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Acute high-intensity interval exercise induces comparable levels of circulating cell-free DNA and Interleukin-6 in obese and normal-weight individuals

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ABSTRACT

Aims: Obesity is associated with lipid aggregation in adipocytes and macrophage infiltration, leading to increased oxidative stress and inflammation. Increased cell-free DNA (cfDNA) concentrations have been observed in clinical conditions of systemic inflammation. While the beneficial effects of regular physical activity on the release of circulating cfDNA still remain unknown, acute intense exercise has been shown to increase inflammatory cytokines and cfDNA concentrations in normal-weight individuals. Therefore, the primary purpose of this study was to examine the effect of acute high-intensity interval Exercise (HIIE) on plasma cfDNA and interleukin-6 (IL-6) responses in obese and normal-weight subjects.

Main methods: Fourteen male subjects (7 obese and 7 normal-weight) participated in an acute HIIE protocol (30 min, 4x4min @ 80% - 90% of VO_{2max}) on a treadmill. Between HIIE intervals, subjects performed 3 min of active recovery at 50–60% VO_{2max}. Blood samples were collected prior to, immediately following exercise, and one hour into recovery for measurements of plasma cfDNA and IL-6.

Key findings: Our results demonstrated a significant elevation in plasma cfDNA immediately following acute HIIE in both obese and normal-weight subjects. A comparable elevation in the concentration of plasma IL-6 was also found between two groups in response to acute HIIE. Furthermore, the level of plasma cfDNA was not correlated with IL-6 either at baseline or in response to acute HIIE.

Significance: These findings may support the utilization of HIIE as a time-efficient exercise protocol to understand the obesity-associated cfDNA and inflammatory responses.

1. Introduction

Obesity is associated with lipid aggregation in adipocytes and macrophage infiltration, leading to the release of numerous pro-inflammatory mediators (e.g., tumor necrosis factor-α [TNF-α], interleukin-6 [IL-6]) [1,13,32,42]. This pro-inflammatory environment contributes to insulin resistance, cardiovascular disease, and atherosclerosis [18,32]. Furthermore, obesity-induced adipose tissue remodeling is associated with adipocyte death [13,15,30,33], resulting in the release of cell-free DNA (cfDNA) [22].

Circulating cfDNA is of pathophysiological relevance as a useful biomarker for patients suffering from various cancers, atherosclerosis, and systemic inflammation [3,9,28,43]. It has been posited that the appearance of cfDNA may be primarily due to a combination of apoptosis and necrosis [27,37,38]. Specifically, the mechanisms of cfDNA release include a unique form of neutrophil-specific cell death, known as neutrophil extracellular traps [11]. Similarly, evidence suggests that blood lymphocytes actively release cfDNA fragments, possibly functioning as an intracellular messenger [2,21,37]. Furthermore, the release of cfDNA may promote macrophage accumulation in adipose tissue via toll-like receptor 9 (TLR9), a sensor to circulating DNA fragments [7,31,35,41]. This finding indicates that obesity-induced adipocyte death and subsequent release of cfDNA may be partially responsible for the observed pro-inflammatory immune activation, via the cfDNA-TLR9 pathway [31].

While the beneficial effects of regular physical activity on the release of circulating cfDNA still remains unknown, increased cfDNA concentrations have been observed in response to various modes of

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acute intense exercise, including short-duration maximal exercise [6], long-duration aerobic exercise [4,8,20], and resistance exercise [5] in healthy normal-weight individuals. The mechanisms of exercise-induced cfDNA response may potentially be due to increased oxidative stress, thereby leading to leukocyte and skeletal muscle cell damage [5,17,29]. Importantly, research has previously shown a greater IL-6 response in obese individuals than normal-weight individuals following acute traditional continuous moderate-intensity exercise [14]. However, high-intensity interval exercise (HIIE) has gained popularity as a time-efficient exercise strategy, offering similar physiological benefits as traditional continuous moderate exercise, especially in patients with cardiometabolic disorders (e.g., obesity and metabolic syndrome) [26]. Therefore, the primary purpose of this study was to examine whether or not acute HIIE could be utilized as a practical model to explore the cfDNA response in obese and normal-weight subjects. It was hypothesized that obese subjects would exhibit a greater expression of plasma cfDNA in conjunction with increased IL-6 following acute HIIE compared to normal-weight subjects. Furthermore, this exercise-mediated cfDNA response would be positively correlated with elevated IL-6.

2. Materials and methods

2.1. Subjects

Fourteen (7 obese and 7 normal-weight) healthy male subjects 18–45 years old were recruited to participate in the study. Subjects with a body mass index (BMI) above 30 kg/m2 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 an informed consent form, a medical history questionnaire, and 7-day physical activity record prior to data collection. The study was approved by the University's Institutional Review Board.

Subjects were excluded from the study if they have any known or suspected cardiovascular, metabolic, rheumatologic, or other inflammatory disease. Subjects were also excluded from the study if they were taking any medication or supplements, users of tobacco products (cigarettes, cigars, chewing tobacco, vapors), or if they consume an average of more than ten alcoholic beverages per week. Subjects were asked to fast overnight for at least eight hours and to abstain from alcohol, caffeine intake, and intense physical activity for at least 24 h prior to each lab visit. To limit the effect of training on physiological responses to acute exercise, those who reported > 150 min of moderate and vigorous physical activity per week were excluded from participation [24].

2.2. Experimental protocol

Two exercise testing sessions comprised the data collection, with a minimum of one week transpiring between each session. Subjects arrived at the Exercise Biochemistry Laboratory between 7:00–7:30 AM. During the first visit, following completion of the informed consent form, medical history questionnaire, and 7-day physical activity questionnaire, height and weight were measured (SECA 769, Chino, CA), along with hip and waist circumference. Additionally, following 20 min of sitting, resting heart rate (HR) was recorded using a HR monitor (Polar T31, Polar Electro, Kempele, Finland) and blood pressure was measured using a sphygmomanometer (752 M-Mobile Series, American Diagnostic Corporation, Hauppauge, NY). Immediately following the assessments above, subjects participated in a graded exercise test (approximately 12 to 15 min) on a treadmill (Norditrack X11i) designed to assess maximal oxygen consumption (VO_{2max}) measured by open-circuit spirometry (ParvoMedics Metabolic Measurement System (ParvoMedics, Sandy, UT, USA) and maximal heart rate (HR_{max}). The maximal exercise protocol began with a three-minute warm-up at 60% age-predicated HR_{max}, followed by an increase in speed until 80% of HR_{max}. Subsequently, the grade was increased by 2% every 2 min until attainment of VO_{2max}. Rating of perceived exertion (RPE, based on the 6–20 Borg scale) and HR were obtained one time during each stage of the VO_{2max} protocol. The validation of VO_{2max} was determined by either the primary criterion of a plateau in VO_2 or two of the three secondary criteria being achieved. The secondary criteria were: [1] reaching predicted HR_{max}, [2] achieving a respiratory exchange ratio of > 1.15, and [3] an RPE ≥ 19.

During the second visit, subjects participated in an acute HIIE following a previously published treadmill protocol [34,39]. To equate the total caloric expenditure between two groups, HIIE consisted of 30-minutes of total exercise, including a 5-min warm-up period of walking/jogging (50–60% of VO_{2max}), followed by 4 intervals of 4 min each at an intensity that elicited 80–90% of VO_{2max}. Between the intervals of HIIE protocol, all subjects performed 3 min of active recovery (walking/jogging) at 50–60% VO_{2max}. Blood collection was performed by a trained phlebotomist prior to (Pre), immediately post (Post), and one hour after the conclusion of acute HIIE (recovery 1 h [R1h]) with a closed IV catheter system (BD Nexiva 20GA, REF 383516, Franklin Lakes, NJ, USA) inserted in the superficial vein of the upper arm. During each blood collection, a 10 mL sample of whole blood was collected into an EDTA coated tube for analysis of plasma IL-6 and cfDNA and centrifuged for 15 min at 2000 x g at 4 °C. All samples were stored at −80 °C for further analyses.

2.3. Measurements of plasma IL-6 and cfDNA

Plasma IL-6 was analyzed in duplicate using high sensitivity enzyme-linked immunosorbent assays (R&D Systems Inc. Minneapolis, MN, USA). cfDNA was extracted from 200 μL of plasma (QIAamp DNA Blood Mini-Kit, Qiagen, Hilden, Germany) and eluted into 50 μL of Qiagen Buffer AE (10 mM Tris-Cl, 0.5 mM EDTA, pH 9.0). The plasma concentration of cfDNA was quantified by real-time PCR (Bio-Rad CFX Connect Real-Time PCR System, Hercules, CA, USA) with the following primers: forward, 5′-AGG TGA ACG TGG ATG AAG TT-3′ and reverse, 5′-AGG GTA GAC CAC CAG CAG CC-3′, using SYBR Green method (iTaq Universal SYBR Green Supermix, Bio-Rad, Hercules, CA, USA). The target sequence was a 189-bp fragment of the β-globin gene. Real-time PCR started with a 2-min denaturation step (95 °C) followed by 40 cycles at 95 °C (5 s) and 60 °C (30s); a melting curve analysis was also included at the end with 95 °C (5 s), 65 °C (5 s), and 95 °C (5 s), with a temperature ramp of 0.5 °C/s. Samples were loaded into a 96-well plate (Bio-Rad, Hercules, CA, USA) and analyzed in duplicate. Each plate included a calibration curve consist of six 4-time serially diluted (26.7 pg/μL to 6.8 ng/μL) human genomic DNA standards (Roche Diagnostics GmbH, Cat. No. 11691112001). DNA values were expressed in pg/uL.

2.4. Statistical analyses

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS version 24.0). Differences between obese and normal-weight groups in baseline variables were conducted by independent *t*-tests. A 2 (group) × 3 (time points: Pre, Post, and R1h) repeated measures analyses of variance (ANOVA) were utilized to examine the effect of acute HIIE on the levels of plasma cfDNA and IL-6. The Greenhouse-Geisser correction of degrees of freedom was used when sphericity assumptions were violated. Significant effects were further analyzed with Bonferroni post hoc comparisons. Furthermore, Pearson's product-moment correlations were used to examine the relationship of plasma cfDNA with IL-6 at baseline and following acute HIIE protocol. Finally, normality of the data was confirmed with a Shapiro-Wilk test. A post-hoc power analysis was conducted using the program G*Power (version 3.1.9.2) for primary outcome measures. Based on the difference of mean and standard deviation (baseline to immediately post-exercise) with an alpha level of 0.05 for plasma cfDNA and IL-6, the overall sample size of 14 subjects in this study

Table 1
Participant anthropometric and metabolic characteristics.

Variable	Obese (N = 7)	Normal-weight (N = 7)	P value
Age (years)	26.0 ± 5.63	22.71 ± 1.60	0.181
Height (cm)	176.14 ± 6.76	179.07 ± 4.80	0.368
Weight (kg)	112.60 ± 17.34	71.51 ± 10.03	* < 0.001
BMI (kg/m^2)	36.09 ± 3.20	22.21 ± 2.0	* < 0.001
HIIE rSBP (mm Hg)	133.47 ± 13.16	114.29 ± 8.04	* 0.006
HIIE rDBP (mm Hg)	81.14 ± 5.52	73.14 ± 6.62	* 0.030
HIIE rHR (BPM)	71.22 ± 5.52	64.71 ± 7.20	0.082
VO_{2max} (mL/kg/min)	38.17 ± 5.48	50.51 ± 5.46	* 0.001
Waist circumference (cm)	108.89 ± 12.91	80.04 ± 7.35	* < 0.001
Hip circumference (cm)	115.79 ± 6.73	95.50 ± 5.10	* < 0.001
WHR	0.94 ± 0.09	0.84 ± 0.06	* 0.027

The * indicates the difference between obese and normal-weight groups. Data are presented as means ± standard deviation (SD). BMI = Body Mass Index; rSBP = Resting Systolic Blood Pressure; rDBP = Resting Diastolic Blood Pressure; rHR = Resting Heart Rate; VO_{2max} = Maximal Oxygen Consumption; WHR = Waist/Hip Ratio.

achieved an adequate power (> 80%) for all outcome variables. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Anthropometric and metabolic measurements of the study participants

Baseline anthropometric and metabolic characteristics of obese and normal-weight participants are reported in Table 1. Differences between obese and normal-weight groups at baseline were statistically significant for weight, BMI, relative VO_{2max}, systolic and diastolic blood pressures, HR, and waist/hip circumferences and ratio. HR, respiratory exchange ratio, oxygen consumption during acute HIIE were recorded (Figs. 1-3).

3.2. Measurement of plasma cfDNA and IL-6

At baseline, our analysis did not observe a difference in plasma level of cfDNA between obese and normal-weight groups, whereas obese subjects exhibited a significantly higher level of plasma IL-6 compared to normal-weight subjects (P = 0.004). Furthermore, repeated measures ANOVA demonstrated a significant increase in the level of plasma cfDNA immediately following acute HIIE in both groups [F [2, 24] = 3.470, P = 0.047] (Fig. 4). A comparable elevation in the concentration of plasma IL-6 was also found between obese and normal-weight subjects in response to acute HIIE protocol [F [2, 24] = 9.213, P = 0.001] (Fig. 5). Finally, the plasma level of cfDNA was not correlated with IL-6 either at baseline (obese group: r = 0.590, P = 0.163; normal-weight group: r = 0.064, P = 0.892) or in response to acute HIIE (percent change [baseline to immediately following exercise]) (obese group: r = −0.227, P = 0.625; normal-weight group: r = 0.524, P = 0.286).

4. Discussion

This study examined the effect of acute HIIE on plasma cfDNA and IL-6 responses in obese and normal-weight subjects. Our results rejected the hypothesis, showing that both groups increased cfDNA concentrations to a similar extent following acute HIIE. Furthermore, the tendency of increased IL-6 following acute HIIE was similar to the cfDNA response, with no difference between obese and normal-weight subjects. To the best of our knowledge, this study is the first to examine the modulatory role of obesity on exercise-induced cfDNA release and to utilize an acute HIIE protocol as a practical model to examine the phenomena of cfDNA release in both obese and normal-weight subjects. These findings may support the utilization of HIIE as a time-efficient exercise protocol to understand the obesity-associated cfDNA and inflammatory responses.

This study observed a significantly greater baseline level of plasma IL-6 in obese versus normal-weight individuals; however, this baseline difference was not mirrored in cfDNA concentrations. These results are in contrast to previous literature demonstrating a positive correlation between BMI and levels of circulating cfDNA [22]. One possible explanation for the absence of significance in baseline cfDNA concentrations between the two groups may be attributed to the relatively low sample size. Nevertheless, increased cfDNA concentrations have been observed in healthy normal-weight subjects in response to various modes of exercise, including acute aerobic exercise [8]. The literature has proposed several mechanisms of exercise-mediated cfDNA release, including apoptosis of myocytes and leukocytes in response to increased oxidative and mechanical stress [10]. Atamaniuk et al. [4] have further demonstrated that the source of exercise-mediated cfDNA is due to apoptosis of mononuclear cells while postulating that oxidative stress and cytokine responses may also originate from damaged skeletal muscle cells following exercise. Importantly, obesity has been associated with increased oxidative stress and immunological modulation [25,40], which is suggestive of increased proneness to exercise-induced oxidative stress and cfDNA release. However, the results of this study demonstrated a similar level of cfDNA concentrations following acute HIIE in both obese and normal-weight subjects. Although increased circulating cfDNA may play an upstream role in the obesity-associated inflammatory environment via activation of TLR9 in adipocyte macrophages [31], our findings indicate that HIIE may be an effective exercise strategy for improving health outcomes, without exacerbating the obesity-associated inflammatory state. Nonetheless, caution should

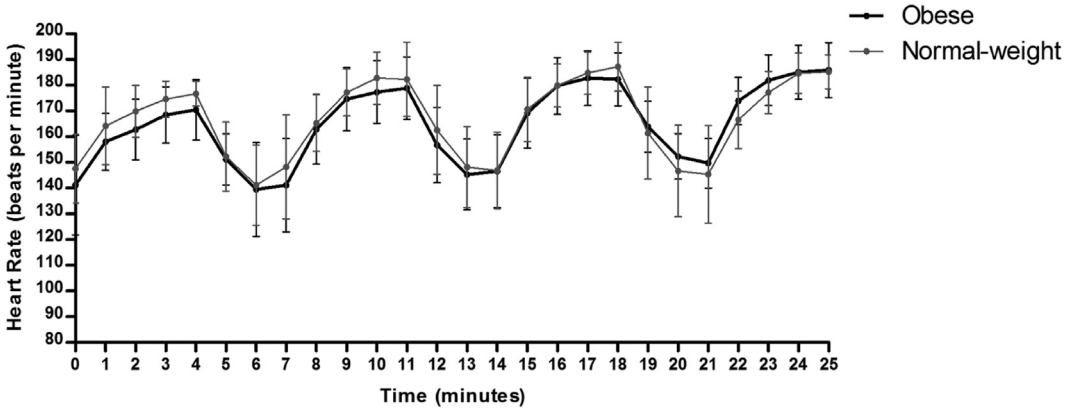

Fig. 1. Heart rate during acute HIIE in obese and normal-weight subjects. Data are presented as means ± SD.

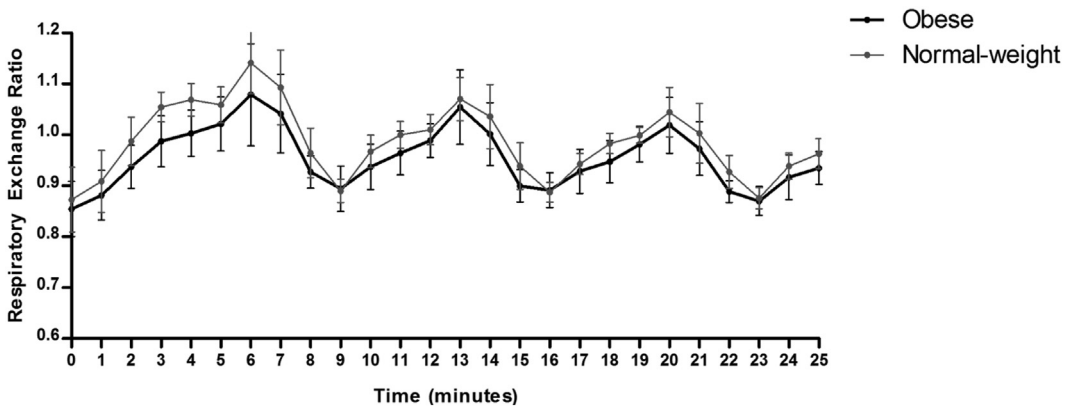

Fig. 2. Respiratory Exchange Ratio during acute HIIE in obese and normal-weight subjects. Data are presented as means ± SD.

be used when interpreting the utility of our findings to promote HIIE as a time-efficient strategy to reduce inflammation in obese individuals as additional markers (IL-10, IL-8, and others) showing a similar response as cfDNA to HIIE would assist in confirming this interpretation.

Recent research has demonstrated that acute HIIE increased the circulating level of IL-6 in healthy normal-weight subjects [12,16]. To elucidate exercise-mediated inflammation in obesity, the current results showed that obese subjects exhibited a similar increase in IL-6 compared to normal-weight subjects following acute HIIE. This result is in agreement with a recent study by Dorneles et al. [19], demonstrating a comparable acute HIIE-induced IL-6 response between obese and normal-weight individuals. Importantly, the HIIE protocol utilized in the present study differs from that of Dorneles et al. [19] with regards to work-to-rest ratio. For example, the present study utilized 4 × 4 min (80–90%VO_{2max}) interspersed with 3 min of recovery (50–60% VO_{2max}), while Dorneles et al. (2016) utilized 10 × 60 *sec* (85–90% P_{max}) interspersed with 75 s of recovery (50%P_{max}). Although acute HIIE protocols utilized differing work-to-rest ratios did not alter the inflammatory response in healthy normal-weight subjects [23], the literature has yet to indicate whether the relative importance of work-to-rest ratio is similar in obese individuals. Additionally, Atamaniuk and colleagues [3] demonstrated that increased cfDNA is associated with the production of IL-6 in human monocytes; however, a relationship with IL-6 was not observed following acute HIIE in the present study. Thus, future research should aim to elucidate the impact of varying HIIE work-to-rest ratios on obesity-associated inflammation, including the potential effects of cfDNA to determine a uniquely optimal protocol for obese populations.

One limitation of this study is that the source of the cfDNA and IL-6 responses may, in large part, be skeletal muscle; however, genes associated with the skeletal muscle response were not currently

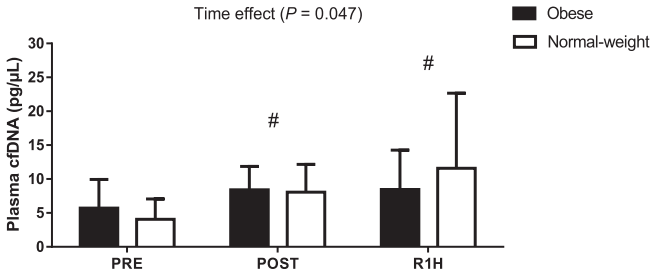

Fig. 4. The expression of plasma cfDNA following acute HIIE in obese and normal-weight subjects. The concentration of plasma cfDNA significantly increased flowing acute HIIE in both obese and normal-weight groups. The # indicates a significant increase from the baseline in plasma cfDNA. Data are presented as means ± SD.

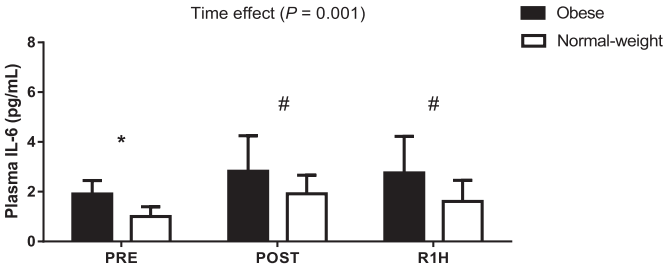

Fig. 5. The expression of plasma IL-6 following acute HIIE in obese and normal-weight subjects. Acute HIIE exhibited an elevation in plasma IL-6 across time in both obese and normal-weight groups. The * indicates a significant difference at baseline (PRE) between groups. The # indicates a significant increase from the baseline in plasma IL-6. Data are presented as means ± SD.

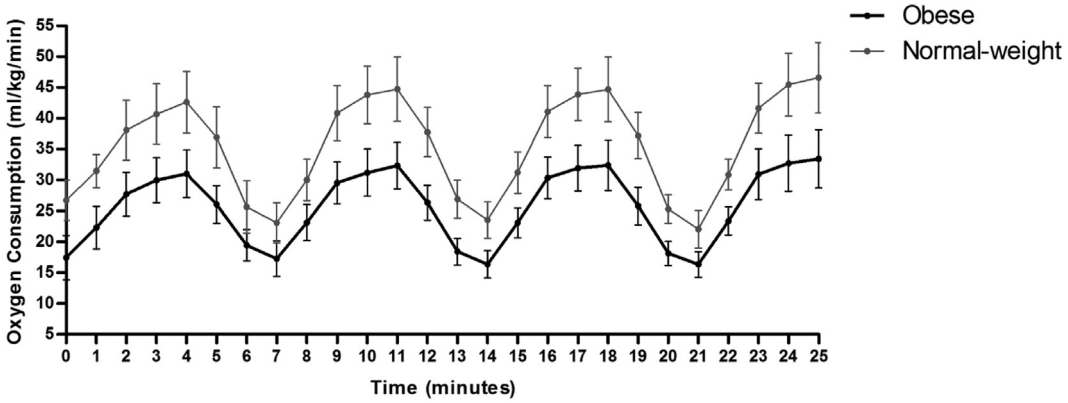

Fig. 3. Oxygen consumption during acute HIIE in obese and normal-weight subjects. Data are presented as means ± SD.

investigated. A second limitation is that treadmill exercise is associated with a greater muscle damage than cycling. However, treadmill exercise was utilized, despite the associated muscle damage, due to it being the primary mode of exercise in clinical settings. In addition, although this study did not fully control for aerobic fitness, an exclusion criteria of > 150 min of moderate and vigorous physical activity per week was included to limit the effect of training on physiological responses to acute exercise. Importantly, it seems unlikely that the disparity in relative VO_{2max} between both groups is solely due to fitness. While fitness may account for some of the difference, lower weight individuals tend to have higher relative VO_{2max} values. Furthermore, HR data in the present study indicates that acute HIIE induced a similar relative exercise intensity between both groups. Our previous study also demonstrated no difference in plasma IL-6 response between obese and normal-weight subjects following 30 min of acute continuous aerobic exercise [36]. Thus, aerobic fitness likely had little to no influence on the findings of this study, with no difference in cfDNA and IL-6 responses between both groups.

5. Conclusion

This study demonstrated that acute HIIE elicited comparable plasma levels of cfDNA and IL-6 between obese and normal-weight subjects. Monitoring circulating cfDNA may provide a greater understanding of obesity-associated inflammation and/or complications along with the measurement of inflammatory cytokines to reveal a more detailed picture of the exercise-induced alteration to the inflammatory profile in obese individuals. Future research with a large sample size is warranted to understand the clinical benefits of obesity-associated cfDNA to potentially predict the effectiveness of exercise interventions. Finally, since skeletal muscle may be the primary source of cfDNA release, other muscle-specific measures such as creatine kinase should be examined to determine whether or not muscle damage is a driver of the cfDNA response.

Conflicts of interest

The authors declare no conflict of interest.

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References

[1] S.U. Amano, J.L. Cohen, P. Vangala, M. Tencerova, S.M. Nicoloro, J.C. Yawe, M. Aouadi, Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation, Cell Metab. 19 (1) (2014) 162–171.
[2] P. Anker, M. Stroun, P.A. Maurice, Spontaneous release of DNA by human blood lymphocytes as shown in an in vitro system, Cancer Res. 35 (9) (1975) 2375–2382.
[3] J. Atamaniuk, C. Kopecky, S. Skoupy, M.D. Säemann, T. Weichhart, Apoptotic cell-free DNA promotes inflammation in haemodialysis patients, Nephrol. Dial. Transplant. 27 (3) (2012) 902–905.
[4] J. Atamaniuk, K.M. Stuhlmeier, C. Vidotto, H. Tschan, A. Dossenbach-Glaninger, M.M. Mueller, Effects of ultra-marathon on circulating DNA and mRNA expression of pro- and anti-apoptotic genes in mononuclear cells, Eur. J. Appl. Physiol. 104 (4) (2008) 711–717.
[5] J. Atamaniuk, C. Vidotto, M. Kinzlbauer, N. Bachl, B. Tiran, H. Tschan, Cell-free plasma DNA and purine nucleotide degradation markers following weightlifting exercise, Eur. J. Appl. Physiol. 110 (4) (2010) 695–701.
[6] J. Atamaniuk, C. Vidotto, H. Tschan, N. Bachl, K.M. Stuhlmeier, M.M. Müller, Increased concentrations of cell-free plasma DNA after exhaustive exercise, Clin. Chem. 50 (9) (2004) 1668–1670.
[7] F.J. Barrat, T. Meeker, J. Gregorio, J.H. Chan, S. Uematsu, S. Akira, ... R.L. Coffman, Nucleic acids of mammalian origin can act as endogenous ligands for toll-like receptors and may promote systemic lupus erythematosus, J. Exp. Med. 202 (8) (2005) 1131–1139.
[8] T. Beiter, A. Fragasso, J. Hudemann, A.M. Niess, P. Simon, Short-term treadmill running as a model for studying cell-free DNA kinetics in vivo, Clin. Chem. 57 (4) (2011) 633–636.
[9] J.I. Borissoff, I.A. Joosen, M.O. Versteylen, A. Brill, T.A. Fuchs, A.S. Savchenko, B.L.J.H. Kietselaer, Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state, Arterioscler. Thromb. Vasc. Biol. 33 (8) (2013) 2032–2040.
[10] S. Breitbach, B. Sterzing, C. Magallanes, S. Tug, P. Simon, Direct measurement of cell-free DNA from serially collected capillary plasma during incremental exercise, J. Appl. Physiol. 117 (2) (2014) 119–130.
[11] V. Brinkmann, A. Zychlinsky, Neutrophil extracellular traps: is immunity the second function of chromatin? J. Cell Biol. 198 (5) (2012) 773–783.
[12] C. Cabral-Santos, C.I.M. Castrillón, R.A.T. Miranda, P.A. Monteiro, D.S. Inoue, E.Z. Campos, F.S. Lira, Corrigendum: inflammatory cytokines and BDNF response to high-intensity intermittent exercise: effect the exercise volume, Front. Physiol. 7 (2017) 662.
[13] S.S. Choe, J.Y. Huh, I.J. Hwang, J.I. Kim, J.B. Kim, Adipose tissue remodeling: its role in energy metabolism and metabolic disorders, Front. Endocrinol. 7 (2016) 30.
[14] T. Christiansen, J.M. Bruun, S.K. Paulsen, J. Ølholm, K. Overgaard, S.B. Pedersen, B. Richelsen, Acute exercise increases circulating inflammatory markers in overweight and obese compared with lean subjects, Eur. J. Appl. Physiol. 113 (6) (2013) 1635–1642.
[15] S. Cinti, G. Mitchell, G. Barbatelli, I. Murano, E. Ceresi, E. Faloia, M.S. Obin, Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans, J. Lipid Res. 46 (11) (2005) 2347–2355.
[16] T. Cullen, A.W. Thomas, R. Webb, M.G. Hughes, Interleukin-6 and associated cytokine responses to an acute bout of high-intensity interval exercise: the effect of exercise intensity and volume, Appl. Physiol. Nutr. Metab. 41 (8) (2016) 803–808.
[17] M. Cury-boaventura, A. Levada-pires, A. Folador, R. Gorjão, T. Alba-loureiro, S.M. Hirabara, T. Pithon-curi, Effects of exercise on leukocyte death: prevention by hydrolyzed whey protein enriched with glutamine dipeptide, Eur. J. Appl. Physiol. 103 (3) (2008) 289–294.
[18] C.E. De Block, L.F. Van Gaal, I.L. Mertens, Mechanisms linking obesity with cardiovascular disease, Nature 444 (7121) (2006) 875–880.
[19] G.P. Dorneles, D.O. Haddad, V.O. Fagundes, B.K. Vargas, A. Kloecker, P.R.T. Romão, A. Peres, High intensity interval exercise decreases IL-8 and enhances the immunomodulatory cytokine interleukin-10 in lean and overweight-obese individuals, Cytokine 77 (2016) 1–9.
[20] I.G. Fatouros, A.Z. Jamurtas, M.G. Nikolaidis, A. Destouni, Y. Michailidis, C. Vrettou, D. Kouretas, Time of sampling is crucial for measurement of cell-free plasma DNA following acute aseptic inflammation induced by exercise, Clin. Biochem. 43 (16) (2010) 1368–1370.
[21] P.B. Gahan, Circulating DNA: intracellular and intraorgan messenger? Ann. N. Y. Acad. Sci. 1075 (1) (2006) 21–33.
[22] M. Haghiac, N.L. Vora, S. Basu, K.L. Johnson, L. Presley, D.W. Bianchi, S. Hauguel-de Mouzon, Increased death of adipose cells, a path to release cell-free DNA into systemic circulation of obese women, Obesity (Silver Spring, Md.) 20 (11) (2012) 2213–2219.
[23] C.R. Harnish, R.T. Sabo, Comparison of two different sprint interval training work-to-rest ratios on acute inflammatory responses, Sports Med. 2 (2016) 20.
[24] U.S. Department of Health and Human Services, Physical Activity Guidelines for Americans, U.S. Department of Health and Human Services, Washington DC, 2008, p. 2008.
[25] C. Huang, M.J. McAllister, A.L. Slusher, H.E. Webb, J.T. Mock, E.O. Acevedo, Obesity-related oxidative stress: the impact of physical activity and diet manipulation, Sports Med. 1 (1) (2015) 1–12.
[26] C.L. Hwang, Y.T. Wu, C.H. Chou, Effect of aerobic interval training on exercise capacity and metabolic risk factors in people with cardiometabolic disorders: a meta-analysis, J. Cardiopulm. Rehabil. Prev. 31 (2011) 378–385.
[27] S. Jahr, H. Hentze, S. Englisch, D. Hardt, F.O. Fackelmayer, R.D. Hesch, R. Knippers, DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells, Cancer Res. 61 (4) (2001) 1659–1665.
[28] K. Jung, M. Fleischhacker, A. Rabien, Cell-free DNA in the blood as a solid tumor biomarker—a critical appraisal of the literature, Clin. Chim. Acta 411 (21) (2010) 1611–1624.
[29] F.C. Mooren, D. Blöming, A. Lechtermann, M.M. Lerch, K. Völker, Lymphocyte apoptosis after exhaustive and moderate exercise, J. Appl. Physiol. 93 (1) (2002) 147–153.
[30] I. Murano, G. Barbatelli, V. Parisani, C. Latini, G. Muzzonigro, M. Castellucci, S. Cinti, Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice, J. Lipid Res. 49 (7) (2008) 1562–1568.
[31] S. Nishimoto, D. Fukuda, Y. Higashikuni, K. Tanaka, Y. Hirata, C. Murata, M. Sata, Obesity-induced DNA released from adipocytes stimulates chronic adipose tissue inflammation and insulin resistance, Sci. Adv. 2 (3) (2016) e1501332.
[32] J.M. Olefsky, C.K. Glass, Macrophages, inflammation, and insulin resistance, Annu. Rev. Physiol. 72 (1) (2010) 219–246.
[33] A. Rigamonti, K. Brennand, F. Lau, C.A. Cowan, Rapid cellular turnover in adipose tissue, PLoS One 6 (3) (2011) e17637.
[34] Ø. Rognmo, E. Hetland, J. Helgerud, J. Hoff, S.A. Slørdahl, High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease, Eur. J. Cardiovasc. Prev. Rehabil. 11 (3) (2004) 216–222.
[35] A. Scharfe-Nugent, S.C. Corr, S.B. Carpenter, L. Keogh, B. Doyle, C. Martin, L.A.J. O'Neill, TLR9 provokes inflammation in response to fetal DNA: mechanism for fetal loss in preterm birth and preeclampsia, J. Immunol. (Baltim., Md.: 1950) 188 (11) (2012) 5706–5712.
[36] A.L. Slusher, M. Whitehurst, R.F. Zoeller, J.T. Mock, M. Maharaj, C.J. Huang, Attenuated fibroblast growth factor 21 response to acute aerobic exercise in obese

individuals, Nut. Metab. Cardiovasc. Dis. 35 (2015) 839–845.
[37] M. Stroun, J. Lyautey, C. Lederrey, A. Olson-Sand, P. Anker, About the possible origin and mechanism of circulating DNA apoptosis and active DNA release, Clin. Chim. Acta 313 (1–2) (2001) 139.
[38] N. Suzuki, A. Kamataki, J. Yamaki, Y. Homma, Characterization of circulating DNA in healthy human plasma, Clin. Chim. Acta 387 (1) (2008) 55–58.
[39] G.A. Tyldum, I.E. Schjerve, A.E. Tjønna, I. Kirkeby-Garstad, T.O. Stølen, R.S. Richardson, U. Wisløff, Endothelial dysfunction induced by post-prandial lipemia: complete protection afforded by high-intensity aerobic interval exercise, J. Am. Coll. Cardiol. 53 (2) (2009) 200–206.
[40] H.K. Vincent, J.W. Morgan, K.R. Vincent, Obesity exacerbates oxidative stress levels after acute exercise, Med. Sci. Sports Exerc. 36 (5) (2004) 772–779.
[41] J. Vollmer, TLR9 in health and disease, Int. Rev. Immunol. 25 (3–4) (2006) 155–181.
[42] S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, J. Ferrante, W. Anthony, Obesity is associated with macrophage accumulation in adipose tissue, J. Clin. Invest. 112 (12) (2003) 1796–1808.
[43] A. Ziegler, U. Zangemeister-Wittke, R.A. Stahel, Circulating DNA: a new diagnostic gold mine? Cancer Treat. Rev. 28 (5) (2002) 255–271.