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Adipose and Skeletal Muscle Expression of Adiponectin and Liver Receptor Homolog-1 With Weight Loss and Aerobic Exercise

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Abstract

Context: Adiponectin is an adipokine mainly secreted by adipocytes that regulates the metabolism of lipids and glucose. Liver receptor homolog-1 (LRH-1), also named NR5A2, is a nuclear receptor that regulates lipid metabolism and homeostasis.

Objective: The purpose of this study was to compare adiponectin and LRH-1 messenger RNA (mRNA) expression in adipose tissue and LRH-1 expression in skeletal muscle between men and women at baseline and to study the effects of aerobic exercise (AEX) training or weight loss (WL) on their expression.

Methods: This hospital and university setting study included 62 overweight and obese men (n = 23) and women (n = 39) older than 45 years, of whom 41 completed 6 months of WL (n = 21) or AEX (n = 20). Outcomes included abdominal and gluteal adipose tissue and skeletal muscle gene expression.

Results: Adiponectin and LRH-1 mRNA expression in adipose tissue and LRH-1 mRNA expression in skeletal muscle is higher in women than in men ($P < .05$). Adiponectin mRNA expression in gluteal and abdominal adipose tissue did not change significantly after AEX or WL. LRH-1 mRNA expression increased both in adipose tissue and skeletal muscle after AEX ($P < .05$) and the change in muscle LRH-1 was different between the groups ($P < .05$). Adiponectin was positively correlated to LRH-1 in adipose tissue ($P < .001$). The change in maximal oxygen consumption related to the change in LRH-1 mRNA ($r = 0.43$; $P = .01$).

Conclusion: LRH-1, as a nuclear reporter, may activate adiponectin mRNA expression in adipose tissue and increases after AEX.

Key Words: adiponectin, liver receptor homolog-1 (LRH-1), adipose tissue, skeletal muscle, obesity

Abbreviations: AEX, aerobic exercise; BMI, body mass index; cDNA, complementary DNA; FFM, fat free mass; HOMA, homeostatic model assessment of insulin resistance; HRR, heart rate reserve; LRH-1, liver receptor homolog-1; mRNA, messenger RNA; PCR, polymerase chain reaction; VO$_2$max, maximal oxygen consumption; WL, weight loss.

Adiponectin is an anti-inflammatory, antiatherogenic, and insulin-sensitizing adipokine [1]. Lower plasma concentrations and expression levels are reported in obese individuals and adults with type 2 diabetes [2]. Adiponectin activates insulin receptor substrate-1 (IRS-1)–mediated phophatidylinositol-3 kinase (PI-3K) and glucose uptake in skeletal muscle cells, enhances muscle beta-oxidation via the activation of adenosine 5′-monophosphate (AMP)-kinase, and suppresses hepatic glucose production [3]. It also has antiatherogenic effects, suppressing monocyte adhesion to endothelial cells by reducing nuclear factor-κB signaling and the messenger RNA (mRNA) expression of adhesion molecules in endothelial cells [4]. Adiponectin mRNA expression is higher in lean individuals compared to obese individuals [5]. There are also sex differences in circulating levels of adiponectin in humans. Adiponectin levels are significantly higher in women than men [6] and negatively associated with obesity, fasting glucose, and insulin levels and insulin

resistance [6-9]. Adiponectin mRNA expression in epicardial adipose tissue is higher in women than men undergoing some type of heart surgery [10]. A reduced level of high-molecular-weight adiponectin levels is more strongly associated with metabolic syndrome in women than men [11]. In animal models, treatment with testosterone reduces plasma adiponectin, and in adipocyte cell culture, testosterone reduces adiponectin secretion [12], suggesting androgens may play a role in sex differences of adiponectin.

Liver receptor homolog-1 (LRH-1) is a monomeric orphan nuclear receptor expressed in the liver, pancreas (islets, β-cells), intestine, ovary, adrenal glands, preadipocytes, adipose tissue, and skeletal muscle [13, 14]. It is mostly recognized for its role in early development, cholesterol homeostasis, and cancer. LRH-1 has emerged as an upstream regulator of the glucokinase-carbohydrate response element binding protein axis. LRH-1 controls the first step of hepatic glucose uptake through direct transcriptional regulation of

the glucokinase gene. Thus, LRH-1 may play a role in insulin sensitivity.

The LRH response element is located downstream of the peroxisome-proliferator response element in the human adiponectin promoter and in mature adipocytes enhances the transcription of adiponectin [3]. It is interesting to speculate that increasing LRH-1 would lead to the increased transcription of adiponectin, which would improve insulin sensitivity. However, the role of LRH-1 and insulin sensitivity has not been examined. Further, there are no studies that have examined the effect of exercise and diet on LRH-1 response. In contrast, there are several studies that use aerobic exercise training (AEX), weight loss (WL), or both that have shown adiponectin mRNA expression increases [2, 15-17] or does not change [18, 19]. The reasons for the discrepancies among studies is unclear; however, participant characteristics such as age may play a role.

The purpose of this study was to compare adipose tissue adiponectin mRNA expression and adipose and skeletal muscle LRH-1 mRNA expression between older overweight and obese men and women before the interventions and study the effects of 6-month WL and AEX interventions on their expression. We hypothesized that adiponectin and LRH-1 mRNA expression would be related to each other and to insulin sensitivity and that 6-month WL and AEX interventions would increase adiponectin and LRH-1 expression in adipose tissue and skeletal muscle.

Materials and Methods

This study was conducted in men and women with a body mass index (BMI) greater than 25 but less than 50. Additional inclusion criteria were age older than 45 years, women had to be postmenopausal for more than 1 year, and nonsmokers (> 5 years). Any medications including insulin, hypoglycemic agents, anti-inflammatory medications, or hormone replacement therapy were exclusionary. Participants needed to be weight stable (< 2 kg weight change in past year) and sedentary (< 20 minutes of structured aerobic activity) to eliminate the influence of individual differences in dietary composition, physical activity, and weight fluctuations on baseline metabolic variables. This study was conducted according to the guidelines of the Declaration of Helsinki and each study participant provided written consent with methods and procedures approved by the institutional review board at University of Maryland School of Medicine and the VA Research and Development Committee (HP-00040472; January 19, 2010). Interested participants were screened and underwent a physical examination including a comprehensive past medical history, fasting blood profile, and a graded exercise treadmill test.

Testing

Participants underwent the following tests before and after the interventions: whole-body dual-energy x-ray absorptiometry, VO_2max, 3-hour (80 mU.m^2.min^{-1}) hyperinsulinemic-euglycemic clamp, vastus lateralis skeletal muscle biopsy (basal and 120 minutes into clamp), and abdominal and gluteal adipose tissue biopsies. Height (cm) and weight (kg) were measured to calculate BMI. Percentage body fat mass was determined by dual-energy x-ray absorptiometry (iDXA, LUNAR Radiation Corp). VO_2max was measured using a continuous treadmill test protocol [20].

Glucose Clamp and Skeletal Muscle Biopsies

Body weight was measured to ensure that all participants were weight stabilized (± 2%) for at least 2 weeks before metabolic testing before and after the interventions and were provided all meals as a eucaloric diet for 2 days before the clamp by a registered dietitian to control nutrient intake [21]. All testing was performed in the morning after a 12-hour overnight fast and 36 to 48 hours after the last exercise bout for AEX group. Whole-body insulin sensitivity was measured using the hyperinsulinemic-euglycemic clamp technique [22]. Arterialized blood was obtained from a dorsal heated hand vein [23]. Blood samples were obtained every 5 and 10 minutes for the determination of plasma glucose and insulin levels. A 10-minute priming and continuous infusion of insulin (80 mU.m^{-2}.min^{-1} Humulin, Eli Lilly Co) was performed for 180 minutes with a continuous infusion of 20% glucose solution starting at 10 minutes. Blood samples were collected in heparinized syringes, placed in prechilled test tubes containing 1.5 mg EDTA/mL of blood, and centrifuged at 4 °C for plasma glucose and stored at –70 °C until analysis for plasma insulin. Plasma glucose concentrations were measured using the glucose oxidase method (2300 STAT Plus, YSI). Plasma insulin was measured in duplicate by radioimmunoassay (Millipore). The homeostatic model assessment of insulin resistance (HOMA) was calculated by ([fasting insulin [μU/mL] × fasting glucose [mmol/L]])/22.5 [24]. M (glucose utilization) was calculated from the amount of glucose infused after correction for glucose equivalent space (glucose space correction). Insulin levels during the clamp were not statistically different before and after each intervention (WL: 1080 ± 87 vs 1175 ± 47 pmol/L and AEX: 1215 ± 42 vs 1171 ± 60 pmol/L). Before the start of the clamp and 120 minutes during the glucose clamp, a vastus lateralis percutaneous needle muscle biopsy was taken from each participant under local anesthesia using a 5-mm Bergström needle (Stille) for the measurement of LRH-1 mRNA expression. Muscle samples were frozen immediately in clamps cooled in liquid nitrogen and stored at –80 °C until assay.

Adipose Tissue Biopsy

On a separate occasion, participants underwent abdominal and gluteal adipose tissue aspirations. Participants were weight stable (± 2%) at least 2 weeks before baseline testing and maintained the Therapeutic Lifestyle Changes diet throughout the study. Participants were provided with an eucaloric diet for 2 days before the fat aspirations, which were performed after a 12-hour overnight fast and 36 to 48 hours after the last exercise bout for the AEX group. Subcutaneous adipose tissue was aspirated under local anesthesia (0.5% xylocaine) from both the abdominal and gluteal regions using a 10-mm mini-cannula. Adipose tissue was rinsed off for blood with saline and was immediately freeze-clamped and stored at –80 °C until assay.

Interventions

Participants were recruited by group and randomly assigned to WL or AEX. Participants in the WL group maintained the American Heart Association diet and reduced calories by 250 to 350 kcal/day. They attended weekly group WL classes led by a registered dietician for instruction in the principles of hypocaloric diet according to the Therapeutic Lifestyle Changes guidelines [25]. Diets were monitored by 7-day food records (or 24-hour recalls) using the American Diabetes

Association exchange list system. Participants in the AEX group walked on motorized treadmills on which the intensity of exercise began at 50% to 60% of heart rate reserve (HRR) for 30 to 40 minutes during week 1, 55% to 65% HRR for 45 to 50 minutes at week 2, 60% to 65% HRR for 50 minutes at week 3, and 65% to 75% HRR at week 4. By week 5 to 8, they exercised for 50 minutes at approximately 70% to 80% HRR. Each exercise session included a 5- to 10-minute stretching and warmup phase and a 5- to 10-minute cool down phase. HR was monitored during exercise training sessions using HR monitors (Polar Electro Inc).

Laboratory Methods

Approximately 1 g of adipose tissue was used for RNA isolation and adiponectin and LRH-1 gene expression. Approximately 50 to 80 mg of muscle was used for RNA isolation and LRH-1 gene expression. Total RNA was extracted by Trizol (ThermoFisher; catalog No. 15596018) method and RNA concentrations were measured in a spectrophotometer. A total of 1 µg of total RNA for each sample was reverse-transcribed into first-strand complementary DNA (cDNA) using Transcriptor First Strand cDNA Synthesis Kit (04 896 866 001; Roche Applied Science) according to the detailed manufacturer's protocol. The random primer was used as the primer and the RT reaction was performed at 10 minutes at 25 °C and then 55 °C for 30 minutes in a volume of 20 µL. The reaction was inactivated by incubating at 85 °C for 10 minutes and stopped by placing the tube on ice. A reverse-transcription control (master mix without reverse-transcription enzyme) was performed.

Quantitative real-time polymerase chain reaction (PCR) and data analysis for adiponectin and LRH-1 were performed in a LightCycler 480 real-time PCR system with LightCycler 480 software (Roche Applied Science). LightCycler 480 Multiwell plate 384 (04 729 748 001; Roche Applied Science), LightCycler 480 Probes Master kit (04 887 301 001; Roche Applied Science), and TaqMan gene expression primer/probe set (ThermoFisher, adiponectin: assay ID HS00605917-m1, LRH-1: assay ID HS00187067-m1) were used. Each quantitative PCR was performed in a final volume of 10 µL, consisting of 2 µL 1:4 diluted template cDNA, 5 µL LightCycler 480 Probes Master, 0.5 µL TaqMan gene expression primer and probe mix, and 2.5 µL nuclease-free water. Water instead of cDNA served as the no template control. According to the manufacturer's instructions, the quantitative PCR protocol was adopted for all samples after incubation at 95 °C for 10 minutes to activate the DNA polymerase, 45 cycles of 95 °C for 10 seconds, and 60 °C for 30 seconds each were performed to facilitate the PCR. For normalization, 36B4 served as an internal control. Data acquisition occurred in real-time during the annealing/elongation incubation at 60 °C. All samples were amplified in triplicate from the same RNA preparation. Gene expression data were analyzed by Roche LightCycler 480 system software version 1.5 advanced relative quantification program. The average of 3 determinations for each sample and the normalized ratio of target-to-reference were used in statistical analyses. The cDNA synthesis and quantitative Real-Time PCR were performed using our standard laboratory methods [26].

Statistical Analyses

The WL group was directly compared to the AEX group using the *t* test (unpaired) on baseline characteristics, within-group change scores (paired-measures *t* tests), and between-groups change scores on measures between baseline and 6 months. An analysis of covariance of changes in outcomes was also performed covarying for sex. Normality was examined using the Shapiro-Wilk test. Nonparametric tests specifically, Spearman correlation coefficients were used to assess relationships between key variables. Statistical significance was set at a 2-tailed *P* value less than .05. Data were analyzed using SPSS (SPSS Inc); results are expressed as mean ± SEM.

Results

Baseline

The study enrolled 92 overweight and obese men (40%) and women (60%). The physical and metabolic characteristics of the 62 individuals (n = 23 men and n = 39 women) who completed the biopsies at baseline are presented in Table 1. At baseline, there were no significant differences between men and women in age, BMI, fat mass, 120-minute glucose, fasting and 120-minute insulin, and HOMA-IR. Women had higher percentage body fat (*P* < .001) and M (expressed in $\mu mol.kg^{-1}.min^{-1}$; *P* < .05 and expressed in $\mu mol.kg_{FFM}^{-1}.min^{-1}$; *P* < .01) than men. Women had lower fat free mass (FFM) (*P* < .001), VO$_2$max (*P* < .001), and fasting glucose (*P* < .05) than men. At baseline, there was no difference between abdominal and gluteal adipose tissue adiponectin expression (Fig. 1A). Abdominal and gluteal adipose tissue LRH-1 mRNA expression were also not significantly different (Fig. 1B). There were, however, sex differences in that women (n = 39) had higher adiponectin mRNA expression both in abdominal (36%; *P* < .001) and gluteal (23%; *P* < .05) adipose tissue regions compared to men (n = 23) (Fig. 1C). Men had 28% lower abdominal adipose tissue LRH-1 expression than women (*P* < .05) and 15% lower gluteal LRH-1 expression than women (*P* = .05; Fig. 1D). Abdominal adipose tissue adiponectin and abdominal LRH-1 expression were highly correlated (*r* = 0.68; *P* < .001; Fig. 2A). Likewise, there was a correlation between gluteal adipose tissue expression of adiponectin and LRH-1 (*r* = 0.64; *P* < .001; Fig. 2B). Basal skeletal muscle LRH-1 was lower in men than women (Fig. 3; *P* < .05). Relationships between gene expression and metabolic variables at baseline (n = 62) indicate that gluteal adiponectin expression was associated with fasting and 120-minute glucose (*r* = –0.27; *P* < .05 and *r* = –0.28; *P* < .05, respectively). Abdominal adipose adiponectin expression was not associated with fasting or 120-minute glucose. There were no relationships between abdominal and gluteal adiponectin with fasting and 120-minute insulin, VO$_2$max (mL/kg/min), or HOMA-IR. Abdominal adiponectin mRNA was associated with M (expressed in $\mu mol.kg^{-1}.min^{-1}$; *r* = 0.27; *P* < .05 and expressed in $\mu mol.kg_{FFM}^{-1}.min^{-1}$; *r* = 0.30; *P* < .05). Likewise, gluteal adiponectin mRNA was associated with M (expressed in $\mu mol.kg^{-1}.min^{-1}$; *r* = 0.25; *P* = .07 and expressed in $\mu mol.kg_{FFM}^{-1}.min^{-1}$; *r* = 0.30; *P* < .05). However, LRH in abdominal and gluteal adipose tissue and LRH-1 expression in skeletal muscle were not related to fasting and 120-minute glucose or insulin, VO$_2$max, HOMA-IR, or M.

Effects of the Interventions

There were 21 participants who completed WL and 20 who completed AEX. Body composition and fitness results are presented in Table 2. There were between-group differences in the change in body weight, BMI, VO$_2$max, fat mass, and FFM (all

Table 1. Baseline physical and metabolic characteristics of all participants (n = 62) and in men (n = 23) and women (n = 39)

	All (n = 62)	Men (n = 23)	Women (n = 39)
Age, y	61 ± 1	61 ± 2	60 ± 1
Body weight, kg	94.4 ± 2.4	103.0 ± 4.1	89.4 ± 2.7[b]
BMI	33 ± 1	33 ± 1	33 ± 1
VO_2max, L/min	2.2 ± 0.1	2.7 ± 0.1	1.9 ± 0.1[c]
VO_2max, mL/kg/min	23.2 ± 0.6	26.6 ± 0.8	21.1 ± 0.6[c]
VO_2max, $mL/kg_{FFM}/min$	40.4 ± 0.8	41.4 ± 1.1	39.8 ± 1.0
% Body fat	42.4 ± 1.1	35.6 ± 1.3	46.6 ± 1.1[c]
Fat mass, kg	41.4 ± 1.8	37.4 ± 2.6	43.9 ± 2.3
FFM, kg	54.5 ± 1.5	65.3 ± 1.7	47.7 ± 1.1[c]
Fasting glucose, mg/dL	99 ± 2	104 ± 2	96 ± 2[a]
120-min glucose, mg/dL	148 ± 6	162 ± 11	139 ± 8
Fasting insulin, pmol/L	116 ± 9	130 ± 14	107 ± 11
120-min insulin, pmol/L	712 ± 64	792 ± 116	654 ± 71
M, $\mu mol.kg^{-1}.min^{-1}$	27.5 ± 1.6	23.2 ± 2.7	30.4 ± 1.7[a]
M, $\mu mol.kg_{FFM}^{-1}.min^{-1}$	47.1 ± 2.8	34.5 ± 3.6	56.4 ± 3.1[c]
HOMA-IR	4.2 ± 0.3	4.9 ± 0.6	3.7 ± 0.4

Values are mean ± SEM.
Abbreviations: BMI, body mass index; FFM, fat free mass; HOMA-IR, homeostatic model assessment of insulin resistance; VO_2max, maximal oxygen consumption.
Statistically significant different pre and post intervention: [a]$P \le .05$; [b]$P < .01$; and [c]$P < .001$.
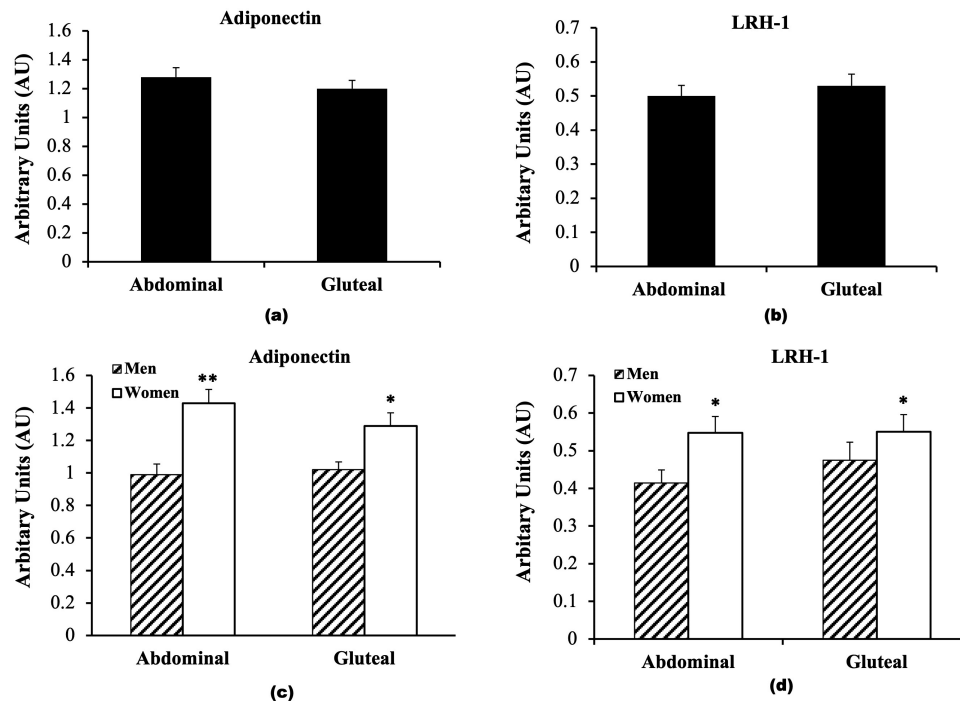

Figure 1. A, Adiponectin, and B, liver receptor homolog-1 (LRH-1) messenger RNA (mRNA) expression in abdominal and gluteal adipose tissue in older adults. C, Abdominal and gluteal adipose tissue adiponectin mRNA expression in men (n = 23, hashed) and women (n = 39, solid white). *$P < .05$; **$P < .001$. D, Abdominal and gluteal adipose tissue LRH-1 mRNA expression in men (n = 23, hashed) and women (n = 39, solid white). *$P < .05$.

$P < .001$). In the WL group, there was a 9% decrease in body weight ($P < .0001$) and an average 3-unit decrease in BMI ($P < .05$). Percentage body fat decreased ($P < .0001$), fat mass decreased ($P < .0001$), and FFM also decreased ($P < .0001$). Percentage body fat significantly decreased in the AEX group ($P < .01$), FFM increased ($P = .05$), fat mass decreased slightly ($P = .09$), and body weight did not change. There was a 12% increase in VO_2 max after AEX ($P < .0001$) with no change in the WL group. Covarying for sex did not change the significance of the body composition and fitness results (Table 3).

The results for glucose metabolism are also presented in Table 2. There were between-group differences in the changes
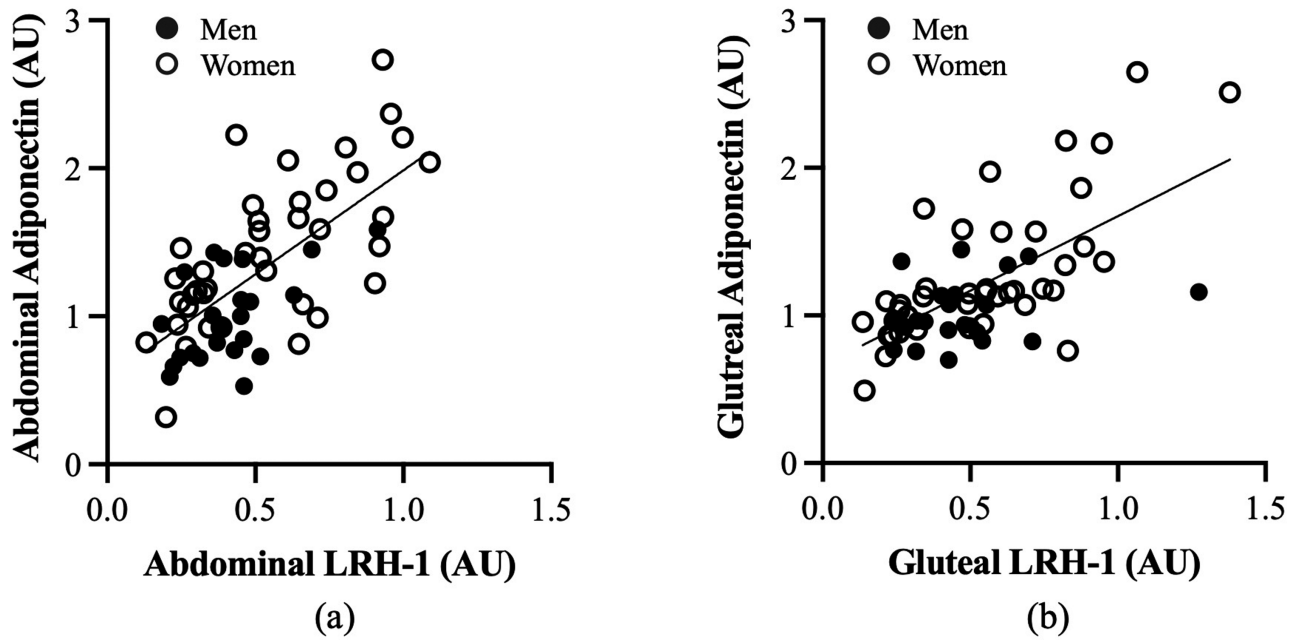
Figure 2. Relationship between adiponectin and liver receptor homolog-1 (LRH-1) expression in A, abdominal adipose tissue ($r = 0.68$; $P < .001$), and B, gluteal adipose tissue ($r = 0.64$; $P < .001$)).

in fasting glucose ($P < .05$) but no other variables of glucose metabolism. With WL, fasting glucose decreased 9% ($P < .0001$), 120-minute glucose decreased ($P < .001$), fasting insulin decreased 22% ($P < .005$), and HOMA-IR decreased. With AEX, fasting insulin decreased 10% ($P = .05$) without significant changes in fasting glucose and in HOMA-IR. M increased when expressed as $\mu mol.kg^{-1}.min^{-1}$ after WL ($P < .0001$) and AEX ($P < .05$) as well as expressed per $\mu mol.kg_{FFM}^{-1}.min^{-1}$ with WL and AEX (both $P < .05$). Covarying for sex did not change the significance of the results (Table 3) except the change in HOMA-IR was significantly different between groups ($P = .04$).

There were no between-group difference in the changes in adipose tissue adiponectin or LRH-1 expression. Adiponectin mRNA expression in the abdominal region did not change significantly (WL: 4.3%; AEX: 7.5%). Likewise, adiponectin mRNA expression in the gluteal region did not change significantly after WL (6.5%) or AEX (4.4%). LRH-1 mRNA expression in abdominal adipose tissue increased after AEX ($P = .05$) but the 12% increase after WL was not statistically significant (Fig. 4C). LRH-1 expression in gluteal adipose tissue tended to increase after AEX ($P = .07$) and did not change after WL (Fig. 4D). There were no significant correlations between changes in abdominal or gluteal adiponectin expression and changes in fasting glucose, insulin, HOMA-IR, or M. Likewise, changes in abdominal and gluteal LRH-1 did not correlate with changes in metabolic variables.

There was a significant group × time interaction in the change in basal LRH-1 ($P < .05$) with an increase after AEX and decrease after WL (Fig. 5). Basal LRH-1 skeletal muscle expression increased after AEX ($P < .05$). There was no change in LRH-1 mRNA expression with insulin stimulation during the clamp in the AEX group (basal vs insulin pre: 18.6 ± 1.3 vs 19.3 ± 1.3 AU; post 22.3 ± 2.0 vs 25.4 ± 2.4 AU) or WL group (basal vs insulin: pre: 20.9 ± 3.0 vs 18.0 ± 1.8 AU; post 16.2 ± 1.4 vs 19.2 ± 1.7 AU). The change in VO$_2$max related to the change in LRH-1 mRNA ($r = 0.43$; $P = .01$). There
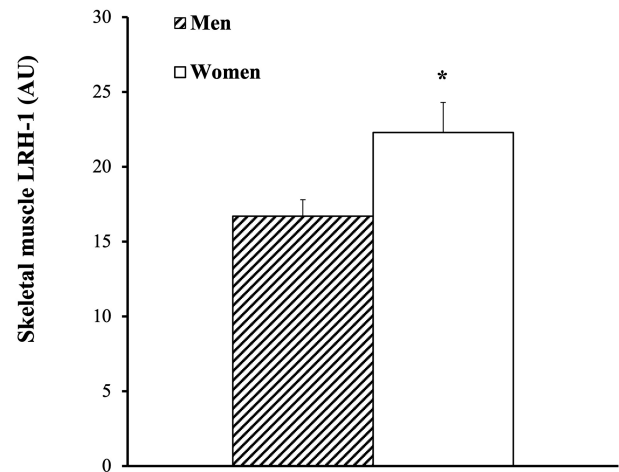

Figure 3. Skeletal muscle liver receptor homolog-1 (LRH-1) messenger RNA expression in men (n = 23, hashed) and women (n = 39, solid white). *$P < .05$.

were no significant relationships between changes in LRH-1 and changes in fasting glucose, insulin, HOMA-IR, or M.

Discussion

The present study indicates that expression of LRH-1 predicts the expression of adiponectin both in abdominal and gluteal human adipose tissue with sex differences in adiponectin and LRH-1 expression. A 6-month AEX program significantly increases abdominal LRH-1 expression and skeletal muscle LRH-1 whereas WL results in a decrease in muscle LRH-1 without changes in abdominal and gluteal adipose tissue LRH-1 expression suggesting differential effects of the interventions on muscle LRH-1. In support of this, improvements in fitness predicted increased skeletal muscle LRH-1 expression. Our results also indicate that WL or AEX did

Table 2. Physical and metabolic characteristics before and after weight loss and aerobic exercise

	WL (n = 21)		AEX (n = 20)	
	Pre	Post	Pre	Post
Age, y	60 ± 1	–	62 ± 2	–
Body weight, kg	102.9 ± 3.9	93.7 ± 3.5[g,a]	92.7 ± 3.6	91.5 ± 4.1
BMI	36 ± 1	33 ± 1[c,a]	32 ± 1	32 ± 1
VO_2max, L/min	2.3 ± 0.1	2.3 ± 0.1[a]	2.4 ± 0.1	2.7 ± 0.1[g]
VO_2max, mL/kg/min	23.0 ± 1.2	24.6 ± 1.3[e,a]	25.1 ± 1.1	29.2 ± 1.3[e]
VO_2max, mL/kg$_{FFM}$/min	40.0 ± 1.0	40.0 ± 1.1	41.7 ± 1.47	44.4 ± 3.0
% Body fat	44.2 ± 1.7	41.2 ± 2.0[g]	38.3 ± 1.8	36.9 ± 2.1[d]
Fat mass, kg	45.7 ± 2.5	38.9 ± 2.4[g,a]	36.3 ± 2.5	35.0 ± 2.9
FFM, kg	57.4 ± 2.8	55.9 ± 2.7[g,a]	57.6 ± 2.4	58.4 ± 2.6[c]
Fasting glucose, mg/dL	101 ± 3	92 ± 2[g,b]	96 ± 2	95 ± 2
120-min glucose, mg/dL	150 ± 10	128 ± 9[e]	131 ± 9	127 ± 9
Fasting insulin, pmol/L	109 ± 11	85 ± 8[f]	100 ± 9	90 ± 9[a]
120-min insulin, pmol/L	685 ± 93	472 ± 57[f]	595 ± 92	505 ± 56
M, μmol.kg^{-1}.min^{-1}	22.1 ± 2.9	31.2 ± 2.8[e]	31.7 ± 2.6	37.3 ± 2.8[c]
M, μmol.kg$_{FFM}$$^{-1}$.min^{-1}	40.8 ± 4.6	55.2 ± 4.7[d]	52.2 ± 4.7	59.6 ± 5.1[c]
HOMA-IR	4.0 ± 0.5	2.8 ± 0.3[e]	3.4 ± 0.3	3.1 ± 0.3

All participants (n = 41) before and after the AEX (n = 20) and WL (n = 21) intervention. Values are mean ± SEM.
Abbreviations: AEX, aerobic exercise; BMI, body mass index; FFM, fat free mass; HOMA-IR, homeostatic model assessment of insulin resistance; VO_2max, maximal oxygen consumption; WL, weight loss.
Statistically significant different pre and post intervention WL vs AEX: [a]$P < .001$; [b]$P < .05$; [c]$P \le .05$; [d]$P < .01$; [e]$P < .001$; [f]$P < .005$; and [g]$P \le .0001$.

Table 3. Changes in physical and metabolic characteristics before and after weight loss and aerobic exercise covarying by sex

Change	WL		AEX		P corrected model	P sex	P arm
	X (SEM)	CI	X (SEM)	CI			
Body weight, kg	−9.51 (0.72)	−11.0 to −8.1	−1.22 (0.75)	−2.74 to −0.30	< .001	.60	< .001
VO_2max, L/min	−0.11 (0.06)	−0.23 to 0.01	0.31 (0.06)	0.18 to 0.43	< .001	.17	< .001
VO_2max, mL/kg/min	0.07 (0.94)	−1.84 to 1.97	3.99 (1.01)	1.94 to 6.04	.008	.19	.008
Fat mass, kg	−6.98 (0.77)	−8.54 to −5.42	−1.29 (0.79)	−2.89 to 0.32	< .001	.86	< .001
FFM, kg	−1.43 (0.37)	−2.18 to −0.69	0.82 (0.39)	0.03 to 1.60	< .001	.42	< .001
Fasting glucose, mg/dL	−8.0 (1.7)	−11.4 to −4.7	−0.9 (1.7)	−4.4 to 2.7	.02	.62	.005
120-min glucose mg/dL	−30.8 (9.6)	−50.3 to −11.4	−2.6 (10.3)	−23.6 to 18.3	.14	.45	.06
Fasting insulin, pmol/L	−25.8 (6.6)	−39.2 to −12.4	−12.4 (6.6)	−25.8 to 1.0	.09	.06	.16
120-min insulin, pmol/L	−191.3 (65.5)	−324.4 to −58.0	−84.2 (65.5)	−217.4 to 49.0	.47	.51	.25
M, μmol.kg^{-1}.min^{-1}	7.2 (2.5)	2.2 to 12.2	5.3 (2.4)	0.4 to 10.2	.62	.47	.58
M, μmol.kg$_{FFM}$$^{-1}$.min^{-1}	13.4 (3.2)	6.8 to 19.9	6.9 (3.1)	0.6 to 13.3	.31	.72	.17
HOMA-IR	−1.4 (0.31)	−2.0 to −0.76	−0.54 (0.31)	−1.16 to 0.09	.04	.04	.06

Abbreviations: AEX, aerobic exercise; BMI, body mass index; FFM, fat free mass; HOMA-IR, homeostatic model assessment of insulin resistance; VO_2max, maximal oxygen consumption; WL, weight loss; X, mean.

not change adiponectin expression in abdominal and gluteal adipose tissue.

Adiponectin, a 29-kDa adipocyte-derived circulating protein, plays a critical role in the maintenance of whole-body homeostasis and metabolism [26, 27]. In visceral fat obesity, the plasma level of adiponectin is lower [28]. WL or AEX increase adiponectin expression, the release in abdominal and gluteal subcutaneous adipose tissue, and circulating adiponectin [16, 29, 30]. Our data show that adiponectin expression was not changed in abdominal and gluteal adipose tissue after AEX intervention, but support findings of sex differences in adiponectin demonstrating that women have higher plasma levels and abdominal adiponectin RNA expression than men [5, 31]. Our data would add that adiponectin RNA expression both in abdominal and gluteal tissue were significantly higher in women than in men and is also consistent with a prior study [17] that there is not a significant difference between abdominal and gluteal adiponectin RNA expression.

Although WL decreased body weight, FFM, and percentage body fat, WL did not change the adiponectin RNA expression in adipose tissue from both abdominal and gluteal regions. We have shown plasma adiponectin levels are negatively related to body fat and positively with insulin sensitivity by the
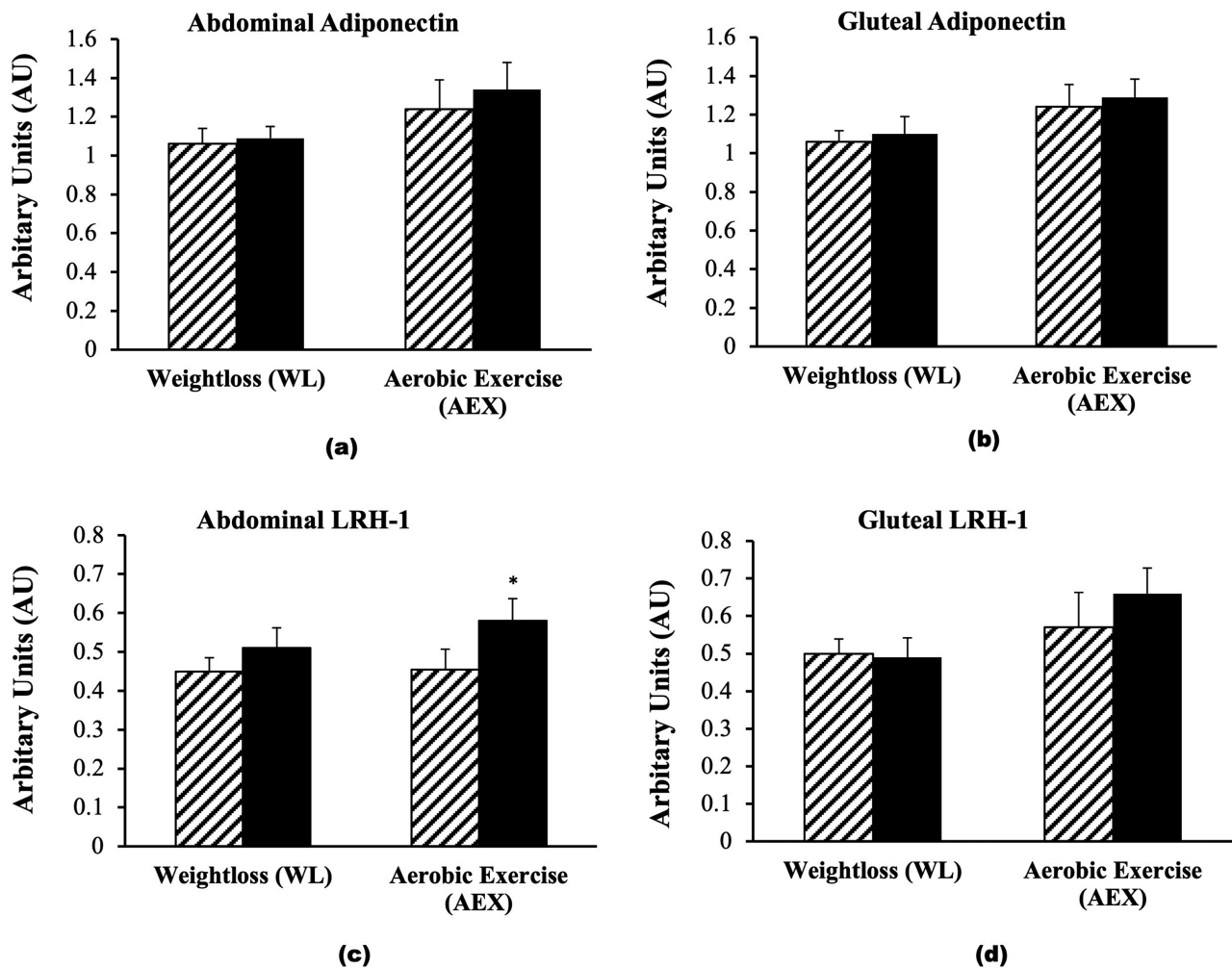
Figure 4. Adipose tissue adiponectin and liver receptor homolog-1 (LRH-1) messenger RNA (A, abdominal adiponectin; and B, gluteal adiponectin; C, abdominal LRH-1; and D, gluteal LRH-1 expression before [hashed] and after weight loss [WL] or aerobic exercise [AEX] [solid black]). *P = .05.
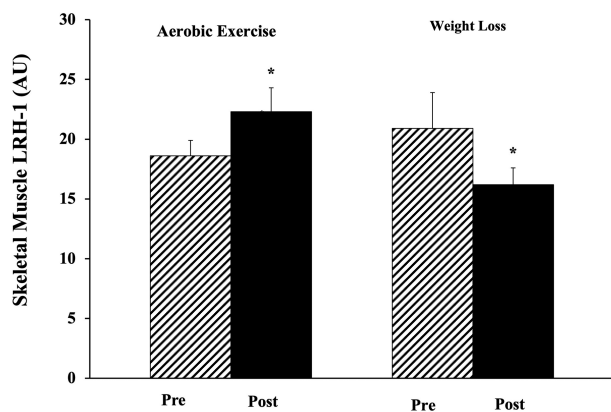

Figure 5. Skeletal muscle liver receptor homolog-1 (LRH-1) messenger RNA expression before (hashed) and after (solid) weight loss (WL) or aerobic exercise (AEX). *P < .05 before vs after AEX and *change between WL vs AEX.

glucose clamp in adult women across a wide range of age and obesity [32]. Furthermore, adiponectin is negatively correlated with truncal fat, both in men and women [33]. It is possible that the amount of WL in this study was not sufficient to induce a significant change in adipose tissue adiponectin gene expression as we have shown that moderate WL did not change circulating adiponectin levels in postmenopausal women [34]. It also may be attributable to whether measures are made in the circulation or tissue, as in our case. Moreover, it is well-known that fat provides the most important form of fuel for aerobic exercise and aerobic sports activities at low to medium intensity exercise (25%-65% of VO_2max) [35]. As AEX increased both VO_2max and LRH-1 mRNA expression in abdominal adipose tissue, it is possible that adipose tissue-derived LRH-1 is involved in the fat utilization during exercise.

LRH-1 is a nuclear receptor that plays an important role in lipid homeostasis and embryogenesis. It is expressed in multiple tissues or organs such as the digestive system, urinary system, and respiratory system. It exists mainly in cells involved in fatty acid/glucose metabolism such as hepatocytes, brown adipocytes, and cardiomyocytes, and neurons involved in the regulation of food intake such as the arcuate nucleus in the hypothalamus and paraventricular nucleus of thalamus [36]. Our data indicate that LRH-1 is expressed both in adipose tissue and muscle. Moreover, we found no significant difference between LRH-1 expressions in abdominal and gluteal adipose tissue. However, we did observe a

significant difference by sex whereby women have significantly higher LRH-1 expression both in adipose tissue and skeletal muscle than men. Iwaki et al [3] reported that LRH-1 is expressed in mature adipocytes and plays a significant role in the transcriptional activation of the adiponectin gene. Our results further show that LRH-1 is positively correlated with adiponectin both in abdominal and gluteal adipose tissues, indicating a relationship between these 2 genes. We are unaware of other studies examining sex differences or the effects of either AEX or WL alone on LRH-1 expression in adipose tissue and skeletal muscle. Since changes in LRH-1 expression were also associated with changes in fitness and LRH-1 increased in skeletal muscle after AEX, it is reasonable to suggest that it acts as a myokine.

Although LRH-1 is involved in hepatic glucose metabolism, we investigated its role in whole-body insulin sensitivity measured by the gold-standard glucose clamp technique. Our results indicate that neither adipose tissue nor skeletal muscle LRH-1 expression were related to fasting glucose or glucose utilization. In IR mouse models, the LRH-1 agonist ligand (dilauroyl phosphatidylcholine) decreases serum glucose and improves glucose homeostasis [37]. In a rodent model with the deletion of liver LRH-1, whole-body glucose infusion rate is not different from control mice but hepatic LRH-1 deficiency does impair the liver's role in glucose metabolism [38]. Our data show AEX significantly improves cardiorespiratory fitness and insulin sensitivity, and increases skeletal muscle LRH-1 RNA expression. This finding indicates that LRH-1 is inducible on AEX training.

We acknowledge a larger sample size in each intervention group might have provided the ability to examine the participants by glucose tolerance status or by sex. Our results did not change after covarying for sex except for changes in HOMA-IR with the interventions. Other limitations are the lack of measurement of circulating total or high-molecular-weight adiponectin and muscle adiponectin expression. To our knowledge, there are no current assays to measure human LRH-1 in plasma. Measurement of protein expression of adiponectin and LRH-1 is another limitation. Future studies could also include the measurement of isotopes to depict changes in liver sensitivity and a no intervention control group. Yet, our well-controlled interventions of long duration, advanced methods for determining insulin action, and measurement of adiponectin and LRH-1 in the specific tissue (adipose and skeletal muscle) are strengths of the study design.

Conclusion

In summary, our study provides evidence that aerobic exercise training of 6 months' duration elicits increases in LRH-1 gene expression both in adipose tissue and skeletal muscle in overweight and obese adults. We suggest that an increase in LRH-1 expression is observed before an increase in adiponectin expression when there are improvements in fitness and that LRH-1 may act as a myokine. Additional studies could aim to examine the adiponectin receptors with lifestyle interventions. Future investigations are also necessary to examine what factors drive changes in tissue LRH-1 with exercise training.

Author Contributions

A.S.R. designed the research, performed the experiments, collected and analyzed the data, and wrote the manuscript; and G.L. performed the experiments and contributed to the writing of the manuscript.

Disclosures

The authors have nothing to disclose.

Data Availability

Data will be provided on reasonable request.

Clinical Trial Information

Clinical trial registration No. NCT00753363 (registered April, 2007).

Conflict of Interest

The authors have no conflict of interest.

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