI-14K, LINCO Research) according to kit instructions. We measured the absorbance in both assays at 450 nm on a plate reader (EL X 800, Universal

microplate reader, BIOTEK Instruments Inc., Winooski, VT). We measured fasting plasma glucose (FPG; mg/dl) using the glucose oxidase procedure (2810-1, Eagle Diagnostics, De Soto, TX) according to kit instructions. We measured the absorbance at 500 nm. We measured the samples in duplicate, and re-assayed those with more than 10% variation from fresh aliquots. The average coefficient of variation using ELISA kits for FPI assays was 3.9% (range: 3.2 – 4.4), for ADPN assays was 3.6% (range: 2.6 – 4.25), and for glucose assays was 1.2% (range: 0.8 – 1.7). We calculated the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) scores using the following equation [25]:

$$\text{HOMA-IR} = [\text{FPI } (\mu\text{U/ml}) \text{ X FPG (mg/dl)}/18]/22.5$$

Anthropometric Measurements

Qualified personnel measured and recorded height (HT, m), body weight (WT, kg), total body fat (BF, %), and waist circumference (WST, cm) and hip circumference (HIP, cm) at 0 and 6 mos between 6 and 7 a.m. on the same day as the blood draw before the long run on Sundays. Anthropometrics were measured with the subjects wearing light running clothes without accessories and footwear. Waist circumference was recorded by placing a tape measure evenly and snugly at the natural waistline in a standing position where a waistline could be clearly identified. In the absence of such clearly identifiable waistline, the waist circumference was measured by placing a tape measure halfway between the top of the hip bone and the bottom of the rib cage. Hip circumference was measured by placing the tape measure evenly and snugly over the widest area of the hips. Waist-to-hip ratio (WHR) and body mass index (BMI) were calculated from the

measurements. Height (m) was measured at baseline (0 mo) using a stadiometer. The sliding part of the stadiometer was lowered so that the subject's hair was pressed flat against the scalp. Total body fat was measured on non-athletic/normal setting using bioelectrical impedance (model: BF-679, TANITA Corporation, Arlington Heights, IL). All measurements were obtained in duplicate, recorded, and averaged.

Statistical Analyses

We used the Statistical Package for Social Sciences (version 17.0) for Windows (SPSS, Inc., Chicago, IL) to perform all statistical analyses. We considered the results statistically significant if a two-tailed p -value was less than or equal to 0.05. We used histograms and the Kolmogorov-Smirnov test for normality to determine whether the variables for the study groups were normally distributed. We log transformed and reassessed the variables that were not normally distributed. We used an independent t-test to assess each dependent variable between the study groups separately for each data collection point (i.e. 0-mo baseline and 6 mos). We also used an independent t-test to assess percent changes in the variables between the groups. We used a paired t-test to assess the difference in each dependent variable separately for each group when the outcome was not controlled for potential confounders. We included potential confounders as covariates in a multi-variable regression analysis using a repeated measure ANOVA to control for the influence of changes in confounders on the outcome variable ADPN only.

Results

Subject Compliance

We enrolled subjects meeting the inclusion and exclusion criteria. Cohort sizes at each stage of the study are shown in Figure 2.2. Of the original 62 enrolled subjects, retention among male subjects was almost three times as high (64%) as females (23%). We carried out data analyses on a total of 20 subjects (i.e. males = 9; females = 11) who completed the 6-mo training and provided complete data sets at baseline (0 mo) and 6 mos.

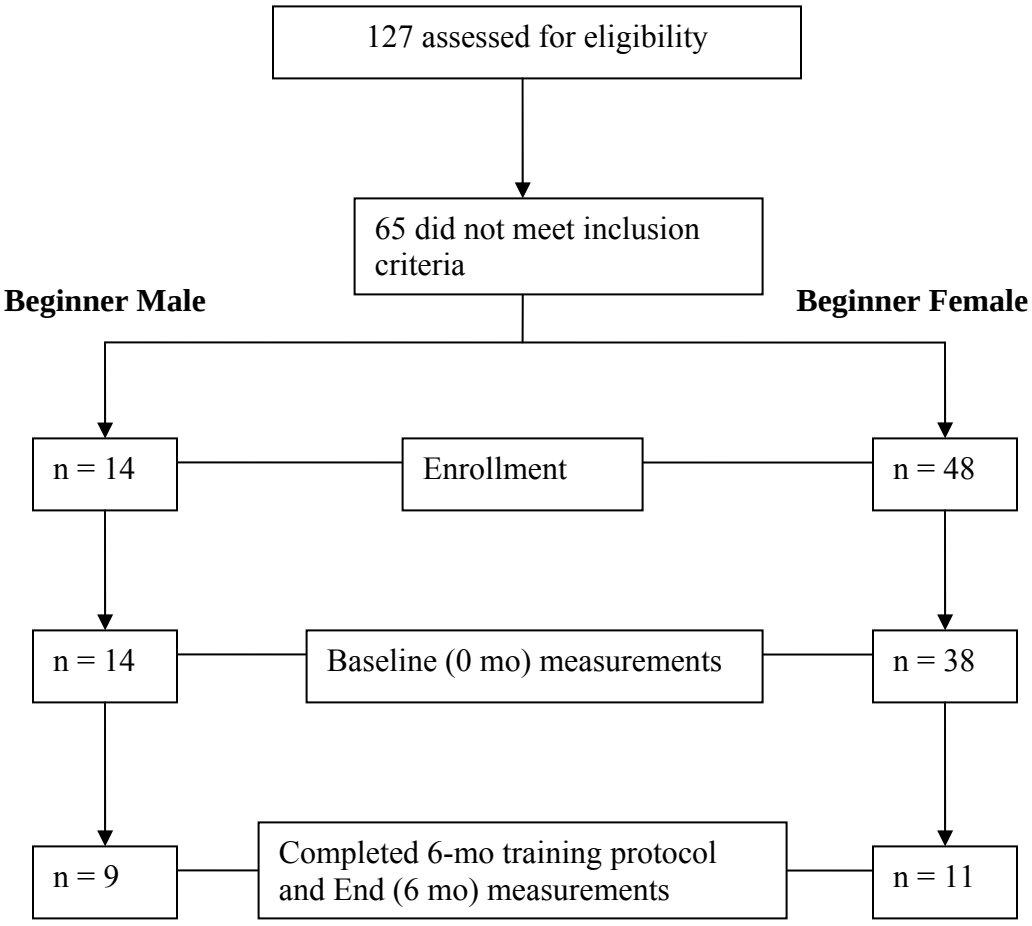

Figure 2.2. Sample size and gender composition at each stage of the study.

Adiponectin Levels

ADPN levels increased significantly at the end of 6 mos in both the male and female groups. At baseline and at the end of 6 mos of training, the ADPN levels in the female group were higher than those in the male group, but not significantly (Table 2.2.). The percent change in ADPN (Δ ADPN %) was not significantly different between males (40.7 ± 50.0%; median, 12.1%) and females (27.0 ± 31.1%; median, 22.3%). After controlling for percent changes in WST and BMI using a repeated-measure ANOVA, the effect of 6-mo exercise on ADPN levels of males and females was significant ($p < 0.05$, Table 2.2., Figure 2.3.).

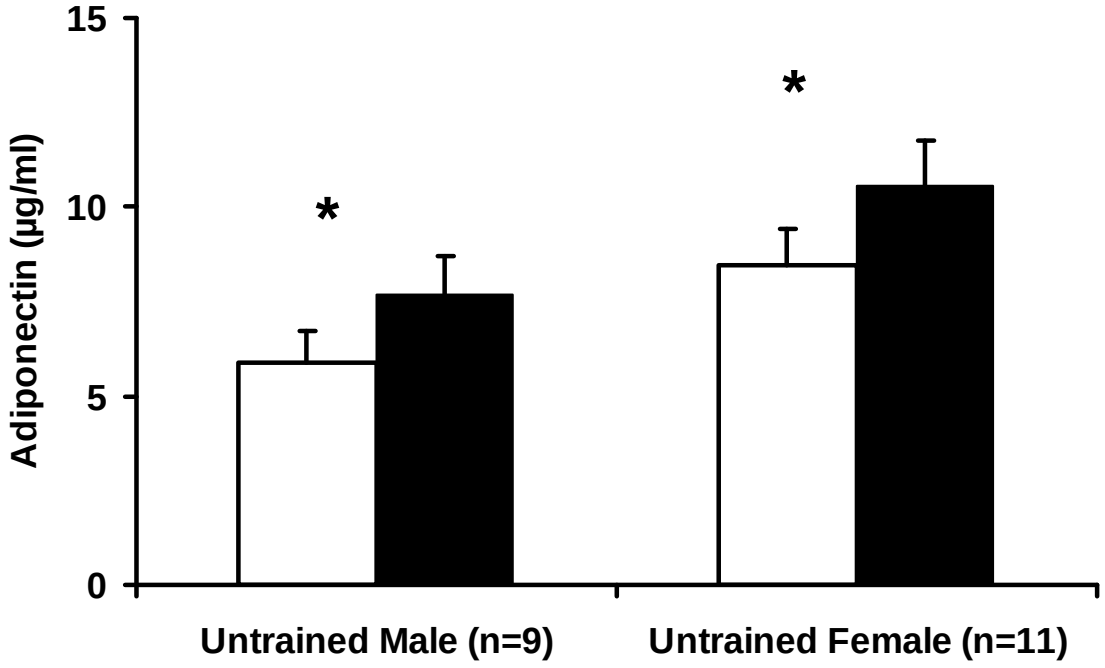

Figure 2.3. Plasma adiponectin levels at baseline and end of 6 mos aerobic training in groups identified by gender. Adiponectin levels (mean ± SEM) at the baseline (0 mo, open bars) and end (6 mos, filled bars) were significant in males and females, as determined by a repeated-measures ANOVA after controlling for percent change in BMI and percent change in WST. Asterisk (*) indicates $p < 0.05$. Sample sizes of each group are indicated in parentheses.

Insulin, Glucose, and HOMA-IR Score

Male and female groups exhibited similar baseline and 6-mo FPI, FPG and HOMA-IR score (Table 2.2.). Furthermore, FPI, FPG, and HOMA-IR score did not change at the end of the 6 mos of training within either group.

Anthropometric Measurements

The male and female groups exhibited similar WT, BMI, and WST measurements at the beginning and at the end of the study, however, the females had significantly higher baseline HIP (Table 2.2.). BF among females was significantly higher than among males at the beginning and at the end of the study. Males exhibited a significant 5% reduction in WST (95% CI, -9.4 to -0.4), while WT, BMI, HIP, WHR decreased non-significantly and BF remained unchanged (Table 2.2.). Females exhibited a significant 7% reduction in WT and BMI (95% CI, -11.8 to -1.4), and a significant 6% reduction in HIP (95% CI, -9.4 to -1.9). In females, the WST decreased by 5% (95% CI, -10.9 to 1.2) and the BF by 3% (95% CI, -11.9 to 5.4), but not significantly, and the WHR remained almost constant.

Discussion

In the present study, we compared the effects of long-term, progressive aerobic exercise training on adiponectin (ADPN) levels between untrained, middle-aged men and women. Previous studies have been mostly conducted on mixed male/female or single-gender cohorts. The influence of gender, if any, on changes in ADPN due to exercise has not been studied. Our results show that long-term, progressive aerobic training

significantly increases circulating levels of ADPN in untrained, middle-aged males and females. These increases were accompanied by significant reductions in some body measurements, but without changes in insulin resistance. The increase in ADPN levels was similar in males and females.

Effects of Reductions in Anthropometrics

In the female group, we observed a 7% decrease in WT and BMI, a 6% decrease in HIP, and a 5% decrease in WST. In the male group, we also observed only a 2% decrease in WT, BMI, and HIP and a 5% decrease in WST. Because there were more obese females than obese males, the decrease in WT in the female group was more than in the male group. Compared to long-term studies [8 – 11] that employed obese, insulin-resistant, or diabetic subjects, the anthropometric changes observed in our middle-aged, untrained subjects were modest. The percent BF decreased non-significantly in both male and female groups. The lack of correlation between changes in ADPN and changes in body fat has been reported [10, 13]. The effect of exercise on ADPN levels cannot be properly assessed unless controlled for concomitant anthropometric changes [26]. The observed increases in ADPN in our male and female groups were significant after adjusting for the percent changes in BMI and WST. Significant increases in ADPN levels have been reported regardless of the extent of body weight loss [8 – 11, 27]. These and our findings suggest that weight loss may not be necessarily associated with exercise-induced increases in ADPN, although weight loss may be obligatorily associated with increases in ADPN in dietary restriction studies [27]. In animal studies, ADPN levels are regulated differently by diet restriction versus exercise [28].

Effects of Gender

Females, in general, exhibit higher ADPN levels than males [29]. As stated previously, most long-term (>3 mos) protocols studying the effects of exercise on ADPN have been conducted on mixed male/female or single-gender cohorts [10, 11, 14, 15, 18, 30]. Long-term exercise interventions have shown increases in ADPN in females [8, 9], but not in males [16]. Progressive exercise studies have shown increases in ADPN in a mixed male/female cohort [11] and a male only cohort [10]. In our long-term, progressive exercise study of untrained, middle-aged males and females, both gender groups exhibited significant percent increases in ADPN. The present study was conducted on subjects with no previous long-term, endurance training experience. The extent to which different exercise training backgrounds play a role in gender-specific exercise-induced changes in ADPN levels remains to be explored. Due to different rates of attrition in the male and female groups, the difference in mean ages diverged from 2.4 years for baseline groups (Table 2.1.) to 3.9 years for the completing groups (Table 2.2.). Differences in age do not affect ADPN levels in females [31 – 33], but aging may increase ADPN levels in normal-weight males over the age of 65 [34 – 35]. However, all of the males in our cohort were under the age of 65. Hence, the mean age difference of 3.9 years between the completing male and female groups is unlikely to significantly affect the comparability of our study groups either in terms of ADPN levels or changes in ADPN levels. The issue of whether the two groups are comparable is complicated by the fact that they are different sexes. For example, the concept of BMI is not directly transportable between the two sexes because of well known gender-based differences in bone mass and fat distribution. In addition, it is well known that ADPN levels in females on average are higher than in

males [24]. However, in terms of mean age, BMI, exercise history, and ethnicity, these two groups are comparable for the main purpose of our study, which is to compare the effects of 6 months aerobic training on changes in ADPN levels between untrained, middle-aged males and females.

Effects of Insulin Resistance

Reported correlations between changes in insulin resistance and changes in ADPN have been inconsistent. Some have shown that ADPN increases with improved insulin sensitivity [36, 37]; others have shown that ADPN increases without improvement in insulin sensitivity [15, 38]; and still others have shown no change in ADPN despite improved insulin sensitivity [12, 13, 39]. In our 6-mo study, significant increases in ADPN levels occurred in both the male and female groups without corresponding reductions in HOMA-IR score, a surrogate measure of insulin resistance. Because insulin sensitivity gradually decreases to control levels within 2 – 4 days after exercise [40, 41], our subjects were asked to refrain from exercise 2 days prior to giving a blood sample. Our findings suggest that exercise-induced increases in ADPN in untrained, middle-aged, overweight males or females occur without changes in insulin sensitivity. Several factors contributed to the relatively small sample size of our study. These were (i) the inclusion of only untrained running club members, (ii) the exclusion of potential subjects on prescription medicines influencing ADPN levels, and (iii) the contribution of long-term, strenuous exercise intervention to injury-related dropouts, especially among the untrained females.

Conclusions

We conclude that long-term progressive aerobic training significantly increases plasma ADPN levels in untrained, middle-aged, overweight males and females to similar extents, but without significantly affecting insulin resistance. The significant reductions in some anthropometric measurements, such as WT and WST, do not appear to influence changes in ADPN. Thus, gender does not seem to significantly affect exercise-induced increases in ADPN due to long-term progressive training in untrained, middle-aged, overweight subjects.

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CHAPTER THREE

LONG-TERM, PROGRESSIVE, AEROBIC TRAINING INCREASES ADIPONECTIN IN AEROBICALLY TRAINED, MIDDLE-AGED FEMALES, BUT NOT MALES

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Abstract

Aerobic training and the adipokine, adiponectin (ADPN), both increase insulin sensitivity. Because the metabolic effects of ADPN and aerobic training are similar, aerobic training has been proposed to increase ADPN. Physically active adolescent females show higher ADPN levels compared to sedentary controls. Long-term aerobic training studies show greater increases in ADPN levels in untrained females than in untrained males, and previously trained young males show no increase in ADPN levels. Consequently, we hypothesized that long-term (6 months), progressive aerobic training would increase ADPN levels in trained, middle-aged females, but not in comparably trained males. We recruited aerobically trained, normal-weight, middle-aged males and females to participate in a marathon training protocol progressing from 9.7 to 88.5 km (6 to 55 miles) per week over 6 months. We collected and stored fasting plasma samples and recorded body measurements at 0 (baseline) and 6 months. Stored samples were analyzed for insulin, glucose, and ADPN. ADPN increased significantly among females [from 18.37 ± 10.39 (mean ± SD) to 22.50 ± 9.87 μg/ml; $P<0.05$], but non-significantly among males (from 6.69 ± 4.47 to 8.10 ± 5.57 μg/ml; $P=0.2$). No significant reduction in insulin resistance or anthropometric measurements occurred in either group. We conclude that long-term, progressive aerobic training increases circulating ADPN levels in trained, middle-aged, normal-weight females, but not in comparable males.

Key Words

adiponectin, exercise, human, intervention studies, sex differences

Introduction

Central obesity is on the rise in middle-aged men and women [1, 2] and increased adipose mass decreases the production of adiponectin (ADPN), an adipokine positively correlated with insulin sensitivity [3]. Mature adipocytes express and secrete ADPN (aka gbp28, Acrp30, and apM1). ADPN knockout mice on a high fat diet develop insulin resistance and coronary artery disease [4], while ADPN over-expression improves insulin sensitivity [5]. Higher levels of ADPN may preserve insulin sensitivity in middle-aged men and women [1, 2] since ADPN levels negatively correlate with obesity, high blood pressure, type II diabetes, hyperlipidemia, and metabolic syndrome [6].

Aerobic exercise improves insulin sensitivity, blood pressure, lipoprotein profile, and metabolic and cardiovascular health [7]. Because some of the effects of aerobic exercise training and ADPN are similar, aerobic training has been proposed to increase ADPN levels [7]. Cross-sectional studies reported significantly higher ADPN levels in physically active versus sedentary adolescent females [8, 9].

At the end of a long-term [24 months (mos)] aerobic intervention involving untrained, middle-aged, obese subjects, ADPN levels increased two-fold more in females than in males [10]. Long-term (6 mos), progressive aerobic training also increased ADPN levels almost twice as much (22.3%) in untrained, middle-aged, overweight females as in untrained males (12.1%) [11], while long-term studies on young, elite male rowers showed no changes in ADPN levels [12, 13]. Thus various studies indirectly suggest that ADPN levels may increase more in physically active females than in males, but no study has yet directly compared the effect of long-term aerobic training on ADPN levels in trained males and females.

We hypothesized that trained, normal-weight, middle-aged females would exhibit significantly greater increases in ADPN in response to long-term, progressive aerobic training than in similarly trained males. To test our hypothesis, we compared the effects of a 6-mo, progressive, marathon-training program on ADPN levels in trained male and female groups that were similar in baseline BMI and age. Consistent with our hypothesis, we found that ADPN levels increased significantly in trained females by the end of 6-mo training, but non-significantly in males.

Methods

Subjects

We recruited subjects from a large ($n \approx 600$) local running club to participate in a 6-mo, progressive, marathon training program. The Institutional Review Board of Loma Linda University approved the research protocol. We presented the objectives and procedures of the study to the club members and gave interested members an informed consent document explaining the procedures and risks and afforded opportunities to ask questions. Potential subjects signed the informed consent document before completing a screening questionnaire. Inclusion criteria were adult males and females that have been training for and completing a minimum of one full-distance marathon per year for each of the past five years. Exclusion criteria were known cardiovascular, renal, hepatic, pulmonary, adrenal, pancreatic, thyroid, or pituitary disorder and smoking.

Demographic Characteristics

We separated the subjects into two groups by gender: trained males and trained females. As shown in Table 3.1., the male and female cohorts were similar in mean age and mean BMI.

During the 6 mos of progressive training, the two groups exhibited similar rates of attrition. However, because younger females dropped out to a greater extent than younger males, the mean ages of the male and female groups diverged slightly, but not significantly (compare Tables 3.1. and 3.2.). Several factors contributed to the relatively small sample size of our study. These were (i) the inclusion of only middle-aged, multi-year trained running club members and (ii) the exclusion of potential subjects on prescription medicines known to influence ADPN levels.

Table 3.1. Physical characteristics in male and female groups at the baseline (0 mo).

	Trained Male (n=14)			Trained Female (n=10)			
Variable	Mean	±	SD	Mean	±	SD	*p**
age	51.5	±	11.1	54.9	±	10.6	NS
HT	1.77	±	0.05	1.63	±	0.09	<0.001
WT	81.1	±	12.8	63.3	±	11.7	<0.005
BMI	25.8	±	3.7	23.9	±	3.5	NS
BF	22.5	±	6.4	28.2	±	8.1	NS
WST	93.1	±	11.6	80.0	±	13.1	<0.05
HIP	99.4	±	7.7	98.9	±	8.9	NS
WHR	0.94	±	0.07	0.81	±	0.09	<0.005

Abbreviation key: age (yr); HT, height (m); WT, total body weight (kg); BMI, body mass index (kg/m^2); BF, total body fat (%); WST, waist circumference (cm); HIP, hip circumference (cm); WHR, waist-to-hip ratio; NS, not significant; *p**, significance between Trained Male and Trained Female groups by independent t-test.

Table 3.2. Physical characteristics and blood analytes in trained male and female groups at the baseline (0 mo) and end (6 mos).
Abbreviation key: age (yr); HT, height (m); WT, total body weight (kg); BMI, body mass index (kg/m^2); BF, total body fat (%); WST, waist circumference (cm); HIP, hip circumference (cm); WHR, waist-to-hip ratio; ADPN, plasma total adiponectin (μg/ml); FPI, fasting plasma insulin (μU/ml); FPG, fasting plasma glucose (mg/dl); HOMA-IR, HOmeostasis Model Assessment of Insulin Resistance; NS, not significant; ^{LT}, tests were conducted on log transformed variable; *p*, significance within Trained Male and Trained Female groups by a paired t-test; *p**, significance between Trained Male and Trained Female groups by independent t-test.

Variable	Time	Trained Male (n=10) Mean	±	SD	Trained Female (n=8) Mean	±	SD	*p**
age	0 mo	50.9	±	11.5	58.8	±	6.3	NS
HT	0 mo	1.75	±	0.03	1.61	±	0.08	<0.005
WT	0 mo	75.8	±	9.6	63.4	±	11.8	<0.05
	6 mo	75.4	±	9.9	64.4	±	12.6	0.06
	p	NS			NS			
BMI	0 mo	24.9	±	3.0	24.4	±	3.5	NS
	6 mo	24.7	±	3.1	24.8	±	3.8	NS
	p	NS			NS			
BF	0 mo	19.3	±	7.7	30.9	±	11.2	<0.05
	6 mo	19.2	±	7.7	30.1	±	8.1	<0.05
	p	NS			NS			
WST	0 mo	86.7	±	11.4	80.4	±	14.1	NS
	6 mo	86.4	±	11.2	79.5	±	10.7	NS
	p	NS			NS			
HIP	0 mo	93.5	±	11.3	99.1	±	8.7	NS
	6 mo	93.0	±	12.4	98.5	±	9.3	NS
	p	NS			NS			
WHR	0 mo	0.93	±	0.10	0.81	±	0.10	<0.05
	6 mo	0.93	±	0.09	0.81	±	0.07	<0.005
	p	NS			NS			
$ADPN^{LT}$	0 mo	6.69	±	4.47	18.37	±	10.39	<0.005
	6 mo	8.10	±	5.57	22.50	±	9.87	<0.005
	p	NS			<0.05			
FPI	0 mo	5.69	±	2.62	4.99	±	3.63	NS
	6 mo	4.97	±	2.94	5.11	±	3.22	NS
	p	NS			NS			
FPG	0 mo	101.4	±	30.2	101.7	±	12.1	NS
	6 mo	97.9	±	15.2	95.6	±	17.4	NS
	p	NS			NS			
HOMA-IR	0 mo	1.43	±	0.73	1.28	±	1.00	NS
	6 mo	1.23	±	0.79	1.25	±	0.90	NS
	p	NS			NS			

Subjects were not restricted to a particular ethnic group, but the proportion of Caucasians to non-Caucasians in the completing cohorts of the study were similar with 80.0% and 87.5%, being Caucasians in the male and female groups, respectively. We included postmenopausal females both on (n=3) and not on (n=4) hormone replacement therapy (HRT) because ADPN levels have been reported to not differ between postmenopausal women on HRT and those not on HRT [14]. We also included two premenopausal women, who did not complete the study, and a perimenopausal female (age 53 yrs) on oral contraceptive (OC) because ADPN levels in females are reported to be stable throughout the menstrual cycle [15, 16] and unaffected by the use of OCs [16]. The ADPN levels have also been reported to not differ between premenopausal women not on OC and postmenopausal women not on HRT [14]. Female participants on oral contraceptives or HRT continued to use these throughout the study.

Training Protocol

All subjects followed the same progressive training protocol for 6 mos, which is described in detail elsewhere [11]. The Sunday distance increased linearly at an average rate of 1.16 km/week (km/week) from 3.2 to 32.2 km (2 to 20 miles) over a period of 6 mos. Weekday and total weekly distances increased non-linearly during the first 8 weeks and thereafter increased linearly until the end of 6 mos (Figure 3.1.). We monitored adherence to the prescribed training protocol using self-reported daily running logs, which were collected each Sunday. Subjects remained in the same pace group throughout the study. We instructed subjects to maintain their pre-intervention dietary habits during the course of the study, but dietary intakes were not monitored.

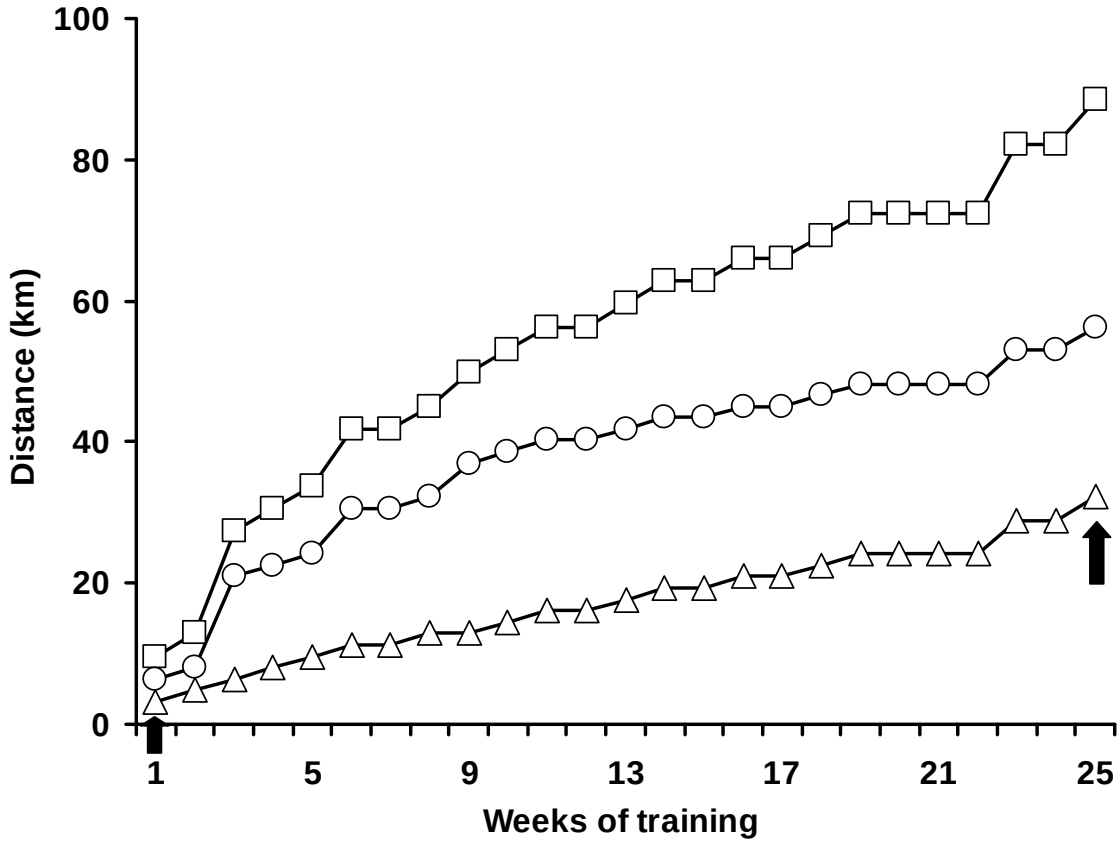

Figure 3.1. Time course of long-term, progressive training protocol. Total weekly distance (□) is the sum of weekday distance (○) and Sunday distance (△). Anthropometric data and blood samples were collected at baseline (0 mo) and 6 mos as indicated by arrows.

Biochemical Measurements

Biochemical measurements are described in detail elsewhere [11]. Briefly, we collected fasting blood samples from the median cubital vein on Sundays, immediately before the start of the 3.2- and 32.2-km (2- and 20-mile), long-run distances corresponding to 0 and 6 mos, respectively. We collected plasma by centrifugation, separated plasma samples into 0.5 ml aliquots, and immediately stored them at -80^{o}C until analyzed. We used spectrophotometric, sandwich ELISA-based assays for fasting total plasma ADPN (mg/ml; EZHADPN-61K, LINCO Research, St. Charles, MO) and fasting plasma insulin (FPI; μU/ml; EZHI-14K, LINCO Research) and the liquid oxidase procedure (2810-1, Eagle Diagnostics, De Soto, TX) for fasting plasma glucose (FPG; mg/dl). We calculated the HOmeostasis Model Assessment of Insulin Resistance (HOMA-IR) scores using the following equation [17]: HOMA-IR = [FPI (μU/ml) x FPG (mg/dl)/18]/22.5

Anthropometric Measurements

Qualified personnel measured and recorded height (HT, m), body weight (WT, kg), total body fat (BF, %), waist circumference (WST, cm) and hip circumference (HIP, cm) as previously described [11]. These measurements were taken at 0 and 6 mos between 6-7 AM on the same day as the blood draw immediately before the long run on Sunday. Waist-to-hip ratio (WHR) and body mass index (BMI) were calculated from the measurements. Height (m) was measured at baseline (0 mo) using a stadiometer. Total body fat was measured on standard adult mode (non-athlete mode) setting using

bioelectrical impedance (model BF-679, TANITA Corporation, Arlington Heights, IL). All measurements were obtained in duplicate, recorded, and averaged.

Statistical Analyses

We used the Statistical Package for Social Sciences (version 17.0) for Windows (SPSS, Inc., Chicago, IL) to perform all statistical analyses. We considered the results statistically significant if a two-tailed P-value was less than or equal to 0.05. We used histograms and the Kolmogorov-Smirnov test for normality to determine whether the variables for the study groups were normally distributed. We log transformed and reassessed the variables that were not normally distributed. We used an independent t-test to assess each dependent variable between the study groups separately for each data collection point (*i.e.* 0-mo baseline and 6 mos). We also used an independent t-test to assess percent changes in the variables between the groups. We used a paired t-test to assess the difference in each dependent variable separately for each group.

Results

Subject Compliance

We enrolled subjects meeting the inclusion and exclusion criteria. Cohort sizes at each stage of the study are diagramed in Figure 3.2. Of the original 27 enrolled subjects, retention among trained male and trained female subjects was the same (66.7%). We carried out data analyses on a total of 18 subjects (*i.e.* males = 10; females = 8) who completed the 6-mo training and provided complete data sets at baseline (0 mo) and 6 mos.

Adiponectin Levels

The levels of ADPN increased at the end of 6 mos of training in both male and female groups, but significantly only among the trained females (Fig. 3.3.). In the female group ADPN levels at the baseline and at the end of 6 mos of training were significantly higher by three-fold than in the male group (Table 3.2.). The percent increases in ADPN were not significantly different between males (22.0 ± 43.4%; median, 11.4%) and females (38.7 ± 53.2%; median, 18.8%).

Insulin, Glucose, and HOMA-IR score

Trained male and female groups exhibited similar FPI and FPG levels and HOMA-IR scores at baseline and at the end of the study (Table 3.2.). Furthermore, FPI and FPG levels and HOMA-IR scores did not change by the end of the 6 mos of training within either group.

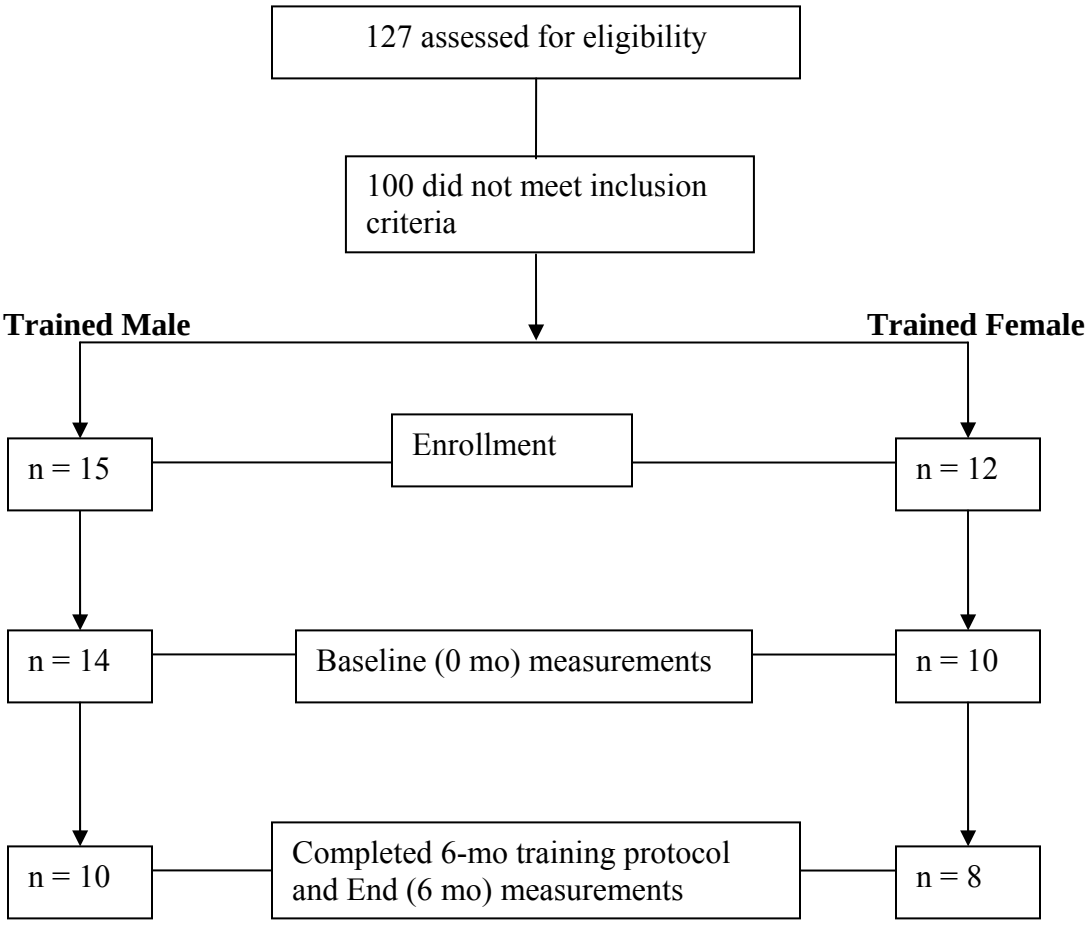

Figure 3.2. Sample size and gender composition at each stage of the study.

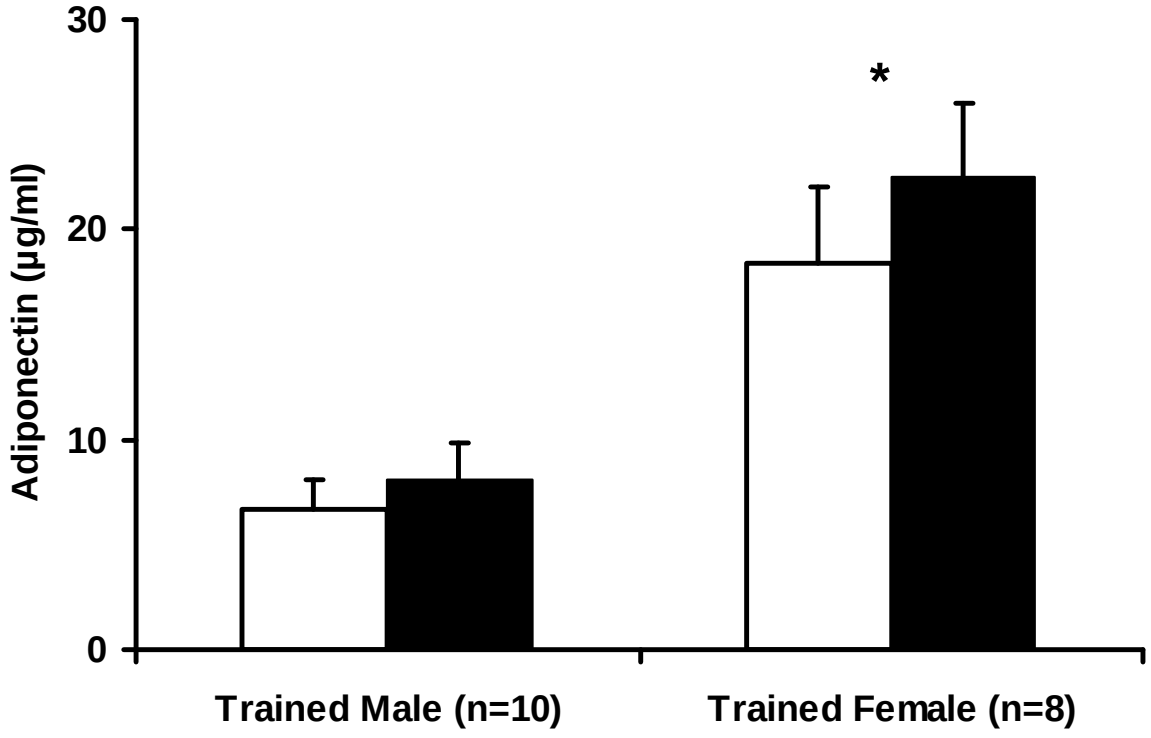

Figure 3.3. Plasma adiponectin levels at baseline and at end of 6 mos aerobic training in groups identified by gender. Increase in adiponectin levels (mean ± SEM) between baseline (0 mo, open bars) and end (6 mos, filled bars) was significant in trained females, but not trained males, as determined by a paired t-test. Asterisk (*) indicates $P<0.05$. Sample sizes of each group are indicated in parentheses.

Anthropometric Measurements

The trained males had higher WT than females. The difference was significant at the baseline, but non-significant at the end of the 6-mo training. The male and female groups exhibited similar BMI, WST, and HIP measurements at the baseline and at the end of 6 mos training. The females had significantly higher BF and lower WHR compared to males at the baseline and at the end of 6 mos training. (Table 3.2.).

Both males and females exhibited negligible and non-significant reductions in mean WST and HIP, and negligible and non-significant increases in mean WHR and BF. Additionally, males exhibited negligible reductions in WT and BMI, whereas females exhibited negligible increases in WT and BMI (Table 3.2.).

Discussion

In the present study, we compared the effects of long-term, progressive aerobic training on adiponectin (ADPN) levels in trained male and female marathoners who were middle-aged and normal-weight. Few exercise interventions measuring changes in ADPN levels have been conducted on middle-aged athletes. Because previous studies have mostly focused on trained, single-gender cohorts, the influence of gender on exercise-induced changes in ADPN has not been adequately addressed. Our results show that long-term, progressive aerobic training of 6 mos duration increases circulating levels of ADPN to a significantly greater extent in trained, middle-aged females than in similarly trained males.

Effects of Gender

Females, in general, exhibit higher ADPN levels than males [18]. In our previous study on untrained, middle-aged subjects undergoing 6-mo, progressive aerobic training, ADPN levels in females were slightly higher both at baseline and at 6 mos compared to males, and both males and females showed significant and similar increases in ADPN levels [11]. However, in the present study on trained, middle-aged marathoners, the male-female differences in ADPN levels at baseline and at 6 mos were significantly larger with the trained females exhibiting ADPN levels three times higher than the comparably trained males. In addition, our 6-mo, aerobic protocol increased ADPN significantly in trained females ($P<0.05$), but non-significantly ($P=0.19$) in trained males, which is similar to a 6-mo study showing no significant increases in ADPN levels among young, trained male rowers [13].

Aerobically exercising females derive proportionately more of their expended energy from free fatty acid oxidation than do males [19, 20]. ADPN expression positively correlates with hormone-sensitive lipase activity in females [21], which regulates lipolysis in adipose tissue. Thus, our observation that trained exercising females exhibit higher levels and greater increases in ADPN than comparably trained males may be metabolically linked to trained females' greater capacity to mobilize and use mobilized free fatty acids to fuel their aerobic exercise.

Effects of Training History

When comparing ADPN levels between untrained subjects from our previous study [11] and trained subjects from our current study, the baseline ADPN levels in the trained males were not higher than those in the untrained males [11]. However, the baseline ADPN levels in the trained females were twice those in the untrained females [11]. Because of differences in mean age, BMI, and ethnicity between the cohorts of these two studies, we cannot readily compare untrained and trained subjects from the same gender. However, it appears that several years of prior aerobic training may increase ADPN levels much more in females than in males.

Effects of Insulin Resistance

Aerobic training lowers resting levels of insulin [12]. However, we did not expect the 6-mo training protocol to produce significant increases in insulin sensitivity because our subjects already were trained and exhibited HOMA-IR scores indicative of healthy insulin sensitivity at baseline. In fact, we did not observe any changes in insulin

sensitivity following 6 mos of training. Our findings suggest that exercise-induced increases in ADPN can occur without measurable reduction in HOMA-IR scores in trained males and females.

Effects of Body Measurement Changes

Because all of our subjects were previously trained, we did not expect the 6-mo aerobic training to produce significant changes in body measurements, nor did we observe any. Elite male rowers also showed no changes in body weight in response to 6-mo training [13]. Weight loss has been considered by some to be a necessary correlate to increased ADPN levels [22-25]. However, studies using long-term aerobic training have reported increases in ADPN levels without weight loss in untrained [10, 11, 26] and trained subjects [12, 13]. These and our current findings with trained subjects suggest that weight loss is not necessary for exercise-induced increases in ADPN.

Conclusions

We conclude that 6-mo, progressive, aerobic training increases circulating ADPN levels in multi-year trained females to a larger extent than in similarly trained males. To our knowledge, our findings are the first to show that only trained females benefit in terms of increased ADPN levels by the additional training. In addition, our findings show that exercise-induced increases in ADPN levels can occur in the absence of changes in insulin-sensitivity or weight loss in previously trained individuals. Comparing the current findings with those from our previous study on untrained subjects, it appears that males on the one hand show little to no increase in ADPN levels beyond what occurs during 6

mos of progressive training. On the other hand, ADPN levels in females increase beyond 6 mos of marathon training if aerobic training continues. Thus, the extent of exercise-induced increase in ADPN levels appears to be gender-specific; limited in males, but extended in females.

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CHAPTER FOUR

DISCUSSION/CONCLUSION

Goal of Dissertation

The overall goal of the dissertation was to elucidate the influence of gender and aerobic training background on exercise-induced increase in adiponectin.

Our **general hypothesis** was that long-term aerobic training increases total circulating adiponectin and that gender and exercise training background strongly influence the extent of the exercise-induced increase in adiponectin.

The general hypothesis included two specific hypotheses –

In Hypothesis 1, we hypothesized that ADPN levels increase significantly in previously untrained males and females in response to long-term, progressive aerobic training. To test this hypothesis, our specific aim was *To compare ADPN levels before and at the end of a long-term, progressive aerobic training regimen in middle-aged, untrained males and females.* We measured ADPN levels in untrained male and female subjects that had never trained for or participated in a full-distance (26.2 mile) marathon. In addition to ADPN levels we measured markers of insulin-resistance and anthropometric parameters.

In Hypothesis 2, we hypothesized that ADPN levels in multi-year aerobic trained females increase significantly in response to long-term, progressive aerobic training, but not in comparably trained males. To test this hypothesis, our specific aim was *To compare ADPN levels before and at the end of a long-term, progressive aerobic training*

regimen in middle-aged, multi-year aerobic trained males and females. We measured ADPN levels in trained male and female subjects that have been training for and completing at least one full-distance (26.2 mile) marathon each year for the five previous consecutive years. We measured markers of insulin-resistance and anthropometric parameters in addition to ADPN levels.

Major Findings of The Dissertation

Our study design and methodology achieved the goal of the project and we conclude that gender and aerobic training background strongly influence exercise-induced increases in ADPN. Our findings also show that exercise-induced increases in ADPN levels can occur independently of insulin-sensitivity changes in both untrained and trained subjects. In addition, we have shown that exercise-induced increases in ADPN can occur independently of weight loss in both untrained and previously aerobic-trained individuals. On the one hand, it appears that males, show little to no increase in ADPN levels beyond that which occurs during 6 mos of progressive marathon training. On the other hand, females show increased ADPN levels beyond 6 mos of marathon training program if aerobic training continues. Thus, the extent of exercise-induced increase in ADPN levels appears to be gender- and training background-specific with ADPN levels increasing in males and reaching a plateau at relatively moderate levels of training, while in females ADPN levels increase with increasing durations of training.

Contributions to The Field

Our comprehensive study fills in the gaps in the literature by answering the following questions:

1. Does long-term aerobic training increase ADPN in healthy subjects?

Most of the studies in the literature have been conducted on overweight [1, 2], obese [3-7], or insulin-resistant or DM2 subjects [3, 8-11]. Although these studies showed an increase in ADPN, studies conducted on untrained and trained healthy subjects showed no changes in ADPN [12, 13]. Our study showed a significant increase in healthy untrained male and female subjects and healthy trained female subjects and a weaker trend of increase P=0.19 in ADPN in healthy trained males. Therefore, we conclude that long-term aerobic training does increase ADPN in healthy subjects.

2. Is weight loss necessary for an increase in ADPN?

The general opinion in the field is that greater than 10% weight loss brought about by caloric restriction, aerobic exercise intervention, and/or liposuction is needed to increase ADPN levels [3, 7]. However, some studies employing progressive training protocols showed increased ADPN levels without significant weight loss [9]. Many studies that observed increased ADPN levels due to exercise training intervention did not control for reductions in weight, body fat, waist circumference [3, 8, 9, 14, 15] to correctly interpret if intervention-induced, specifically exercise intervention-induced, increases in ADPN levels were due to intervention alone or due to reductions in anthropometric measurements.

Our untrained male and female subjects showed a reduction in body weight and waist circumference [16]. After statistically controlling for these changes in a repeated

measure ANOVA, the increase in ADPN was still significant. The trained subjects in our study showed no decrease in anthropometric measures, but the trained females showed a significant increase in ADPN and the trained males showed a trend of increased (P=0.19) ADPN levels at the end of 6-mo progressive aerobic training (submitted). Therefore, we conclude that weight-loss is not a prerequisite for exercise-induced increases in ADPN.

3. Does gender influence aerobic exercise-induced increase in ADPN?

Most studies were conducted on cohorts with male-female subjects together [1, 6, 7, 9, 10, 12, 14, 17] or male [4, 13, 15, 18] or female [2, 8] subjects. Studies employing female-only cohorts reported increases in ADPN while most studies employing male-only cohorts reported no changes. Ring-Dimitriou *et al.* reported two-times higher increases in ADPN in females compared to males [9]. Until our two studies, no study had compared males and females during same exercise intervention. Our untrained and trained females showed a significant and two times greater increase in ADPN levels compared to our untrained and trained males. Our untrained males showed a significant increase in ADPN, but not the trained males. Therefore, we conclude that exercise-induced increase in ADPN is gender specific.

4. Is aerobic exercise-induced increase in ADPN affected by different training backgrounds? Are there differences in aerobic exercise-induced increases in ADPN in people with and without aerobic training background?

Most long-term exercise intervention studies have been conducted on untrained subjects [1-11, 14, 15, 17-20] with only a few on trained subjects [13, 21]. No previous study, however, compared changes in ADPN levels in trained and untrained subjects undergoing the same exercise training protocol. Our comprehensive study sought to

answer the question: Do untrained subjects show higher increases in ADPN levels, as they have not been conditioned to exercise training? Our trained and untrained females both showed similar (median, ~20%) increases in ADPN. Our trained and untrained males also showed similar (median, ~10%) increases in ADPN. Therefore we conclude that aerobic exercise-induced increase in ADPN does not differ according to training background.

Assumptions and Limitations of The Study

Our study groups were based on self-reported information concerning the subjects' exercise training and medical history. The results of our studies should be interpreted with attention to the following limitations –

1. Body composition –

We used the bioelectric impedance method (model: BF-679, TANITA Corporation, Arlington Heights, IL) for measuring total body fat as a percent of total body weight. This method does not give differential body fat measurement data associated with different compartments of body fat, subcutaneous and visceral. Hence, we could not associate the increase in ADPN levels with loss of a specific compartment of fat, particularly visceral fat.

We measured body fat of both trained and untrained subjects using the standard mode of the TANITA® model. According to our trial runs with the TANITA® model, athlete mode understates the total body fat measurements whereas standard mode overstates the total body fat measurements of a trained subject. To compare body fat composition of the untrained group to that of the trained group, it was not advisable to

measure the untrained subjects on the standard mode and the trained subjects on athlete mode, as it would yield a much larger difference in the mean percent body fat of two groups and introduce error.

2. Family medical history -

We did not record the family medical histories of our subjects for hypertension, DM2, or cancer. Offspring of DM2 patients have lower circulating ADPN levels [22] as compared to those without the family history of DM2. But short-term (7-weeks) aerobic training reported similar increases in ADPN levels in the offspring of DM2 patients and those without the family history of DM2 [23].

3. Intensity of exercise training –

VO_2max is a measure of a subject's aerobic capacity. Exercise studies also use VO_2max as a measure of intensity of exercise training. The scope of our training protocol was not to improve the endurance capacity of subjects, but to train them to complete a full-distance marathon. Our study measured the effect of increasing duration (distance) of training, but not intensity of training, on changes in ADPN levels. We measured the intensity of aerobic training as pace (minutes taken to complete a mile distance) of the subject. Our subjects were instructed to follow the given training protocol and trained independently, hence the best estimate of the intensity of their aerobic training was determined by pace measurement. There were no significant differences in the pace at baseline and at the end of 6 mos in the trained or untrained subjects.

4. Ethnic composition of the cohorts –

Non-Hispanic whites (Caucasians) have higher ADPN levels compared to other ethnic groups, *e.g*. non-Hispanic blacks, Asians, and Hispanics [24]. Because our study

measured changes in ADPN levels in response to the long-term aerobic training, we did not restrict the study cohorts to a specific ethnic group. The effect of ethnicity, if any, on the changes in ADPN levels due to exercise (aerobic or resistance) training have not been noted. When we separated our subjects into two groups based on their ethnicity: Caucasians and other-than-Caucasians, the ratio of Caucasians to other-than-Caucasians was similar within each gender for the trained and untrained study cohorts. Thus, ethnicity was not a likely confounding factor to the results of the study.

5. Menopausal status or day of menstrual cycle -

Our study included premenopausal women both on and not on oral contraceptives and postmenopausal females both on and not on hormone replacement therapy (HRT). ADPN levels do not differ between pre and postmenopausal women nor between postmenopausal women on HRT and not on HRT [25]. We also included premenopausal women both on and not on oral contraceptives because ADPN levels in females are reported to be stable throughout the menstrual cycle implying that changes in estrogen levels do not affect ADPN levels [26].

It has been noted that the luteal phase of the menstrual cycle is associated with weight gain in women [62]. The weight gain is anticipated due to premenstrual increases in appetite and eating and/or fluid retention [63]. The changes in appetite, water retention and the resulting weight gain is reported to be minor [27] and higher for women using monophasic oral contraceptives than bi or triphasic oral contraceptives [28]. We did not control for the menstrual phases of the premenopausal females in the study for blood draws and anthropometric measurements. This may account for some of the lack of anthropometric reductions in some premenopausal females in the study.

6. Age matching of the cohorts -

We did not match individual subjects by age between comparison groups, but the mean ages of male and female groups from trained and untrained cohorts were similar. Differences in age do not affect ADPN levels in females [29-31], but aging may increase ADPN levels in normal-weight males over the age of 65 [32, 33]. However, males from both trained and untrained cohorts that completed the 6-mo study and provided the complete dataset were under the age of 65. Although the subjects were not age-matched, the numerically small mean difference between the ages of completing male and female groups is unlikely to significantly affect the comparability of our study groups either in terms of ADPN levels or changes in ADPN levels.

7. Cohort size -

The sample sizes of our study groups are similar to the sample sizes of other exercise-intervention study cohorts. But the specific reasons for the low sample size in our study were –

(i) Inclusion of only middle-aged, multi-year trained or untrained running club members

(ii) Exclusion of potential subjects on prescription medicines known to influence ADPN levels

(iii) Program attrition/drop out –

We experienced a lower drop out rate (36%) in untrained male subjects, than in untrained female subjects (77%). We know anecdotally that repetitive stress injuries disproportionately afflicted the untrained female subjects, but injuries did not account for all who withdrew. We conjecture that untrained female subjects were especially

motivated to train in order to lose weight and that untrained male subjects were more motivated to train in order to become more "fit" or "healthy". About 81% of the untrained females that dropped out did so by half way through the study. We speculate that when some of the overweight untrained females found little or no weight loss part-way into the training program, they lost their primary incentive for training and withdrew from the program.

The drop out rate was much lower in the trained subjects (males and females = 33.3%). The trained subjects withdrew from the study mainly due to repetitive stress injuries, which are common among many full-distance marathon runners during longer training runs [34].

8. Pro-inflammatory adipokines –

We did not measure pro-inflammatory cytokines, such as IL-6 or TNF-α, in addition to FPI, FPG and ADPN. Hence, we can not connect increases in ADPN levels observed in our study to reductions in pro-inflammatory adipokines. Out of many adipokines, ADPN has closest structural resemblance to TNF-α. An inverse correlation has been reported in TNF-α and ADPN levels [35]. Studies have reported a lack of reduction in TNF-α [36] and reductions in TNF-α along with increases in ADPN levels [37]. It is possible that increases in ADPN occur independently of reductions in other pro-inflammatory adipokines.

9. Dietary control in the study –

Dietary intervention significantly increased ADPN levels, but not by 3-mo aerobic exercise training in male rats [38]. The results of this study suggested that ADPN levels may change differently by diet and exercise intervention. We did not regulate the

dietary intake of our subjects. We now believe that controlling dietary intake in an exercise intervention study would be advisable because having a similar diet among subjects would help eliminate the possible influence of dietary variation on ADPN levels. A controlled diet in an exercise-intervention study can be achieved by: 1) recruiting subjects from the same baseline BMI group, *e.g.* overweight, obese, or normal-weight subjects only and keeping total caloric intake constant between the study groups or 2) If the cohort has a range of baseline BMI, the total number of calories can be assigned based on a subject's baseline BMI and physical activity while keeping the proportion of major nutrients *e.g.* carbohydrates, proteins, fats, and fiber same in the subjects' diet during the period of the study.

10. Measurement of insulin resistance using HOMA-IR equation –

We calculated insulin resistance using the HOMA-IR equation, which is a surrogate measure of insulin resistance [39]. Considering the long-term aerobic exercise intervention of our study, it was difficult to use the gold standard, hyperinsulinemic euglycemic clamp technique to measure insulin resistance of the subjects. The clamp procedure is invasive, laborious, requires drawing multiple blood samples and has to be conducted in a hospital setting. The insulin resistance values obtained by the HOMA-IR method correlate with those obtained from the clamp technique and HOMA-IR method is thus a reliable alternative to the clamp method of insulin resistance measurement [40]. Currently, tissue-specific insulin resistance is measured in three major organs: Liver, skeletal muscle, and adipose tissue. While the HOMA-IR technique is reliable, it does not provide information about organ-specific insulin resistance. It would be beneficial to know if ADPN levels in our study increased due to improvement in insulin sensitivity in

whole body or a specific organ. Hyperinsulinemic euglycemic clamp method in combination with radio-labeled glucose, allows quantification of the organ-specific contribution insulin resistance to the defect in whole-body insulin-mediated glucose disposal [41].

Recommendations for Changes in Methodology

1. Measurement of Insulin levels –

Consider using an ELISA kit with a standard curve range similar to the physiological FPI range (3-20 μU/ml). Ultrasensitive assay kits such as Insulin Ultrasensitive ELISA (80-INSHUU-E01, Alpco Diagnostics, Salem, NH) offers the standard curve range similar of the physiologic range of FPI (0.15 – 20 μU/ml). We used EZHI-14K (LINCO Research, St. Charles, MO) ELISA kit to measure FPI levels. The standard curve of this assay ranges from 2 – 200 μU/ml. As a result, this kit is not sensitive to measure physiological FPI levels (3-20 μU/ml) in healthy subjects. Alpco Diagnostics' ultrasensitive insulin kit was not available when we were conducting assays in year 2007.

2. Measuring insulin resistance –

Consider using the hyperinsulinemic euglycemic clamp technique to measure insulin sensitivity in place of a surrogate measure of insulin resistance such as the HOMA-IR score. Also consider employing glucose utilization technique to measure organ-specific insulin resistance. We could not use the glucose clamp technique, as it requires dedicated facility, which is not currently available at Loma Linda University.

3. Measuring body fat –

Consider using Dual energy X-ray absorptiometry (DXA, formerly known as DEXA), to measure body fat. This also is beneficial for knowing body fat composition and changes in different body fat compartments. DXA measurements are expensive and time consuming. In addition, the goal of the study was not to correlate increases in ADPN through long-term aerobic exercise training to changes in body fat composition. Hence, we used the BIA (bioelectric impedance) method of measurement of body fat.

4. Measurement of aerobic capacity –

Consider using VO_2max measurement technique in place of "pace" of a subject to measure the "fitness" or aerobic capacity of a subject. This measure will indicate if long-term aerobic training improved the subject's aerobic capacity. However, measuring VO_2max requires an operational human performance laboratory which is not available at Loma Linda University.

Future Studies

My hope is that the successes and challenges of the present research project will open doors in new directions to further understand the connection between exercise and adiponectin levels.

1. Increase the number of time points for sample acquisition between baseline and the end of training protocol in a long-term aerobic training program. This will help in understanding if there is a dose-response relationship between increase in exercise training and ADPN levels.

2. Continue fasting sample acquisition after end of the 6-mo aerobic training program. This will help in understanding the time-course of changes in ADPN levels after training distance is significantly reduced.

3. Acquire one-time, fasting blood samples from untrained and trained subjects and separate the subjects based on ethnicity in a larger cross-sectional study. This will help in understanding the influence of ethnicity on exercise-induced increases in ADPN levels.

4. Conduct an exercise-intervention study with controlled dietary intake (not restricted). This will help in understanding if changes in ADPN are as a result of exercise intervention alone and not due to dietary changes that might occur during a long-term aerobic exercise-intervention.

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