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Acute insulin response following exercise and its association to lipid changes in sedentary African-American women

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Abstract: Sedentary African-American (AA) women are at increased risk of hypertension, dyslipidemias, metabolic syndrome, and impaired insulin response to exercise. The purpose of the study was to determine the effects of a single bout of aerobic exercise on fasting serum insulin and glucose concentrations following 1464 kJ (350 kcal) of exercise and to determine if this response was associated with serum lipid concentrations in overweight AA women. Premenopausal AA women (n = 11, mean ± SD, age = 32.5 ± 4.8 yr., BMI = 29.8 ± 4.8 kg·m^{-2}, % fat = 35.6 ± 6.3, VO_2peak = 21.5 ± 3.6 ml·kg^{-1}·min^{-1}, total cholesterol = 4.8 ± 0.6 mmol·L^{-1}, triglycerides = 0.60 ± 0.2 mmol·L^{-1}, HDLC = 3.3 ± 0.5 mg·dL^{-1}) performed 1464 kJ (350 kcal) of treadmill exercise at 60%-70%VO_2peak. Fasting plasma insulin and glucose concentrations were collected 24-h prior to, and immediately, 24-h, and 48 h following exercise. Fasting insulin concentration increased immediately following exercise (Baseline=77.1 ± 10.42 vs. Immediately=117.4 ± 15.28 µU·mL^{-1}, 95%CI= 32.71, 47.89; P<0.05). The change in insulin concentration from 24-h pre- to 24-h post-exercise was correlated with BMI (r= 0.51), VO_2peak (r= -0.47), and the change in lipoprotein lipase activity (r=0.37) (*P*<0.05 for all). In conclusion, in sedentary AA women, the insulin response immediately following exercise may be elevated, and is not suppressed below pre-exercise concentrations during the 48-h following exercise. The insulin response 24-h following exercise is modestly associated with markers of lipoprotein metabolism.

Keywords: Aerobic exercise, Blood lipids, Insulin sensitivity, Metabolism, Sedentary

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1. Introduction

Cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality in USA, disproportionately affecting African-American (AA) women [1]. While physical inactivity and consumption of energy-dense foods undoubtedly contribute to CVD, AA women are more likely to be obese, have hypertension and poor insulin sensitivity compared to European-American women [1-4]. These findings are consistent with studies showing that at least 40% of AA women have metabolic syndrome (MetS) [5, 6]. Lipid metabolism plays a significant role in the development of MetS in adults as shown by the elevated triglyceride (TG) and low high-density lipoprotein cholesterol (HDL_C) values.

There is an unresolved debate regarding the acute effect and chronic adaptations of exercise training in reducing cardiometabolic risk factors [7]. Several studies have focused on the effects of exercise on lipid profile, insulin response and blood pressure changes. Indeed, there is evidence describing the chronic adaptations of exercise on lipids [8] and insulin sensitivity [9]; however, the evidence describes the acute effects of exercise on TG, lipoprotein, insulin sensibility, and blood pressure responses [10, 11]. For instance, a study was designed to determine the effect of three consecutive days of 90-min treadmill exercise performed at 60% of participant's maximal aerobic

consumption (VO_2max) on TG, HDL_C, and low-density lipoprotein cholesterol (LDL_C) in overweight and obese [7]. Significant differences were found between exercise sessions on HDL_C and LDL_C, with reductions in TG concentrations 24-h following the third exercise session; therefore, three days of consecutive exercise have a higher effect on increasing HDL_C and lowering TG than a single exercise session.

The acute effect of exercise on TG and lipoprotein subclass concentration have been studied in eight lean women [body mass index (BMI) < 25 $kg \cdot m^{-2}$] [10]. Participants were measured at a basal breakfast, during and following 90-min cycling exercise performed at 50% of their individual peak oxygen consumption (VO_2peak). Both, HDL_C and LDL_C concentrations were significantly increased during exercise despite the reduction of TG concentration. The authors concluded that acute exercise elicited beneficial changes on HDL_C and LDL_C lipoproteins, which are similar to those produced by chronic exercise. There is also evidence for an acute effect of exercise on insulin resistance in seven overweight/obese women [12]. Insulin sensitivity response was compared in participants that performed acute high-intensity continuous exercise (HICE) versus high-intensity interval training exercise (HIIT) [12]. These groups were compared to a non-exercise control. The insulin resistance (IR) was estimated by the homeostasis model assessment (HOMA-IR), and there was a significant reduction in HOMA-IR following HIIT compared to a control condition. Recent meta-analytical evidence confirms that HIIT might produce positive changes in MetS patients [13].

In adults, low levels of physical activity and high caloric diets can lead to obesity and type 2 diabetes, which is associated with IR and increased risk of cardiovascular disease, especially in susceptible populations such as AA women [14]. Acute bouts of exercise positively affect metabolic health by eliciting similar post-training adaptations of chronic exercise such as those reported after 12 weeks of training in obese black women [15]. These adaptations include improvements in the insulin sensitivity, lipid profile and glycemic responses by increasing their ability to shift between carbohydrate and fat as fuel [16].

Fasting insulin concentration is inversely related to lipoprotein lipase activity (LPLa) in men and women [17]. Similarly, IR is inversely related with post-heparin LPLa [18], and this association may be mediated through the down regulation of muscle LPL mRNA noted during insulin infusion [19]. Untrained individuals show a hyperinsulinemic response to a glucose challenge relative to trained individuals [20, 21]. Given the stimulatory effect of aerobic exercise on LPLa [22], the association between fasting insulin and lipid markers should be evaluated following exercise.

Very few studies have determined whether there are differences in lipid profile and acute insulin response to a moderate exercise bout in AA women; therefore, the purpose of the study was to examine the acute response of fasting insulin and glucose concentrations following ~1464 kJ (350 kcal) of exercise performed at 60-70% VO_2peak, and to determine whether this response was related to variables associated to serum lipid concentrations in obese premenopausal AA women.

2. Methods

2.1 Participants

Adult AA female volunteers from East Central Alabama were recruited by posted flyers, advertisements in local newspapers, presentations to civic organizations and churches. All volunteers were initially screened via phone or face-to-face interviews and considered for the study if they were between 25 and 45 years of age, apparently healthy, physically inactive and reporting normal menstrual cycles for the previous 6 months. Volunteers were considered healthy if they reported being free from metabolic disease, diagnosed CVD, were not taking any medication known to affect glucose or lipid metabolism and were free from any contraindication to exercise. Volunteers were characterized as physically inactive if they participated in less than three exercise sessions per week at moderate intensity with a total weekly exercise duration < 90 min [23].

2.2 Preliminary Procedures

On participants initial laboratory visit, they completed the informed consent document and a health history and gynecological questionnaires. A small blood sample at this visit was collected to ensure that the total cholesterol and TG were ≤ 240 $mg \cdot dl^{-1}$ and 200 $mg \cdot dl^{-1}$, respectively. On the second laboratory visit, participant's height, weight, waist and hip circumferences were measured, and body composition (i.e., % body fat) was estimated via seven-site skinfold [24]. All participants underwent a physical examination by a physician before VO_{2peak} and work rate were determined from a standardized treadmill graded exercise test [25]. Throughout the test, heart rate (HR) was monitored continuously by using a 12-lead electrocardiogram, blood pressure was

determined manually, and ratings of perceived exertion (RPE) were obtained during the last 30 seconds of every stage and at maximal exercise. Respiratory gas exchange, oxygen consumption (VO_2) and carbon dioxide (CO_2) production were measured on a breath-by-breath basis and averaged over 30 second intervals using an automated gas analysis system (CPX/D Exercise Stress Testing System, Medical Graphics, Minneapolis, MN). The test was considered a maximal effort if two of the following criteria were met: the achieved maximum HR was within 10 beats·min^{-1} of the individual's age-predicted maximum (HRmax), the respiratory exchange ratio (RER) was ≥ 1.10, the RPE was ≥ 18, or a plateau for VO_2 was achieved despite a further increase in workload [26].

2.3. Experimental Procedures

The experimental exercise session was scheduled to take place during their early follicular phase of each participant's menstrual cycle. Based on their gynecological questionnaire, we estimated approximately when their menstrual cycle will start. Participants were contacted over the telephone and asked to confirm when their menstrual cycle started. Based on that information, they were scheduled to attend the laboratory for the blood draw and exercise session.

The experimental exercise consisted of continuous walking-jogging at 60 to 70% of VO_{2peak}. The work rate and exercise duration needed to expend ~1464 kJ (350 kcal) was estimated from VO_2, RER and kilocaloric equivalent data obtained from the preliminary graded exercise. After a brief warm-up period, the treadmill speed and grade were increased to meet a predicted intensity of 60 to 70% of VO_{2peak} for each participant. Respiratory gas-exchange data and exercise HR were obtained initially and at 15-min intervals throughout the exercise session to verify the participant's exercise intensity and rate of energy expenditure. During the exercise session, the total energy expenditure and remaining exercise time were calculated from the respiratory gas exchange data at each interval, and adjustments were made in either speed or grade to maintain the prescribed exercise intensity.

Blood samples were obtained 24 h before (baseline), immediately post (IPE), 24 h, and 48 h after the experimental exercise session. Participants reported to the laboratory at approximately the same time each morning after a 12 h fast in which water was allowed *ad libitum*. All blood samples were obtained from an antecubital vein using a venous catheter and vacutainer system. First, blood was drawn into two chilled 10 ml vacutainer tubes containing Na-EDTA and immediately placed on ice. Next, 75 IU·kg^{-1} heparin (1000 IU·mL^{-1}) were administered and allowed to circulate for 10 min to release endothelial-bound lipoprotein lipase. A 10 ml post-heparin blood sample were drawn into a chilled vacutainer tube containing Na-heparin. Plasma from pre- and post-heparin blood were isolated by centrifugation at 1500x g for 20 min at 4°C, and 0.01% NaN_3 solution were introduced into all plasma samples (4 μl·mL^{-1} plasma) for preservation. Aliquots of pre- and post-heparin plasma were sealed separately in 2 mL centrifuge tubes and stored at -70°C for later analysis.

2.4. Biochemical Analysis

Before freezing, HDL and HDL_3 subfractions were separated from fresh aliquots of plasma as described elsewhere [27, 28]. Plasma samples were analyzed enzymatically for TC, HDL_C, HDL_{3C}, and TG concentrations. HDL_{2C} were calculated as the difference between HDL_C and HDL_{3C}, and LDL_C were calculated from TC, TG and HDL_C. Insulin was measured by ELISA (Sigma-Aldrich, RAB0327). Total plasma lipase activity and hepatic lipase activity (HLa) were assayed according to standard procedures [24, 29, 30].

Measures of hematocrit and hemoglobin were determined from fresh blood samples and used to estimate changes in plasma volume relative to the baseline blood sample [31]. All plasma lipid concentrations and enzyme activities were then adjusted for the estimated plasma volume changes that occurred over the blood sampling period. The order in which participant plasma samples were analyzed was randomly selected before each biochemical analysis. Inter-assay variation was avoided by analyzing all samples from each participant within a single assay run. Average absorbances in all spectrophotometric analyses were calculated from replicate measurements within an absorbance range of 0.01 nm. When differences between replicate absorbances were > 0.01 nm, all four samples for the subject were reanalyzed. Absorbances for normal and abnormal control plasma were determined at regular intervals throughout each assay run. Replicate measurements of radioactivity within a range of 10% were accepted for enzyme assays. When differences between replicate counts per minute were >10%, all samples for the subject were reanalyzed. Control

samples were included at regular intervals throughout each assay run. Coefficients of variation (CVs) for spectrophotometric and radiolabeled assays were calculated from the control absorbances and counts per minute, respectively, and used as indicators of inter- and intra-assay reliability. Intra-assay CVs, determined from control serum measured multiple times within each assay run were as follows: TC = 1.4%, HDLC = 1.5%, HDL_3C = 2.1%, TG = 1.7%, insulin = 4.2%, total plasma lipase activity = 4.5%, and HLa = 4.2%.

2.5. Physical Activity and Diet Records

Physical activity and diet record keeping procedures were explained to participants. Before undergoing experimental procedures, participants recorded their diet over a three-day period (two weekdays and one weekend day) before data collection. The food records were analyzed for caloric intake and nutrient composition using a commercially available software (Auto-Nutritionist IV, Hearst Corp, San Bruno, CA). Participants were provided with an individualized diet plan based on their three-day dietary report. Participants were asked to adhere to their individualized diet plan and record their dietary intake starting two days before the baseline blood sample and continuing until they completed the experiment. Participants were asked to avoid any strenuous exercise other than the experimental exercise session. Participants recorded their physical activity and diet on specific recording forms and provided this information to laboratory technicians on each laboratory visit during experimental procedures. Self-reported physical activity and dietary records were used to assess the daily variation in each participant over the experimental blood sampling period.

2.6. Statistical Analysis

All data were analyzed using the Statistical Analysis System (SAS for Windows, version 9.3, SAS Institute, Cary, NC). Experimental data were analyzed using a one-way, repeated-measures ANOVA. The lipid-dependent variables of interest were plasma volume-adjusted concentrations of TC, TG, LDL_C, HDL_C, HDL_{2C}, and HDL_{3C} as were insulin, LPLa and HLa. Dietary information was analyzed for daily caloric intake and nutrient composition of fat, carbohydrate, protein, and cholesterol in the same manner.

Table 1. Descriptive characteristics for African-American women (n = 11).

Variable	Mean ± SD
Age (yr.)	32.5 ± 4.8
Body mass (kg)	77.9 ± 15.2
Body height (cm)	164.0 ± 6.5
Body mass index ($kg \cdot m^{-2}$)	29.8 ± 4.8
Body fat mass (%)	35.6 ± 6.3
Waist/Hip ratio	0.8 ± 0.1
VO_2peak ($L \cdot min^{-1}$)	1.6 ± 0.2
VO_2peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	21.5 ± 3.6
Homeostatic model assessment (HOMA) score	2.4 ± 1.2

Note: Values are means ± SD.

Table 2 Mean blood pressure and metabolic variable scores.

Variable	Baseline	Immediately	24-h post	48-h post
SBP (mm Hg)	110.6 ± 8.9	113.0 ± 9.3	112.3 ± 8.6	114.0 ± 8.4
DBP (mm Hg)	73.0 ± 5.0	73.6 ± 9.0	73.6 ± 9.8	75.6 ± 5.1
MAP (mm Hg)	85.5 ± 5.7	86.7 ± 8.2	86.5 ± 8.4	88.4 ± 5.5
TC ($mmol \cdot L^{-1}$)	4.8 ± 0.6	4.8 ± 0.6	5.0 ± 0.3	4.95 ± 0.4
TG ($mmol \cdot L^{-1}$)	0.60 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.52 ± 0.2
LDL_C ($mmol \cdot L^{-1}$)	3.3 ± 0.5	3.2 ± 0.6	3.4 ± 0.6	3.3 ± 0.6
HLa ($\mu mol \cdot FFA \cdot mL^{-1} \cdot h^{-1}$)	22.1 ± 8.8	21.8 ± 9.2	22.6 ± 8.8	23.8 ± 12.7
Glucose ($mmol \cdot L^{-1}$)	4.8 ± 0.3	4.7 ± 0.4	5.0 ± 0.5	4.9 ± 0.4

Note: Values are means ± SD.

Abbreviations: SBP = Systolic blood pressure; DBP = Diastolic blood pressure; MAP = Mean arterial pressure; TC = Total cholesterol; TG = Triglycerides; LDLC = Low-density lipoprotein; HLa = Hepatic lipase activity

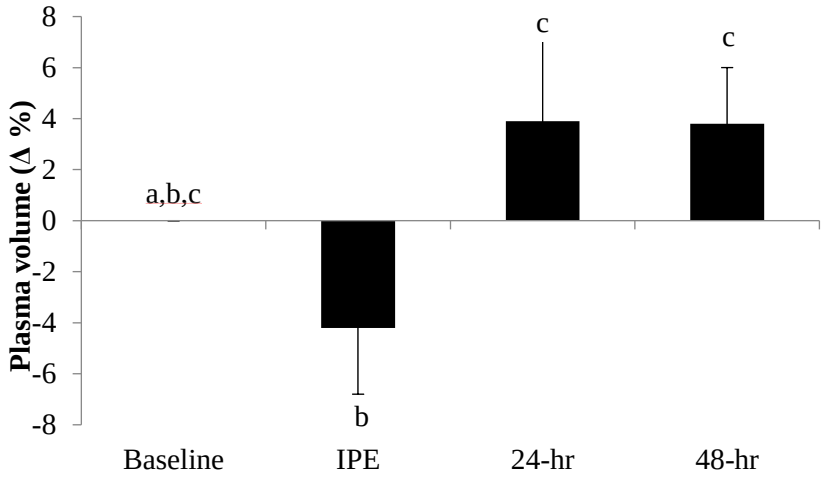

Figure 1. Plasma volume changes in African-American women (n = 11). Bars with different superscripts are significantly different at $P \leq 0.05$. IPE = immediately post-exercise.

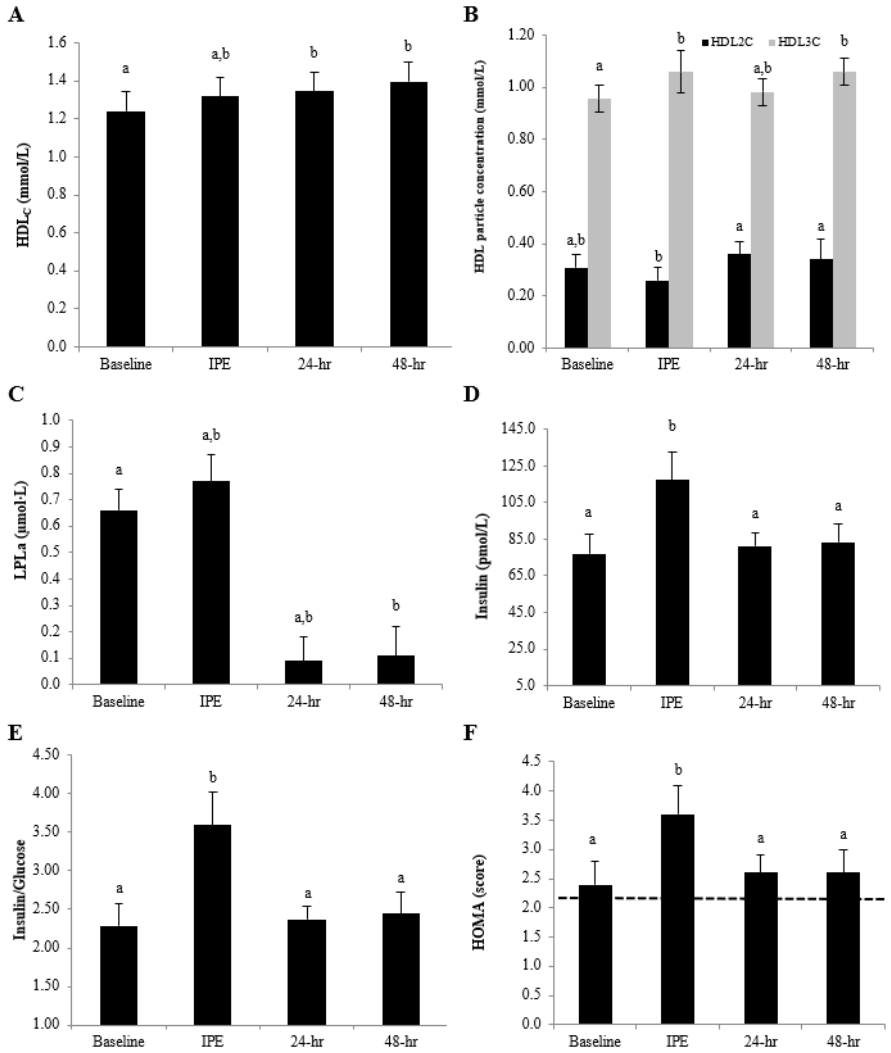

Figure 2. HDLc (panel A), HDL particle (panel B), LPLa (panel C), insulin particle (panel D), Insulin/Glucose ratio concentrations (panel E), and HOMA score (panel F) in African-American women (n = 11). Bars with different superscripts are significantly different at $P \leq 0.05$. IPE = immediately post-exercise. Values above the discontinuous line in HOMA score (panel F) are indicative of elevated cardiometabolic risk (sensitivity = 0.79, specificity = 0.70) [32].

This investigation was exploratory in nature; therefore, the comparison-wise error rate was set at $P < 0.05$. Each test for significance was conducted independently; therefore, it is recognized that the experiment-wise error rate may be somewhat higher than $P < 0.05$. Duncan's New Multiple Range Test was used to determine significant global findings. Relationships among the physiological characteristics, baseline plasma lipid, glucose, insulin, and enzyme values with exercise were determined by using Pearson correlations.

3. Results

Descriptive statistics for the study participants are shown in Table 1. Participants expended a mean of 1458.5 ± 17.2 kJ (348.6 ± 4.1 kcal) during the 64.6 ± 16.8 min exercise session and achieved 140.5 ± 15.6 beats·min^{-1} (~75% age-predicted HRmax) and a RER = 0.95 ± 0.05.

Significant changes were found on plasma volume between baseline and exercise ($P = 0.009$; Fig 1); therefore, immediate and post-exercise mean values were adjusted for plasma volume changes for further statistical analyses. No significant differences were found for mean TC ($P = 0.20$), TG ($P = 0.40$), LDL-$_C$ ($P = 0.26$), HLa ($P = 0.61$), and glucose ($P = 0.17$) concentrations (Table 2).

Significant mean differences were found for HDL_C ($P = 0.013$), HDL_{2C} ($P = 0.046$), HDL_{3C} ($P = 0.024$), LPLa ($P = 0.046$), insulin ($P = 0.010$), and I/G ratio ($P = 0.002$) concentrations, as well as HOMA scores ($P = 0.044$) (Fig 2).

4. Discussion

This study was designed to examine the acute response of fasting insulin and glucose concentrations following ~1464 kJ (350 kcals) of exercise performed at moderate intensity, and to determine whether this response was associated with serum lipid and lipoprotein concentrations in overweight sedentary AA women. The main finding of the study was that plasma insulin concentration increased (~50%) immediately following a single bout of aerobic exercise.

In the present study, plasma glucose concentration did not vary following exercise, suggesting that the elevation of plasma insulin concentration immediately following exercise effectively controlled glucose concentration. The insulin response has been shown to be similar between normal weight and obese subjects following an acute bout of 30-min of continuous submaximal aerobic exercise (75% VO_2max) [25]. Previous meta-analytic evidence has shown a small effect of exercise in improving insulin sensitivity [33]. Our findings in plasma glucose concentration might be explained since acute exercise (~60 min, moderate intensity exercise) enhances insulin sensitivity by increasing insulin-stimulated microvascular perfusion, molecular signaling and glycogen synthase in muscle [26]. Furthermore, a single bout of 40 min moderate intensity aerobic exercise has been found to produce similar GLUT4 translocation responses in skeletal muscle in obese and polycystic ovary syndrome women [29].

In the present study, the change in plasma insulin concentration was positively correlated with BMI. This association suggests that with a greater degree of overweightness, post-exercise insulin concentration increases. AA women with higher BMI than their counterparts of the present study (32.2 vs. 29.8 kg·m^{-2}) who expended more energy during an acute exercise session (425 kcal/75 min vs. ~350 kcal/~60 min), also showed an improvement in insulin response following exercise [34].

The weak, but significant, positive correlation between the change in insulin concentration and the post-exercise change in LPL activity indicates that insulin could have a small influence on lipolytic activity in this sample of AA women. Acute exercise produces gender-specific responses in muscle and adipose tissue LPL activity [35], which are usually higher in men than in women within the first 6-h following exercise. In women, LPL activity has been found to remain unchanged following an acute bout of 90 min of high-intensity aerobic exercise [35]. The inverse association between fasting insulin concentration and LPL activity reported previously may be offset by the stimulatory effect of aerobic exercise on LPL activity. Acute exercise using a similar paradigm (i.e., moderate intensity, energy expenditure ~1500-2500 kJ) in sedentary and obese women has shown equivocal findings in lipoprotein responses [36, 37].

An inverse association between aerobic power and CVD risk was found in two studies on overweight and obese AA women [38, 39], which reinforces the concept that having a sedentary behavior increase the risk of metabolic diseases. AA women might benefit from acute bouts of moderate intensity exercise to reduce and/or control MetS biomarkers associated to cardiovascular disease morbidity and mortality. Indeed, a recent meta-analysis shows that regular (median duration = 12 weeks) aquatic moderate-intensity endurance exercise elicited positive changes in HDL_C, LDL_C, and TC in women [40]. Future interventions

should include different modalities of acute and chronic exercise in this population [41].

5. Conclusion

In conclusion, sedentary AA women had increased insulin responses immediately following exercise of moderate intensity, and the response is unsuppressed below pre-exercise concentrations during the 48-h following exercise. Modest associations between insulin responses 24-h following exercise and markers of lipoprotein metabolism were found. Given that HIIT has not consistently demonstrated a positive effect on insulin and lipid profile in overweight populations, acute moderate intensity exercise might be a positive intervention strategy to improve physical activity and metabolic profile in young AA women.

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Ethics Approval Statement

The study was conducted according to the principles expressed in the Declaration of Helsinki and the study protocol was approved by the Auburn University institutional review board.

Informed Consent

Volunteers who met these requirements were invited to participate in this study.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Author's contribution & Statement

Sofiya Alhassan and Peter W. Grandjean contributed to the study conception and design. Material preparation, data collection and analysis were performed by Sofiya Alhassan, J. Kyle Taylor, Peter W. Grandjean, and José Moncada-Jiménez. The first draft of the manuscript was written by Sofiya Alhassan, Luis M. Gómez-Miranda, Ivan Rentería, José Moncada-Jiménez and Peter W. Grandjean and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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