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The impact of obesity on pentraxin 3 and inflammatory milieu to acute aerobic exercise

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ABSTRACT

Pentraxin 3 (PTX3) has recently been linked to obesity-associated inflammation, serving as a cardioprotective modulator against cardiovascular disease (CVD). Aerobic exercise has been shown to enhance plasma PTX3 levels; however, the impact of obesity on PTX3 response to exercise remains unknown.

Objective. Therefore, this study sought to examine whether obese subjects would have an attenuated plasma PTX3 response compared to normal-weight subjects following acute aerobic exercise. The relationship of plasma PTX3 with pro-inflammatory cytokines (IL-6 and TNF-α) was also examined.

Methods. Twenty healthy subjects (10 obese [4 males and 6 females] and 10 normal-weight [4 males, 6 females]) performed 30 min of continuous submaximal aerobic exercise.

Results. At baseline, obese subjects exhibited approximately 40% lower plasma PTX3 and a 7-fold greater IL-6 concentration compared to normal-weight subjects. In response to exercise, no difference was observed in PTX3 or IL-6 as indicated by area-under-the-curve "with respect to increase" (AUCi) analyses. Furthermore, PTX3 AUCi was positively correlated with cardiorespiratory fitness levels (VO_{2max}) ($r = 0.594$, $p = 0.006$), even after controlling for body mass index.

Conclusion. These findings suggest that in addition to obesity-associated complications, low cardiorespiratory fitness levels could impact exercise-induced PTX3 elevations, thereby potentially diminishing PTX3's effects of anti-inflammation and/or cardioprotection.

1. Introduction

Adult obesity prevalence rates in the United States are 35.7% [1], contributing to the increased risk and pathology of obesity-associated metabolic diseases including type 2 diabetes mellitus and cardiovascular disease (CVD). Obesity is a chronic low grade systemic inflammatory state associated with elevated levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [2]. This increased pro-inflammatory profile has been shown to be the direct result of infiltrating M1, *classically activated*¸ macrophages deriving from the increased migration of blood monocytes into adipose and skeletal tissues under certain inflammatory conditions [3–5].

A novel biomarker, pentraxin 3 (PTX3), primarily released by adipocytes [6], monocytes/macrophages [7,8], and neutrophils [9] has been linked to obesity-associated inflammation. For example, PTX3 has been found in the atherosclerotic plaques in humans, potentially contributing to the pathogenesis of atherosclerosis [8,10,11]. However, decreased plasma PTX3 levels are demonstrated in both obese individuals and patients

Abbreviations: AUC, area-under-the-curve; BMI, body mass index; VO_{2max}, cardiorespiratory fitness; CVD, cardiovascular disease; IL-6, interleukin-6; IL-10, interleukin-10; PTX3, pentraxin 3; TNF-α, tumor necrosis factor-alpha.

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with metabolic syndrome [12,13]. These findings clearly implicate that PTX3 deficiency promotes vascular inflammation, atherosclerosis, and heart damage [14,15].

It has been well established that increased cardiorespiratory fitness provides anti-inflammation and cardioprotection, thereby reducing the risk of CVD [16–19]. Interestingly, plasma levels of PTX3 are elevated in endurance trained individuals compared to controls [20]. Similarly, Nakajima et al. [21] demonstrated that acute moderate and intense aerobic exercise elevates PTX3 concentrations in untrained individuals. Collectively, these findings suggest that exercise-induced PTX3 elevations may play a role in modulation of obesity-associated inflammation and/or the incidence of insulin resistance [22]. To understand the potential effect of exercise on PTX 3 response in obesity, our study sought to examine whether obese subjects would have an attenuated plasma PTX3 response compared to normal-weight subjects. We further examined whether elevated plasma PTX3 would be negatively associated with increased pro-inflammatory cytokines such as IL-6 and TNF-α following acute aerobic exercise.

2. Materials

2.1. Subjects

Twenty healthy subjects (10 obese [4 males and 6 females] and 10 normal-weight [4 males and 6 females]) ages 18 to 30 years were recruited to participate in the study. Subjects with a body mass index (BMI) above 30 kg/m^2 were classified as obese, and those with a BMI between 18.5 and 24.9 kg/m^2 were classified as normal-weight. All subjects completed the informed consent process, a medical history questionnaire, and 7-day physical activity record prior to data collection. The study was approved by the Florida Atlantic University's Institutional Review Board.

Subjects were excluded from the study if they presented inflammatory diseases/conditions (e.g., cardiovascular disease, chronic kidney or liver disease, diabetes), were taking medication known to affect the results, were users of tobacco products (cigarettes, cigars, chewing tobacco), or consumed an average of ten or more alcoholic beverages per week.

Subjects were instructed to undergo an overnight fast for at least eight hours and to abstain from alcohol, caffeine intake, and intense physical activity for at least 24 h prior to each laboratory visit. Finally, women who were pregnant or nursing also were excluded from the study because of the potential effects on immune responses [23].

2.2. Procedures

Subjects were asked to arrive at the laboratory between 7:00 and 9:00 on the morning of the testing session. Session consisted of completion of informed consent, familiarization with all instruments and procedures, anthropometric measures, and an assessment of maximal oxygen consumption (VO_{2max}) test administered in gradation on a treadmill with the intention of reaching maximal exertion within 12 to 15 min. $VO_{2\ max}$ was determined using ParvoMedics Metabolic Measurement System (ParvoMedics, Sandy, UT, USA). HR and rating of perceived exertion (RPE) were recorded every exercise stage. Exhaled carbon dioxide (VCO_2) and inhaled oxygen (VO_2) were averaged every 15 s to calculate respiratory exchange ratio (RER: VCO_2/VO_2). Criteria for attaining $VO_{2\ max}$ included a plateau in O_2 consumption and two of the following secondary criteria: RER ≥1.15, HR within 10 bpm of subject's age-predicted maximum heart rate (220 − age), and an RPE ≥19. HR and blood pressure were assessed by HR monitors (Polar T31, Polar Electro, Kempele, Finland) and sphygmomanometer (752 M-Mobile Series, American Diagnostic, Hauppauge, NY) prior to exercise and during recovery.

The second exercise testing session consisted of 30 min of continuous exercise at 75% VO_{2max} as determined during session one, with HR and BP assessment prior to and immediately post exercise. A 10 ml blood sample was drawn from each subject's anticubital vein prior to, immediately post, and at 1 and 2 h into recovery using a 21G butterfly needle into a tube containing K_2 ethylenediaminetetraacetic acid (K_2EDTA) (BD Vacutainer, Franklin Lakes, NJ). Blood samples were immediately centrifuged at 3000 rpm for 20 min at room temperature. Plasma was collected and immediately stored at −80 °C in cryogenic tubes in 500 μL aliquots for analysis of PTX3 using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) according to manufacture instructions.

2.3. Multiplex protein assay

Plasma concentrations of inflammatory cytokines IL-6 and TNF-α were quantified by high sensitivity multiplex protein assay (Luminex Performance Assay, R&D Systems, Minneapolis, MN) according to manufacturer's instructions. Analyte concentrations were measured using Luminex MAGPIX analyzer (Luminex, Austin, TX, USA). At least 100 beads per analyte per well were counted and concentrations calculated from mean fluorescent intensity using Luminex xPONENT software, Luminex Corporation (Version 4.2).

2.4. Statistical analyses

Data analysis was performed using the SPSS version 21.0. Independent t-tests were conducted to compare baseline levels on all variables between obese and normal-weight subjects. A two group (obese and normal-weight) by four time point (pre, post, R1, R2) repeated measures analysis of variance (ANOVA) was used to examine the effect of acute aerobic exercise on PTX3, IL-6, and TNF-α responses. If the Mauchly's test indicated violation of the sphericity assumption, the degrees of freedom were corrected by using Huynh–Feldt estimates. To assess overall release of PTX3, IL-6, and TNF-α in response to exercise, integrated trapezoidal area-under-the-curve "with respect to ground" (AUCg) was calculated according to the previously published formula by Pruessner et al. [24]. Additionally, to assess the intensity of response relative to baseline, AUC "with respect to increase" (AUCi) was also calculated. The differences in both AUCg and AUCi for PTX3, IL-6, and TNF-α between the two groups were conducted using independent t-tests. Finally, Pearson's correlation was utilized to examine the relationships of PTX3 AUCg and AUCi with BMI, $VO_{2\ max}$, as well as AUCg and AUCi values for IL-6 and TNF-α. All data are presented as means ± S.E.M. with statistical significance being defined as a P-value ≤0.05.