A single session of aerobic exercise influences paraoxonase 1 activity and concentration

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Abstract. Our purpose was to examine changes in paraoxonase 1 (PON1) concentration and activity following a single aerobic exercise session. Sixteen men (32 ± 8 yrs.; BMI = 29.4 ± 6.8 kg/m²; % fat = 29 ± 13; VO₂max = 38.3 ± 11.9 ml/min·kg⁻¹; waist circumference = 93.7 ± 16.0 cm; HDL-C = 1.19 ± 0.21 and triglycerides = 1.22 ± 1.04 mmol·L⁻¹; direct LDL = 2.69 ± 0.73 mmol·L⁻¹) expended 400 kcaLs by treadmill walking at 65% of VO₂max.

Fasting blood samples were collected before (PRE), immediately post-exercise (IPE), 24 hours post-exercise, and 48 hours post-exercise. PON1 concentration, PON1 activity, lipids, apolipoprotein A1 (Apo A1), apolipoprotein B (Apo B), and thiobarbituric acid reactive substances (TBARS) were analyzed for each time point. The men were divided into two groups based on their body mass index (BMI): Normal weight (NW) and Obese Group. At baseline, PON1 concentration and activity were significantly higher in the NW group as compared to the obese group. In addition, PON1 activity was significantly higher in the NW group as compared to the obese group for all time points. Furthermore, PON1 concentration and activity were significantly increased in the combined group immediately post-exercise and returned to baseline levels within 24 hours. PON1 activity was significantly increased in the Obese group IPE and this was observed with increases in HDLc, Apo A1, and TBARS.

Key words. PON1 activity, Exercise, Lipids, Lipoproteins.

Resumen. Nuestro objetivo fue examinar los cambios en la concentración y actividad de la paraoxonasa 1 (PON1) luego de una sola sesión de ejercicio aeróbico. Dieciséis hombres (32 ± 8 años; IMC = 29.4 ± 6.8 kg/m²; % grasa = 29 ± 13; VO₂max = 38.3 ± 11.9 ml/min·kg⁻¹; círculo de cintura = 93.7 ± 16.0 cm; HDL-C = 1.19 ± 0.21 y triglicéridos = 1.22 ± 1.04 mmol·L⁻¹; LDL directo = 2.69 ± 0.73 mmol·L⁻¹) hicieron ejercicio en una banda sin el 65% del VO₂max hasta gastar 400 kcal. Se recolectaron muestras sanguíneas en ayuno antes (PRE), inmediatamente finalizado el ejercicio (IPE), 24 y 48 horas posteriores al ejercicio. Para cada una de esas muestras, se analizó la concentración y la actividad de PON1, y la concentración de lipidos, apolipoproteína A1 (Apo A1), apolipoproteína B (Apo B), y sustancias reactivas al ácido tiobarbitúrico (TBARS). Los participantes fueron asignados a dos grupos con base en un índice de masa corporal (IMC): grupo de peso normal (NW) y grupo de personas obesas. Los resultados de línea base indicaron que la concentración y actividad de PON1 fueron significativamente mayores en el grupo NW en comparación con el grupo de personas obesas. También se encontró que la actividad de PON1 fue significativamente mayor en el grupo NW en comparación con el grupo de personas obesas en las demás mediciones. Es más, la concentración y actividad de PON1 aumentaron significativamente en ambos grupos combinados inmediatamente luego del ejercicio y regresó a sus niveles basales en 24 horas. La actividad de PON1 aumentó significativamente en el grupo de personas obesas IPE y esto se observó con aumentos en HDLc, Apo A1 y TBARS.

Palabras claves. actividad de PON1, ejercicio, lipidos, lipoproteínas.

Introduction

Over 32% of U.S. adults do not participate in leisure time physical activity and women were reportedly less active than their male counterparts (Go et al., 2013). Physical activity guidelines provided by the American College of Sports Medicine (ACSM) and American Heart Association (AHA) state that adults between the ages of 18-65 years should accumulate at least 30 minutes of moderate-intensity aerobic exercise on 5 days per week (Haskell et al., 2007). This weekly volume has been shown to be the minimum level of physical activity needed to prevent hypokinetic diseases (Haskell et al., 2007). However, according to the ACSM and Centers for Disease Control and Prevention (CDC), nearly half of all adults do not meet the ACSM and AHA guidelines mentioned above («Adult participation in recommended levels of physical activity—United States, 2001 and 2003», 2005; Haskell et al., 2007).

Health benefits of regular exercise include decrease blood pressure (BP), improved insulin sensitivity, improved glucose regulation, decreased body weight, improved antioxidant defenses, and improved dyslipidemias (Dufaux, Order, Muller, & Hollmann, 1986; Durstine et al., 2001; Grandjean, Crouse, & Rohack, 2000; Lesgards et al., 2002; Miller et al., 1979; Nakamura, Uzawa, Maeda, & Inomoto, 1983; Sztok & Laurant, 2011). Aerobic exercise improves lipid profiles by increasing plasma high-density lipoprotein (HDL) concentrations (Dufaux et al., 1986; Durstine et al., 2001; Grandjean et al., 2000; Miller et al., 1979; Nakamura et al., 1983) and lowering plasma TG levels (Grandjean et al., 2000; Kantor, Cultilane, Herbert, & Thompson, 1984). Even though regular exercise has many benefits, an acute bout of exercise generates free radicals leading to increased oxidative stress (Ji, 1995). Oxidation of low-density lipoprotein (LDL) and HDL can be attenuated by an important enzyme, PON1 (Aviram et al., 1998; M. I. Mackness, Arrol, Abbott, & Durrington, 1993; M. I. Mackness & Durrington, 1995; Sorensen et al., 1999; Watson et al., 1995), which protects LDL and HDL by hydrolyzing the oxidized phospholipids formed on LDL and HDL particles (Aviram et al., 1998; M. I. Mackness, Arrol, & Durrington, 1991). PON1 is a calcium-dependent enzyme and is the most abundant of the PON family of enzymes that circulates exclusively with the HDL particle (Aviram & Rosenblat, 2004; Bornna, 1980; Deakin et al., 2002; Khersonsky & Tawfik, 2005; La Du, 1996; Mackness, Durrington, & Mackness, 2004; Primo-Parmo, Sorensen, Teiber, & Da Du, 1996). PON1 was first recognized for the ability to detoxify organophosphate compounds and its name was derived from a commonly used substrate, paraoxon (Aldridge, 1953a, 1953b; Mazur, 1946). Recently, two meta-analyses observed an increased risk of coronary heart disease (CHD) in subjects with low PON1 activity regardless of age (Wang et al., 2012; Zhao et al., 2012).

The limited research determining the influence of a single session of aerobic exercise on PON1 concentration and activity have yielded conflicting results (Benitez et al., 2002; Ibora et al., 2008; Otocka-Kniecik et al., 2010; Tomas et al., 2002). For instance, PON1 activity was significantly higher immediately following a single session of aerobic exercise (Otocka-Kniecik et al., 2010; Tomas et al., 2002). In contrast, PON1 activity was not altered following a single session of aerobic exercise (Benitez et al., 2002; Ibora et al., 2008). In each of the studies above, the investigators did not report concentration of PON1 or caloric expenditure. Increases in PON1 activity have been reported to counter the free radicals produced on lipoproteins associated with exercise (Otocka-Kniecik et al., 2010; Tomas et al., 2002), but exercise may influence the concentration of PON1 leading to changes in activity.
Furthermore, a single session of aerobic exercise is well documented to improve lipoprotein profiles and increases in HDLc have been seen with caloric expenditures as low as 350 kcal (Crouse et al., 1995). Since PON1 is an HDL-associated enzyme, it may be important to examine if caloric expenditure plays a role in altering PON1.

Therefore, the purpose of this study was to examine the changes in the concentration and activity of PON1 following a single session of aerobic exercise on a treadmill expending 400 kcals of energy at an intensity of 60 to 70%VO2max.

Methods

Subjects

Sixteen apparently healthy adult male volunteers participated in this study. They were recruited by email and flyers that were posted around campus. Participants were screened and risk stratified by the use of a health history questionnaire. They were excluded if they were smokers or on lipid-altering medications. The subjects that met inclusion criteria were equally assigned to one of two groups based on their body mass index (BMI). A subject with a BMI of < 25 kg/m² would be assigned to the NW group and a BMI of ≥ 30 kg/m² would be assigned to Obese group. The Auburn University at Montgomery Institutional Review Board approved the protocol and all participants voluntarily signed an informed consent.

Maximal Graded Exercise

Each participant was asked to complete a maximal graded exercise test (GXT) on a treadmill (Trackmaster, Newton, KS). The Bruce Protocol was employed to determine maximal oxygen consumption (VO2max). Expired gas (oxygen and carbon dioxide) fractions were sampled continuously using a pneumotach, mixing chamber, and gas analyzers through a Parvo Medics cart (Sandy, UT). Heart rate and blood pressure was assessed throughout the GXT.

Intervention

A schematic is presented in Figure 1. All participants underwent testing on four separate days. The participants were asked to avoid strenuous activity 24 hours prior to coming into the laboratory. The initial testing (Day 1) included preliminary screening of subjects and collection of anthropometric variables. The participants reported to the Human Performance Laboratory at Auburn University at Montgomery seven days following the initial GXT (Day 2).

Blood Sampling

Blood samples were drawn 10 to 12 hours following an overnight fast. Blood samples were drawn prior to the exercise session (PRE), immediately post-exercise (IPE), 24 hours post-exercise (24), and 48 hours post-exercise (48) under fasting conditions. Blood samples were centrifuged at 3000 rpm for 15 minutes. An aliquot of serum was prepared and placed into a -80°C ultralow freezer until testing.

Laboratory Testing

Lipids and Lipoproteins. Lipid profiles, Apo A1, and Apo B were determined by an automated analyzer in a Siemens ADVIA chemistry platform (Malvern, PA). The coefficient of variation for these variables was less than 2%. PON1 concentration. Enzyme linked immunosorbent assay (ELISA) was used to determine the PON1 concentration in serum. PON1 was determined using commercially available ELISA kit (USCN Life Science Inc.; Wuhan, China). The samples and standards were analyzed in duplicate. A 96 well plate washer and plate reader (Biotek Instruments Inc.; Winooski, VT) was used and programmed according to manufacturers’ instructions. The PON1 concentration was reported in ng/mL. The coefficient of variation was 5.3%.

PON1 activity. PON1 activity was determined by using a commercially available enzymatic kit (Zeptometrax Corporation; Buffalo, NY). The samples and standards were analyzed in duplicate. The absorbance was measured at 270 nm using a Spectronic UV1 spectrophotometer by Thermo Scientific. The PON1 activity was reported in kU/L. The inter-assay and intra-assay variation was 2.3% and 2.6%, respectively.

TBARS. Thiobarbituric acid reactive substances (TBARS) were determined by using a commercially available enzymatic kit (Zeptometrax Corporation; Buffalo, NY). TBARS were used as a marker of lipid peroxidation. The samples and standards were analyzed in duplicate. The absorbance was measured at 532 nm using a Spectronic UV1 spectrophotometer by Thermo Scientific. TBARS are expressed as malondialdehyde equivalents and reported in nmol/mL. The inter-assay and intra-assay variation was 5.9% and 5.1%, respectively.

Lipids, lipoproteins, PON1 activity, PON1 concentration, and TBARS were adjusted for plasma volume changes (Dill & Costill, 1974).

Statistical Analysis

Statistical analysis were performed using IBM® SPSS® version 19, 2010. All measurements were expressed as mean ± standard deviation (SD). The changes in the variables: PRE, IPE, 24, and 48 were compared using a 2 (group) x 4 (sampling points) repeated measures ANOVA. Independent and paired T-tests were performed to analyze differences between groups and sampling points, respectively. Statistical significance was determined as p < 0.05.

Results

The descriptive characteristics of the subjects are presented in Table 1. There were no significant group differences in age and height. Weight, BMI, and waist circumference were significantly lower in the Normal Weight (NW) versus Obese group (p < 0.001, for all). NW group showed significantly higher VO2max values compared to the Obese group (p = 0.01).

Table 1: Baseline Characteristics

| Variable            | Normal Weight (n = 8) | Obese (n = 8) | p  
|---------------------|----------------------|--------------|------
| Age (yr)            | 24.8 ± 8.5           | 29.0 ± 7.9   | 0.170
| Weight (kg)         | 77.8 ± 5.9           | 102.2 ± 13.9 | <0.001
| BMI (kg/m²)         | 25.1 ± 3.9           | 30.9 ± 5.3   | <0.001
| Waist Circumference (cm) | 80.6 ± 9.1        | 106.7 ± 9.5 | <0.001

Values are presented as mean ± standard deviation. BMI, Body Mass Index. Statistical significance (p < 0.05)* between groups.

Table 2: Baseline values for blood lipids and oxidative stress markers.

| Variable            | Normal Weight (n = 8) | Obese (n = 8) | p  
|---------------------|----------------------|--------------|------
| TC                  | 197.4 ± 86.0         | 293.4 ± 103.4| 0.031
| TG                  | 4.7 ± 1.85           | 7.4 ± 6.874  | 0.272
| HDLc                | 57.8 ± 1.71          | 48.2 ± 1.55  | 0.069
| LDLc                | 2.8 ± 0.44           | 4.4 ± 0.42   | 0.002
| Apo A1              | 1.1 ± 0.11           | 1.0 ± 0.15   | 1.602
| Apo B               | 0.8 ± 0.18           | 0.8 ± 0.20   | 0.765
| PON1                | 103.6 ± 28.9         | 160.0 ± 29.0 | 0.003
| PON1c               | 222.4 ± 73.8         | 284.7 ± 70.4 | 0.048
| TBARS (nmol/mL)     | 4.0 ± 1.93           | 5.2 ± 2.13   | 0.224

Values are presented as mean ± standard deviation. TC, total cholesterol; TG, triglycerides; HDLc, HDL-cholesterol; LDLc, low-density lipoprotein; Apo A1, Apolipoprotein A1; Apo B, Apolipoprotein B; HDLc, high-density lipoprotein disassociated; PON1a, Paraoxonase 1 activity; PON1c, Paraoxonase 1 concentration; TBARS, Thioarbituric acid reactive substances. Statistical significance (p < 0.05)* between groups.

Retos, número 27, 2015 (1º semestre)
Lipids, lipoproteins, PON1, and TBARS were measured at baseline for NW and Obese groups and results are presented in Table 2. There were no differences for lipids, lipoproteins, and TBARS at baseline between groups (p > 0.05). However, PON1 concentration and activity were both significantly higher at baseline in the Obese group (p = 0.031 and p = 0.048).

A single session of aerobic exercise on a treadmill expending 400 kcal at an intensity of 60 to 70% VO_2max was performed by the NW and Obese groups. The results for lipids, lipoproteins, PON1, and TBARS are presented in Table 3. Total cholesterol (TC), triglycerides (TG), direct low-density lipoprotein (dLDL), and lipoprotein-associated lipase (LPL) were significantly increased in the combined group, exercise-induced changes HDLc and Apo A1 were not significantly altered following a single session of exercise (p = 0.003) and 24 hours post exercise (p = 0.003) as compared to PRE. The dLDL were significantly increased IPE (p = 0.007) and returned to pre-exercise levels within 24 hours (Figure 4).

PON1 concentration was significantly higher in NW than Obese for all time points (p < 0.05). In the NW group, PON1 concentration was not altered by a single session of exercise immediately post-exercise (Exercise 2) (p < 0.05). This was similar in the Obese group.

In the NW group, PON1 activity was not altered following a single session of exercise immediately post-exercise (Exercise 2) (p < 0.05). This was similar in the Obese group.

Apo A1 was not altered in the NW group following the exercise session. However, in the Obese group Apo A1 were significantly increased IPE as compared to PRE (p = 0.021). In the NW and Obese groups, Apo B were significantly increased IPE as compared to PRE (p = 0.041 and p = 0.049). TBARS were significantly increased in NW (p = 0.026) and Obese groups (p < 0.001) IPE and returned to pre-exercise levels within 24 hours (Exercise 2).

The NW and Obese groups were combined and the data is presented in Table 3. TC, TG, dLDL, and Apo A1 were significantly increased IPE (p = 0.007), but not in the NW group (p > 0.05). HDLc were significantly increased IPE (p = 0.006) in the combined group and returned to pre-exercise levels with 24 hours (Figure 4).

Pearson-Product Moment Correlations were performed to evaluate relationships following exercise-induced changes between PON1 activity, HDLc, Apo A1, and TBARS. In the NW group, HDLc and Apo A1 were significantly correlated with one another following a single session of aerobic exercise (r = 0.917, p = 0.001). In the Obese group, HDLc and Apo A1 were significantly correlated following the exercise session (r = 0.931, p < 0.001). In the Obese group, exercise-induced changes in TBARS were significantly correlated with exercise-induced changes in PON1 activity (r = 0.730, p = 0.040), HDLc (r = 0.799, p = 0.017), and Apo A1 (r = 0.773, p = 0.024). Furthermore, PON1 activity had a positive correlation with HDLc (r = 0.462, p = 0.249) and Apo A1 (r = 0.458, p = 0.254) following the bout of exercise, but they were not significant. In the combined group, exercise-induced changes HDLc and Apo A1 were significantly correlated (r = 0.917, p < 0.001).

**Discussion**

Aerobic exercise is generally prescribed to control weight and improve overall health. There is limited research on the influence of aerobic exercise on PON1 status (concentration and activity) as previously described. Mackness et al. (B. Mackness et al., 2001) suggests that determining the concentration and activity of PON1 are critical in determining the status of PON1. This study examined changes in the concentration and activity of PON1 following a single session of aerobic exercise in NW and Obese men.

**Figure 2. Paraoxonase 1 Activity**

<table>
<thead>
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<th>NW</th>
<th>IPE</th>
<th>24 Post</th>
<th>48 Post</th>
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<td>180</td>
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<td>HDLc (mg/dl)</td>
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<td>80</td>
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<tr>
<td>Apo B (mg/dl)</td>
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<td>30</td>
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**Table 3.**

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<th>IPE</th>
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<th>48 Post</th>
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<td>3</td>
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<tr>
<td>HDLc</td>
<td>mmol/l</td>
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<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Apo A</td>
<td>mmol/l</td>
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<td>2</td>
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<td>4</td>
</tr>
</tbody>
</table>

Results are presented mean ± SD.

**Figure 3. Paraoxonase 1 Activity**

**Figure 4. TBARS Concentration**

Baseline, before exercise; IPE, immediately post-exercise; 24 Post, 24 hours post-exercise; 48 Post, 48 hours post-exercise; Statistical significant (p < 0.05), *Significantly higher in NW as compared to Obese at each time point. † within group significantly higher IPE as compared to PRE. Results are presented mean ± SD.

In the NW group, PON1 activity was not altered following a single session of exercise immediately post-exercise (Exercise 2) (p < 0.05). This was similar in the Obese group.

Apo A1 was not altered in the NW group following the exercise session. However, in the Obese group Apo A1 were significantly increased IPE as compared to PRE (p = 0.021). In the NW and Obese groups, Apo B were significantly increased IPE as compared to PRE (p = 0.041 and p = 0.049). TBARS were significantly increased in NW (p = 0.026) and Obese groups (p < 0.001) IPE and returned to pre-exercise levels within 24 hours (Exercise 2).

The NW and Obese groups were combined and the data is presented in Table 4. TC, TG, dLDL, and Apo A1 were significantly increased IPE (p < 0.001) following a single session of exercise immediately post-exercise as compared to PRE (p = 0.002).

References


MacKinney, M. R., & Orlowska-Majdak, 2013; Otocka-Kmiecik et al., 2010; Tomas, et al. (2002). The first study to examine changes in PON1 activity following a single session of aerobic exercise consuming 400 kals of energy. Apo A1, a key HLD-associated lipoprotein, is not necessary for the attachment of PON1 to HLD or phospholipids (Sorenson, et al., 1999).

However, the stability and activity of PON1 is enhanced in the presence of Apo A1 as compared to without Apo A1 (Sorenson, et al., 1999). Investigators have suggested that Apo A1 is determined along with HDLC in the Obese group. In our study, a single session of exercise significantly increased Apo A1 in the Obese group and, but not in the NW group. Correlations between Apo A1 and PON1 activity exhibited a modest positive association in the Obese group. When the Obese and NW groups were combined the Apo A1 was significantly increased IPE. Our results are similar to investigators that reported an increase in Apo A1 following exercise (Rector, et al., 2007).

TBARS was analyzed to determine lipid peroxidation following a single session of aerobic exercise. In our cohort, TBARS was significantly higher at baseline in the Obese group as compared the NW group. TBARS was significantly increased in the both groups IPE indicating an increase in production of free radicals from the exercise session. The increased TBARS was positively correlated with PON1 activity indicating the activity may be in response to lipid peroxidation. This is consistent with findings from other studies (Otoka-Kmiecik et al., 2010; Tomas, et al., 2002). Additionally, the same was true when the two groups were combined.

In conclusion, PON1 concentration was not altered by a single session of aerobic exercise in either group. However, a single session of aerobic exercise was shown to significantly increase PON1 activity in the Obese group IPE, but not in the NW group. However, our data may suggest that Apo A1 may be a potential mechanism to explain the increased PON1 activity following aerobic exercise. We did not investigate this potential mechanism in this study. Furthermore, accumulating 400 kcal of exercise per day may be effective intervention for individuals that are Obese to reduce weight and provide additional health benefits as well as improve PON1 status.