Original Research

Exercise reduced pentraxin 3 levels produced by endotoxin-stimulated human peripheral blood mononuclear cells in obese individuals

Aaron L Slusher[1,2], Yoshimi Shibata[3], Michael Whitehurst[1], Arun Maharaj[1], Justin M Quiles[1] and Chun-Jung Huang[1]

[1]Exercise Biochemistry Laboratory, Department of Exercise Science and Health Promotion, Florida Atlantic University, Boca Raton, FL 33431, USA; [2]Department of Kinesiology and Health Sciences, Virginia Commonwealth University, Richmond, VA 23284, USA; [3]Department of Biomedical Science, College of Medicine, Florida Atlantic University, Boca Raton, FL 33431, USA
Corresponding author: Aaron L Slusher. Email: slusheral@vcu.edu

Impact statement

Our laboratory has previously demonstrated that obese individuals present with lower plasma concentrations of the anti-inflammatory protein pentraxin 3 (PTX3), whereas acute aerobic exercise increases plasma PTX3 levels similarly compared to normal-weight individuals. As a follow-up, the present study demonstrates that PBMCs isolated from obese and normal-weight individuals produce comparable amounts of PTX3 *ex vivo* in response to lipopolysaccharide (LPS). Furthermore, given that acute aerobic exercise reduced the *ex vivo* production of PTX3 in both groups, our results clearly indicate that plasma PTX3 levels are relatively independent of those produced by PBMCs *ex vivo*. In addition, our findings suggest that the mechanisms associated with PTX3-mediated production of the anti-inflammatory cytokine interleukin 10 may be impaired in obese individuals, and thus provides a key finding necessary for the elucidation of PTX3's role in the mediation of anti-inflammatory profiles and the subsequent amelioration of inflammatory disease during obesity.

Abstract

The purpose of this study was to determine whether obesity would reduce the capacity of peripheral blood mononuclear cells (PBMCs) to produce the anti-inflammatory protein pentraxin 3 (PTX3) in response to *ex vivo* stimulation with lipopolysaccharide (LPS), and if acute aerobic exercise would enhance this PTX3 production capacity. In addition, the inter-relationships of LPS-induced PTX3 with the inflammatory cytokines (interleukin 6 [IL-6], IL-10, and tumor necrosis factor alpha) were examined. Twenty-one healthy subjects (10 obese and 11 normal-weight) performed an acute bout of aerobic exercise at 75% VO_{2max}. The capacity of PBMCs to produce PTX3 *ex vivo* following LPS stimulation was the same in obese and normal-weight subjects at rest, and decreased equally in both subject groups following acute aerobic exercise. This is in contrast to plasma PTX3, which is lower in obese subjects at rest and increased equally in both obese and normal-weight subjects following exercise. In addition, *ex vivo* PTX3 production was positively associated with IL-6 and IL-10 in response to acute aerobic exercise ($r = 0.686$, $P = 0.020$; $r = 0.744$, $P = 0.009$, respectively) in normal-weight, but not in obese individuals ($r = 0.429$, $P = 0.249$; $r = 0.453$, $P = 0.189$, respectively). These findings indicate that concentrations of PTX3 observed in plasma are relatively independent of those produced by PBMCs *ex vivo* and the mechanisms associated with PTX3-mediated anti-inflammatory signaling may differ during obesity.

Keywords: Pentraxin 3, aerobic exercise, cardiovascular disease, inflammatory cytokines, monocytes, obesity

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Introduction

Pentraxin 3 (PTX3), an acute phase response protein, plays an anti-inflammatory role in obesity-related inflammation.[1] Rapid increases in plasma PTX3 are reported in endotoxemia.[2] It has also been demonstrated that plasma lipopolysaccharide (LPS) endotoxin levels originated from the gut gram-negative bacteria are increased in obesity,[3] and as a result, LPS generally induces pro-inflammatory mediators as well as the production of the anti-inflammatory protein PTX3 by peripheral blood mononuclear cells (PBMCs).[4,5] More specifically, PTX3 is expressed and released from adipocytes, monocytes, and monocyte-derived macrophages upon cellular activation with LPS and the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α).[1,4,5] During obesity, expression of PTX3 mRNA is increased in visceral adipose tissue.[1,6] To the contrary, circulating concentrations of PTX3 are lower in obese compared to normal-weight individuals,[7,8] indicating that plasma PTX3 levels are

distinct from those produced at local inflammatory sites in obesity.

PTX3 synthesis and production following cellular activation by LPS is mediated by the transcription factors nuclear factor (NF)-κB and activator protein 1 (AP-1).[9–11] Consequently, PTX3 has been shown to serve as a counter-regulatory protein which inhibits pro-inflammatory signaling,[5] protects the hosts from inflammatory damage of the heart (i.e. reduced lesion size within the coronary artery and aorta),[12,13] and improves survival from toxic shock caused by LPS *in vivo*.[14] In addition, the anti-inflammatory cytokine interleukin 10 (IL-10) produced by PTX3-stimulated PBMCs *ex vivo* is considered a key anti-inflammatory mechanism of PTX3.[15] Therefore, increased PTX3 levels may be important to protect against obesity-associated inflammatory diseases, such as cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM).

Importantly, aerobic exercise training has been shown to promote cardiorespiratory fitness and reduce the risk of obesity-associated inflammatory diseases by conferring an anti-inflammatory effect.[16,17] For example, resting levels of plasma PTX3 are elevated in aerobically trained men and women,[18,19] and in obese individuals following aerobic exercise intervention.[20] We have recently demonstrated that acute aerobic exercise enhances plasma PTX3 levels in obese and normal-weight subjects, but obese PTX3 levels are still less than 50% of the normal-weight subject levels.[8] Therefore, the purpose of this study was to determine whether or not obesity would reduce the capacity of PBMCs to produce PTX3 in response to *ex vivo* stimulation with LPS, and if acute aerobic exercise would enhance this PTX3 production capacity. In addition, the inter-relationships of LPS-induced PTX3 with the inflammatory cytokines (IL-6, IL-10 and TNF-α) were examined.

Materials and methods

Subjects

Twenty-one healthy subjects (10 obese [4 males and 6 females] and 11 normal-weight [5 males and 6 females]) 18 to 35 years of age were recruited to participate in the study. Subjects with a body mass index (BMI) above or equal to 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 provided informed consent and completed a medical history questionnaire prior to data collection. Additionally, a seven-day physical activity record was obtained indicating that all subjects participated in less 150 min of physical activity per week prior to their participation in this research investigation. The study was approved by the University's Institutional Review Board.

Subjects diagnosed with inflammatory diseases/conditions (e.g. CVD, chronic kidney or liver disease, diabetes), under the current administration of medication known to alter inflammatory and/or metabolic profiles, who were users of tobacco products (cigarettes, cigars, chewing tobacco), and/or consumed an average of 10 or more standard alcoholic beverages per week were excluded. All subjects were instructed to undergo an overnight fast for at least 8 h 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.[21]

Procedures

Subjects arrived at the laboratory between 7:00 and 9:00 on the morning of two separate testing sessions separated by a minimum of one week. During session one, subjects provided informed consent and were familiarized with all instruments and procedures. Thereupon, anthropometric measures (BMI and waist-to-hip ratio) were obtained and a maximal oxygen consumption (VO_{2max}) test was administered in gradation on a treadmill beginning with a 3-min warm-up at 3 mph with 0% grade. Speed was increased to elicit 80% ± 5 bpm of the subject's age predicted maximal heart rate (HR) within the first 2 min (stage one), and allowed to reach a steady-state during the next 2 min (stage two). After 4 min, grade was increased 2% every 2 min while speed remained constant until the subjects reached voluntary exhaustion within 12 to 15 min. $VO_{2\ max}$ was determined using ParvoMedics Metabolic Measurement System (ParvoMedics, Sandy, UT). HR and rating of perceived exertion (RPE) were recorded during the final 15 s of every exercise stage. Rates of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were assessed and 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 HR (220-age), and an RPE ≥ 19. HR and blood pressure (BP) were assessed by HR monitors (Polar T31, Polar Electro, Kempele, Finland) and sphygmomanometer (752M-Mobile Series, American Diagnostic Corporation, 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 whole blood sample was drawn from each subject's anticubital vein prior to, immediately post, and at 1 and 2 h into recovery (R1h and R2h) using a 21G butterfly needle into a tube containing K_2 ethylenediaminetetraacetic acid (K_2EDTA) (BD Vacutainer, Franklin Lakes, NJ).

Assessments of LPS-induced PTX3 and inflammatory cytokine secretion

LPS-stimulated PTX3 and inflammatory cytokine production by PBMCs *ex vivo* in obesity was assessed by the previously published method.[22] Whole blood samples were immediately centrifuged at 3000 rpm for 20 min at room temperature. The buffy coat was collected and carefully layered over equal volume of Ficoll-Paque ($\rho = 1.077$ g/mL; Sigma-Aldrich, St. Louis, MO) in a conical tube and centrifuged at $400 \times g$ for 30 min at room temperature. The mononuclear cell layer was isolated, and washed with saline three times. PBMC cells at 1.0×10^6 cells/mL were cultured in RPMI 1640 media plus 5% fetal bovine serum

(FBS) and 1% penicillin and streptomycin in a 96-well cultured plate (Corning Incorporated, Corning, NY). Some cultures were stimulated with LPS (10 ng/mL; Phenol extraction from *E. coli*, Sigma-Aldrich). After incubation at 37°C for 24 h, culture supernatant was isolated and analyzed to assess cellular secretion of PTX3, IL-6, IL-10, and TNF-α.

PTX3 (R&D Systems, Minneapolis, MN) and cytokine concentrations (IL-6, IL-10, and TNF-α; Biolegend, Inc. San Diego, CA) were quantified in duplicate by ELISA methods according to manufacturer's instructions. PTX3 and cytokine levels from PBMC cultures without LPS were lower than minimum detection levels, and therefore not included in the analysis.

Statistical analyses

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS version 22.0). Independent *t*-tests were conducted to compare anthropometric and cardiorespiratory profiles, as well as LPS-stimulated PTX3, IL-6, IL-10, and TNF-α concentrations between obese and normal-weight subjects at rest. A two group (obese and normal-weight) by four time point (pre, post, R1h, R2h) repeated measures analysis of variance (ANOVA) was used to examine the effect of acute aerobic exercise on LPS-stimulated PTX3, IL-6, IL-10, and TNF-α responses. If the Mauchly's test indicated violation of the sphericity assumption, the degrees of freedom were corrected by using Greenhouse-Geisser estimates. Furthermore, the percent change (pre to immediately post-exercise) in PTX3, IL-6, IL-10, and TNF-α concentrations were calculated to examine the relationship among these variables and with BMI, waist-to-hip ratio, and relative VO_{2max} by Pearson's correlations. Finally, data on plasma PTX3 concentrations from our current subjects (9 obese and 9 normal-weight only) have been previously published.[8] Thus, this study also examined the relationship of plasma and LPS-stimulated *ex vivo* production of PTX3 with LPS-stimulated *ex vivo* IL-6, IL-10, and TNF-α production at rest and in response to acute exercise. All data are presented as means ± SEM unless otherwise stated with statistical significance being defined as a P value ≤0.05.

Results

Participants' anthropometric and cardiovascular characteristics

Table 1 demonstrates anthropometric characteristics of obese and normal-weight subjects. Obese subjects exhibited significantly greater measures of body weight (t [19] = 5.51, $P < 0.001$), BMI (t [11.42] = 9.98, $P < 0.001$), waist and hip circumferences (t [19] = 7.11; t [11.98] = 7.20, $P < 0.001$, respectively), waist-to-hip ratio (t [19] = 3.32, $P = 0.004$), and resting systolic and diastolic blood pressures (t [19] = 4.10, $P = 0.001$; t [19] = 4.21, $P < 0.001$, respectively). Cardiorespiratory fitness levels (relative VO_{2max}) were significantly lower in obese compared to normal-weight subjects (t [19] = −5.19, $P < 0.001$). Although male subjects weighed more (t [19] = 2.25, $P = 0.037$), were taller (t [19] = 2.386, $P < 0.001$), and presented with greater waist-to-hip ratios (t [19] = 3.45, $P = 0.003$) compared to female subjects, no differences in any other variables, including BMI or VO_{2max}, were observed between male and female subjects.

LPS-induced PTX3 and inflammatory cytokine secretion following acute aerobic exercise

Resting levels of LPS-stimulated PTX3, IL-6, IL-10, and TNF-α are shown in Figure 1. Obese subjects showed an attenuated production of LPS-stimulated IL-10 concentrations compared to normal-weight subjects (t [14.18] = −2.59, $P = 0.021$), while no differences were observed in PTX3, IL-6, and TNF-α concentrations between two groups. In response to acute aerobic exercise, repeated measures ANOVA revealed that LPS-stimulated cellular secretion of PTX3 (F [3, 57] = 6.298, $P = 0.001$), IL-6 (F [2.248, 40.462] = 7.062, $P = 0.002$), and IL-10

Table 1 Participant descriptive characteristics.[a]

Variable	Normal-weight ($n = 11$: 5M/6F)	Obese ($n = 10$: 4M/6F)	P value
Age (y)	23.27 ± 0.68	22.8 ± 1.50	0.77
Weight (kg)	63.42 ± 3.74	100.92 ± 5.84	< 0.001*
Height (m)	1.69 ± 0.04	1.67 ± 0.03	0.59
BMI	21.89 ± 0.49	36.08 ± 1.33	<0.001*
Waist (cm)	71.36 ± 2.17	99.50 ± 3.41	<0.001*
Hip (cm)	94.64 ± 1.26	118.44 ± 3.06	<0.001*
Waist-to-hip ratio	0.75 ± 0.02	0.84 ± 0.02	0.004*
Resting HR (bpm)	68.36 ± 2.19	74.2 ± 3.04	0.131
Resting SBP (mmHg)	110.36 ± 3.04	127.0 ± 2.64	0.001*
Resting DBP (mmHg)	72.00 ± 2.02	82.60 ± 1.43	<0.001*
Relative VO_{2max} (mL/kg/min^{-1})	46.14 ± 2.28	30.61 ± 1.88	<0.001*

BMI: body mass index; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; VO_{2max}: maximal oxygen uptake.
[a]Data are presented as means ± SEM.
*A significant difference between normal-weight and obese groups at baseline ($P < 0.05$).

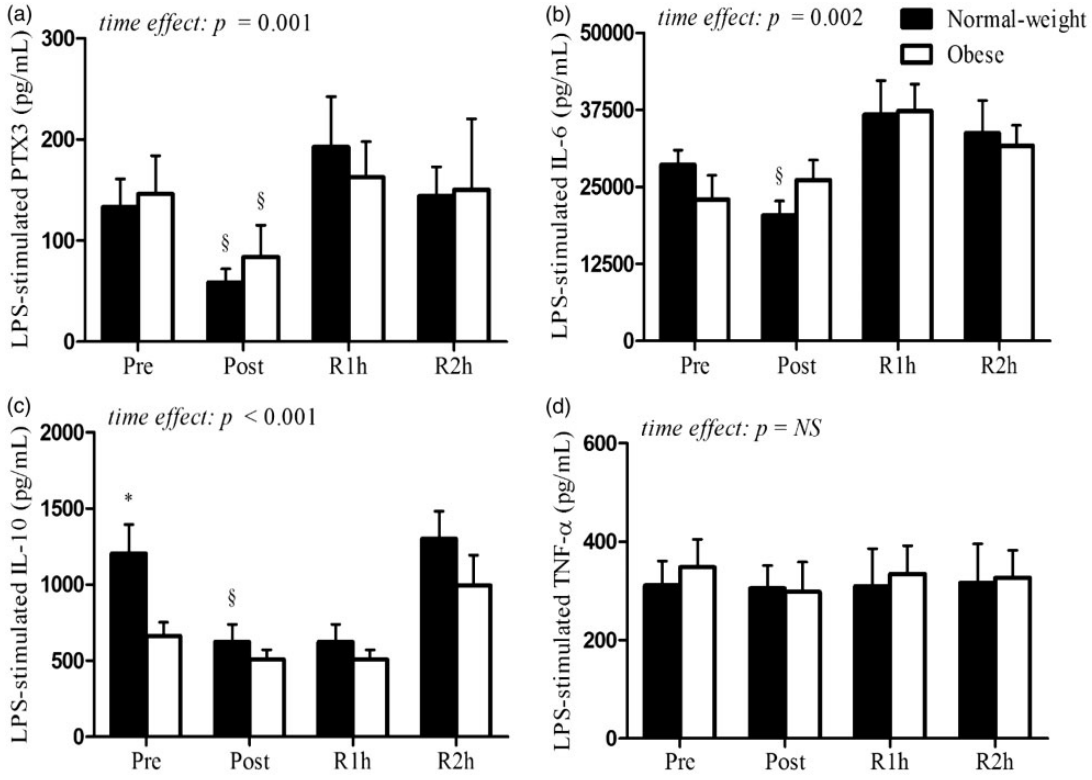

Figure 1 LPS-stimulated PTX3, IL-6, IL-10, and TNF-α concentrations produced *ex vivo* from isolated PBMCs of normal-weight and obese subjects at rest and following acute aerobic exercise. Although no difference in LPS-stimulated PTX3, IL-6, and TNF-α concentrations were observed between obese and normal-weight subjects at rest (panels a, b, and d), LPS-stimulated IL-10 concentrations were attenuated in obese compared to normal-weight subjects (panel c). A significant time effect for LPS-stimulated PTX3, IL-6, and IL-10 (panels a–c), but not TNF-α (panel d), was observed in obese and normal-weight subjects. The * indicates a significant difference between normal-weight and obese groups at rest and following exercise. The § indicates a significant difference compared to resting concentrations ($P < 0.050$). Data are presented as means ± SEM. Pre, prior to exercise; Post, immediately post-exercise; R1h, 1 h into recovery from exercise; R2h, 2 h into recovery from exercise

(F [3, 57] = 9.185, $P < 0.001$), but not TNF-α, was lower in both obese and normal-weight subjects (Figure 1(a)–(d)).

Correlations among variables at rest and following acute aerobic exercise

At rest, LPS-stimulated PTX3 was positively correlated with IL-10 ($r = 0.863$, $P = 0.001$), but not IL-6 or TNF-α in normal-weight, whereas no associations were observed with IL-6, IL-10, or TNF-α following LPS stimulation in obese subjects (Figure 2(a)–(d)). Additionally, waist-to-hip ratio was negatively correlated with resting levels of LPS-stimulated PTX3 and IL-10 ($r = -0.429$ $P = 0.050$; $r = -0.475$, $P = 0.029$, respectively), while BMI was negatively correlated with LPS-stimulated IL-10 ($r = -0.526$, $P = 0.014$) in all subjects.

In response to acute aerobic exercise, LPS-stimulated PTX3 percent change (pre to immediately post-exercise) was positively correlated with IL-6 and IL-10 percent change ($r = 0.686$, $P = 0.020$; $r = 0.744$, $P = 0.009$; Figure 3(a) and (c)) in normal-weight, but not obese ($r = 0.429$, $P = 0.249$; $r = 0.453$, $P = 0.189$; Figure 3(b) and (d)). However, no association was observed between LPS-stimulated TNF-α and other variables. Furthermore, LPS-induced PTX3 concentrations at rest and in response to exercise were neither associated with BMI and relative VO_{2max}. Finally, no correlations were found in plasma and LPS-stimulated *ex vivo* production of PTX3 with LPS-stimulated *ex vivo* IL-6, IL-10, and TNF-α production at rest and in response to exercise.

Discussion

This is the first study to compare the LPS-stimulated *ex vivo* production of PTX3 from PBMCs and its association with pro-inflammatory and anti-inflammatory cytokines in obese and normal-weight individuals following acute aerobic exercise. It is speculated that PBMCs, mainly monocytes,[23] in obesity would be exposed to higher concentrations of plasma LPS than in normal-weight individuals.[3] Thus, although our LPS concentration is higher than actual plasma levels (10 ng/mL vs. ~0.1–0.2 ng/mL),[3] our *ex vivo* LPS stimulation would mimic such an *in vivo* peripheral blood condition. Nonetheless, our important findings demonstrate that LPS-induced PTX3 production levels from PBMCs at rest, which are similar in obese and normal-weight individuals, do not appear to contribute to the reduced plasma PTX3 levels that our laboratory has previously observed in obesity.[8] These results also indicate that the capacity of PBMCs to produce PTX3 *ex vivo* as a counter-regulatory protein following inflammatory challenge is intact in obesity. Furthermore, in contrast to our laboratory's previous finding that acute

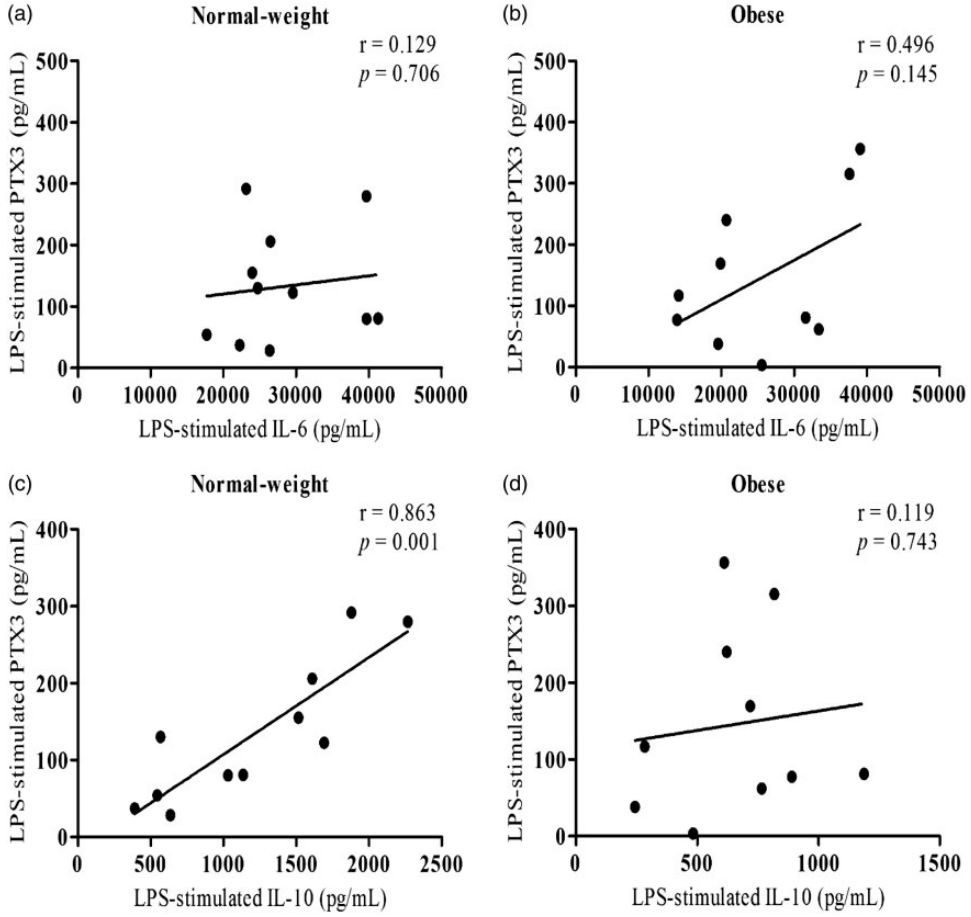

Figure 2 Associations of LPS-stimulated PTX3 with IL-6 and IL-10 concentrations in obese and normal-weight subjects at rest. Although LPS-stimulated PTX3 concentrations were not associated with IL-6 (panel a), a positive association was observed with IL-10 in normal-weight subjects (panel c). LPS-stimulated PTX3 concentrations were not associated with IL-6 or IL-10 in obese subjects at rest (panels b and d) ($P \leq 0.050$).

exercise slightly increases plasma PTX3 levels in both groups,[8] the LPS-stimulated *ex vivo* production of PTX3 in both obese and normal-weight groups was decreased immediately following acute aerobic exercise. Therefore, these results clearly show that the post-exercise plasma PTX3 levels seem to be independent of the reduced *ex vivo* PTX3 production by PBMCs, and may be more dependent upon other cell types, such as neutrophils, which have been shown to store and release PTX3 into circulating during acute aerobic exercise.[24]

The other important finding in our study is that the positive associations of PTX3 with IL-6 and IL-10 observed in normal-weight individuals supports the posit that endogenous PTX3 regulates the balance between pro- and anti-inflammation in normal-weight individuals.[12–14] However, these associations were not observed in obese individuals. Therefore, despite comparable production levels of PTX3, IL-6, and TNF-α by PBMCs at rest, IL-10 production was significantly diminished, suggesting that the endogenous anti-inflammatory mechanisms of PTX3 which enhance IL-10 expression is impaired in obese PBMCs. This is particularly worrisome given that our laboratory and others have recently began to elucidate the mechanisms by which PTX3 preferentially increases IL-10 production from isolated PBMCs in normal-weight individuals, down-regulates the pro-inflammatory signaling cascade, and protects monocyte-derived macrophages from premature cellular death.[5,15,25,26] These findings have provided evidence to suggest that PTX3 aids in the polarization of monocyte-derived macrophages from an M1, pro-inflammatory, to an M2, anti-inflammatory phenotype, and thus, important mechanistic targets that may aid in the prevention in obesity-related pro-inflammatory disease, including CVD and T2DM.

Another possible explanation for the impaired production of IL-10 at rest may be due to the redistribution of monocyte subsets between obese and normal-weight individuals. Monocytes are a heterogeneous population, and the ligation to LPS by the pattern recognition receptor toll-like receptor 4 (TLR4) is facilitated by either classical monocytes ($CD14^+/CD16^-$) or pro-inflammatory monocytes ($CD14^+/CD16^+$) that results in the elevated cellular secretion of IL-6/IL-10, and TNF-α, respectively.[27–29] Although the subset of monocytes responsible for PTX3 production is currently unknown, it is possible that the lower resting levels of LPS-stimulated IL-10 observed in obese subjects is a consequence of reduced proportions of classical relative to pro-inflammatory monocytes in obese individuals.[30–32]

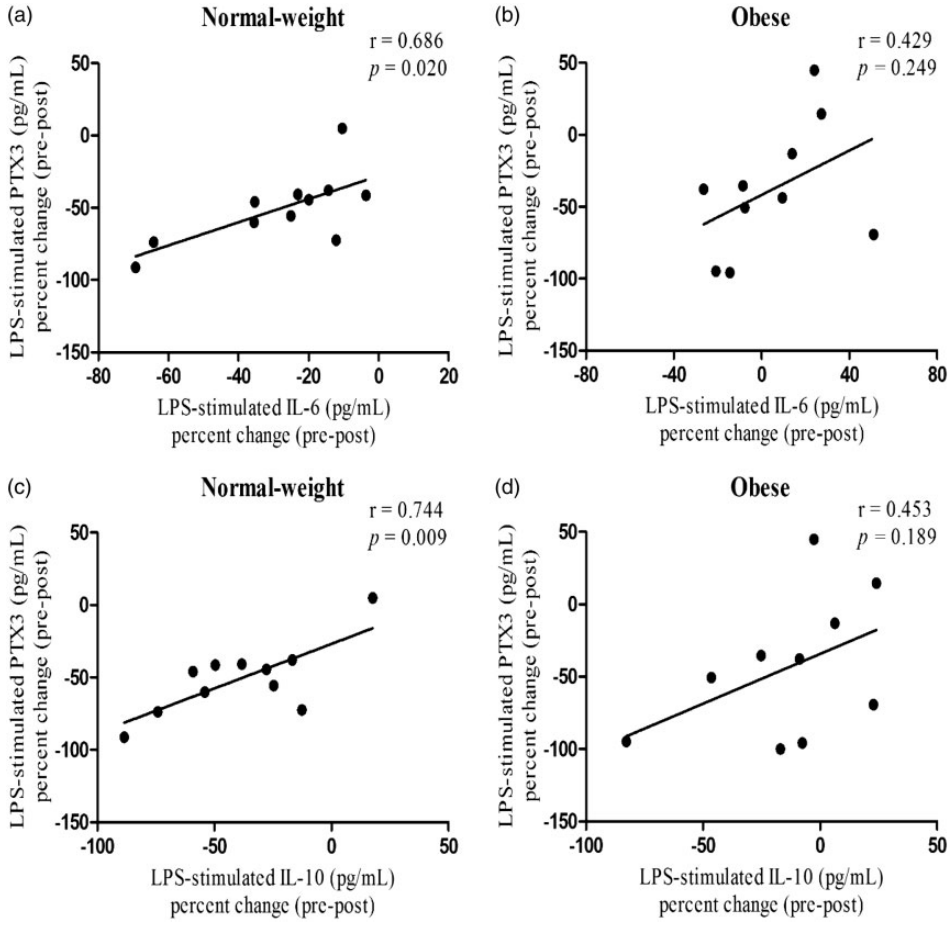

Figure 3 Associations of LPS-stimulated PTX3 percent change (pre–post) with IL-6 and IL-10 percent change following exercise in obese and normal-weight subjects. The LPS-stimulated PTX3 percent change was positively associated with IL-6 and IL-10 percent change in normal-weight (panels a and c), but not obese subjects (panels b and d) ($P \leq 0.050$).

Furthermore, acute aerobic exercise is purported to induce an anti-inflammatory response by decreasing the capacity of circulating monocytes to produce inflammatory proteins.[33–35] While findings from these studies suggest that circulating monocytes may temporarily be in an anergic state (hypo-responsive) that potentially contributes to the reduced capacity of monocytes to produce and secrete PTX3 following LPS stimulation,[36] other studies indicate that the exercise exerts an anti-inflammatory response via the downregulation of TLR4 surface expression on $CD14^+$ monocyte.[17,37] Interestingly, Hong and Mills demonstrated that 20 min of treadmill running at 65–70% VO_{2max} resulted in the preferential mobilization of pro-inflammatory monocytes, and that while TLR4 expression was reduced on classical monocytes, pro-inflammatory monocytes expressed elevated levels of TLR4.[38] Although the percentage of monocytes in the present study are unknown, and therefore the authors cannot verify whether the percentage of monocyte subsets are different among obese and normal-weight individuals at rest or in response to acute aerobic exercise, a recent study has demonstrated that obesity does not influence the distribution of monocyte subsets immediately following acute exercise.[32] Therefore, further studies are necessary to examine the phenotypic changes of monocytes in response to acute aerobic exercise as a mechanism to explain the suppressed production of PTX3 and IL-10 that was observed in both obese and normal-weight groups in the present study.

In conclusion, this study demonstrates that PBMCs isolated from obese and normal-weight individuals produce comparable amounts of PTX3 *ex vivo* in response to LPS. Furthermore, given that acute aerobic exercise reduced the *ex vivo* production of PTX3 in both groups, our results clearly indicate that plasma PTX3 levels are relatively independent of those produced by PBMCs *ex vivo* and further suggest that the mechanisms associated with PTX3-mediated anti-inflammatory signaling is impaired in obese individuals. Therefore, additional research focusing on obesity-related mechanistic consequences related to PTX3 signaling may provide comprehensive knowledge necessary for the elucidation of PTX3's role in the mediation of anti-inflammatory profiles and the subsequent amelioration of inflammatory disease during obesity.[39]

Authors' contributions: All authors participated in the design, interpretation and analysis of the data, and the review of the manuscript. Conception and design: ALS, MW, C-JH. Data collection: ALS, MW, AM, JBQ, C-JH.

Data analysis and interpretation: ALS, YS, C-JH. Manuscript writing: ALS, YS, MW, AM, JMQ, C-JH. Final approval of manuscript: ALS, YS, MW, AM, JMQ, C-JH.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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