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The effects of the combination of resistance exercise and heat stress on cell production and plasma levels of cytokines

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ABSTRACT

Global warming and its effects on human health encourage the examination of the effect of resistance exercise and heat stress on cytokines production. The aim of this study was to investigate the interaction effect of resistance training and heat on the immune system of healthy active men. Six trained males (27.83±1.9 years, 86.45±10.3 kg) completed a 60-minute resistance exercise with 75% 1RM in two conditions (~25 °C, normal or ~35 °C, heat). The following were evaluated: LPS-stimulated cytokine production, plasma cytokine levels, and cortisol. The results showed that heat stress increased (P=0.027) plasma IL-6 and remained high after 1 hr (P=0.026). Upon stimulation with LPS, IL-6 cell production in heat and normal conditions increased immediately (PH=0.026; PN=0.048), decreased 1 hr after exercise, and returned to baseline (PH=0.005; PN=0.033). However, post-exercise LPS-TNF-α release decreased immediately only in a heated environment (P=0.029). The concentration of blood cortisol was unaffected by normal or heat conditions. The findings of this research suggest that other factors beyond cortisol regulated cytokines during heat-exposed exercise. Furthermore, the IL-6 had a regulatory function on TNF-α release. These cytokine changes showed that real work in similar temperatures did not negatively affect the immune system.

KEYWORDS

Resistance Exercise; Warm Condition; LPS-IL-6; LPS-TNF-α

1. INTRODUCTION

Resistance exercise training is performing static or dynamic muscle contractions against external resistance of varying intensities. It has many functional benefits by improving overall health and well-being. Some studies recommended resistance exercise for healthy adults or adults with pathological issues (Haskell et al., 2007).

The impacts of physical training on the immune system based on the physiology of stress were investigated in a pioneering work (Nieman & Wentz, 2019). Moreover, the immune response is affected by stressors such as temperature and exercise (Walsh & Whitham, 2006). However, hypothalamic-pituitary-adrenal (HPA) axis can respond to these stressors by regulating homeostasis (Mastorakos, Pavlatou, Diamanti-Kandarakis & Chrousos, 2005; Wang, Liu, Luo, Zhu & Li, 2015).

In addition, monocyte stimulation by bacterial cell wall components activates pro-inflammatory cytokines such as IL-6 and TNF-α. Besides, LPS-stimulated cytokines are influenced by cortisol's suppressive and simulative effects (DeRijk et al., 1997). During an infection, pro-inflammatory cytokines such as TNF-α and IL-6 throughout the HPA axis stimulate the release of glucocorticoids. In turn, the production of pro-inflammatory cytokines is suppressed (Malek, Ebadzadeh, Safabakhsh, Razavi & Zaringhalam, 2015). Moreover, studies demonstrated that acute resistance exercise as a stressor could enhance cortisol concentration (Walker et al., 2020).

TNF-α productively causes a local inflammatory response and aids in controlling infections (Dimitrov, Hulteng & Hong, 2017). On the other hand, the systemic release of TNF-α can lead to sepsis, shock, and death, while IL-6 causes lymphocyte activation and antibody production (Petersen & Pedersen, 2005). Furthermore, IL-6 usually refers to a pro-inflammatory cytokine. However, it may be more reasonable to call it an anti-inflammatory cytokine (Scheller, Chalaris, Schmidt-Arras & Rose-John, 2011) since it can inhibit TNF-α production (Petersen & Pedersen, 2005).

Generally, some studies indicated a considerable increase in plasma IL-6 and LPS stimulated-induced IL-6 production over exercise (Costello et al., 2018; Phillips et al., 2008). In contrast, other studies had distinctive findings (Starkie, Hargreaves, Rolland & Febbraio, 2005; Starkie, Rolland, Angus, Anderson & Febbraio, 2001), which have caused challenging debates. Studies on the production of TNF-α demonstrated its decrease after aerobic or anaerobic exercise (Dimitrov et al., 2017; Phillips, Flynn, McFarlin, Stewart & Timmerman, 2010). Yet, some studies find other results (Docherty et al., 2022).

The effects of exercise and elevated temperature on stimulated cytokine production were inspected, and it was found that elevated temperature was a significant regulator of cytokine production like IL-6 and TNF-α (Eskandari et al., 2020; Heled, Fleischmann & Epstein, 2013; M. Phillips et al., 2008; Presbitero, Melnikov, Krzhizhanovskaya & Sloot, 2021). On the other hand, nowadays, the world is facing global warming, and many people are forced to do heavy work in this extreme environment. Therefore, the current study implemented these conditions to understand the possible changes in our immune system.

Most previous studies only investigated the effects of resistance exercise and heat stress on improving strength and performance (Martínez-Sobrino, Leibar, Calleja-González & del Campo-Vecino; Miles et al., 2019). However, there is a crucial need to study health conditions and immune system reactions affected by these circumstances. Thus, the present study evaluated the response of plasma IL-6 and cortisol. Moreover, the changes of LPS stimulated mononuclear cells, IL-6 (LPS-IL-6) and TNF-a (LPS-TNF-a), to exercise resistance were examined in two conditions of normal and heat stress in trained adult men. Therefore, the aim of this study is to investigate the interaction effect of resistance training and heat on the immune system of healthy active men.

2. METHODS

2.1. Participants

Six healthy trained adult men volunteered to complete this study. They were 27.83±1.9 years old, 179.16±3.54cm tall, and with an 86.45±10.39 kg body mass. In addition, they had a 22.30±2.68 body fat percentage, a 26.90±2.95 kg/m2 body mass index, and a 67.00±6.6 kg lean body mass. Moreover, 1.10±0.9 was their 1RM bench press to weight ratio, and 1.57±0.21 was their 1RM squat to weight ratio. The subjects who had been consistently doing resistance exercise (1:30 hrs per session, three days/week for two years), were all healthy, they did not smoke or drink alcohol, had no infections, and didn’t have symptoms of respiratory illness for four weeks before the study were selected.

Additionally, none of the subjects took exogenous anabolic-androgenic steroids, drugs, medications, dietary supplements, cafe, and tea with potential effects on physical performance and immune system 48 hrs before the experiments. All participants were informed of the experimental protocol, and the risks involved in the study were explained in detail. Then, the subjects delivered their written informed consent to participate. The graduate department of Tarbiat Modares University approved the research protocol. It was based on the declaration of Helsinki.

2.2. Study Procedure

2.2.1. Resistance Exercise Protocol

The subjects visited the laboratory for three sessions (one week apart). At first session anthropometric measurements of height, body mass, body fat percentage, and fat-free mass were made using bioimpedance (Olympia co., Korea). Also, maximal strength was determined using the 1RM test in the following free-weight exercises: bench press, squat, leg flexion, leg extension, lat pull-down, biceps curls, rowing, and shoulder press. The 1RM test assessed maximal strength in 8 free-weight exercises. The following Brzycki Formula (1993) provides a reasonable estimate of the maximum load providing the number of repetitions: 1RM = Weight / (1.0278 – (0.0278 × Number of repetitions)).

The exercises, volume, and intensity of the protocol were chosen due to the influence of the large muscle groups' use and the hypertrophic nature of the training in stimulating anabolic and catabolic hormones.

At second and third session Before the exercises, the individuals performed a general warm-up on an ergometric cycle and a specific warm-up for each exercise. After the warm-up, the subjects completed the protocol. In this stage, the exercise intensity was 75% of the 1RM, at either ~25 ℃ (normal) or ~35 ℃ (heat) in a randomized, counterbalanced fashion. Subjects executed ten repetitions in each set for three sets, and the rest between each set and each exercise (set) was 60 seconds, for a total of 55 minutes.

2.2.2. Heat Stress Protocol

Participants participated in two separate sessions with two weeks' intervals including normal (~25 ℃) and heat (~35 ℃) conditions. They were required to stay in a warm environment provided by a heater and core temperature of subjects was assessed by rectal thermistor. It is worth noting that both trials were conducted at 50% humidity. Temperature and humidity were measure using hot-wire anemometer (aTES-1341, Taiwan). In addition, the participants in both groups consumed 4 ml/kg of body weight every 15 minutes during performing exercise. Changes of temperature in pre, post and 1 hr later in two condition was shown in Figure 1.

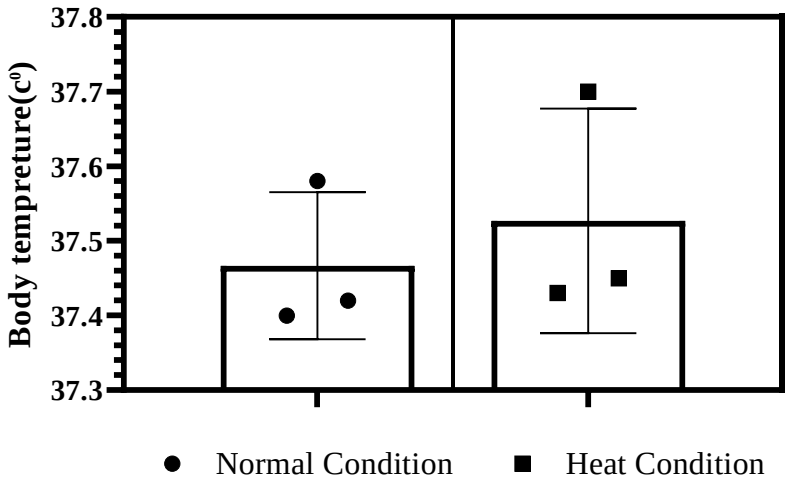


Figure 1. Body temperature at 25℃ (NORMAL) or 35℃ (HEAT). Dates are presented as mean±SD (n= 6).

2.2.3. Sampling

Venous blood samples were collected at pre-exercise (Pre), immediate post-exercise (Post), and 1-hour post-exercise (1 hr later) stages. All blood samples were analyzed for alterations in LPS-stimulated cytokine production. Additionally, all samples were analyzed for plasma cortisol.

2.2.4. Plasma Preparation and ELISA Measurement

Two milliliters of blood were placed in K2-EDTA tubes and then centrifuged for 10 minutes at 3000 g. Moreover, plasma was removed and frozen at –70 ℃ for plasma cytokine measurement and cortisol analysis. Then, the amount of cortisol and IL-6 were measured by ELISA kits (Monobind, USA and eBioscience, Austria, respectively) according to the manufacturer's instructions. All samples were analyzed within the same assay batch to eliminate inter-assay variance. Besides, all intra-assay variances were <6.4% for cortisol and <5.2% for IL-6. Plasma sensitivity was also 0. 37 μg/dl for cortisol and 0. 92 pg/ml for IL-6.

2.2.5. Stimulation of Whole Blood

Five milliliters of blood were collected in sodium heparin tubes and kept at room temperature. The blood was slowly added into tubes containing 3 ml of ficoll (BaharAfshan, Iran) and centrifuged for 15 minutes in 400 × g at 22 ℃. Furthermore, mononuclear cells were harvested, and after two times washing with PBS, viability was measured using trypan blue. The viability measurement showed more than 95% viable cells. Besides, the cells were suspended in a complete culture RPMI medium. The medium was supplemented with 11 mM sodium bicarbonate, two mM L-

glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum.

Additionally, 100µl of the 3×106 cell/ml suspensions were seeded into 96-well flat-bottomed plates (Nunc, Denmark), and 5 µg/ml of *Escherichia coli* LPS (Sigma St. Louis, MO) were added to each well giving a final volume of 200µl (triplicated wells). They were incubated for 48 hrs at 37 °C with 5% CO2. After incubation, the supernatant was harvested for cytokine assay. The IL-6 and TNF-α in the cell culture supernatant were measured using the ELISA kit (Diaclone, France) according to the manufacturer's instructions. Antibody sensitivity was less than two pg/ml for IL-6 and eight pg/ml for TNF-α. Moreover, the overall intra-assay coefficient was calculated, which was 4.2% for IL-6 and 3.3% for TNF-α.

2.3. Statistical Analysis

Cytokines and cortisol amount (means±SD) were determined. In addition, the Shapiro-Wilk test was used to ensure normal data distribution. Mauchly's sphericity test also evaluated the homogeneous variances. An analysis of variance (ANOVA) repeated measures within-between was used to evaluate the two variables (Time×Condition) at three different times (pre-exercise vs. post-exercise vs. c). Bonferroni post hoc procedures were used to locate the pairwise differences and an independent sample t-test was used to evaluate the differences in the variables between both Condition (heat vs. normal) when a significant F value was achieved. Furthermore, it is worth mentioning that $p \leq 0.05$ was considered significant. Data analysis was performed using SPSS software (version 26, SPSS, Inc., Chicago, IL).

3. RESULTS

3.1. Cytokines Release from LPS Stimulated Blood Mononuclear Cells

Changes in variables released from LPS-stimulated blood mononuclear cells are presented in Table 1. The IL-6 release from LPS stimulated blood mononuclear cells in heat condition was significant (F= 13.72, P=0.001, η coefficient=0.73). The LPS-IL-6 Significantly increased post-exercise compared with pre-exercise (P=0.026). However, this cytokine decreased after 1 hr compared with post-exercise (P=0.005) and had returned to near baseline within 1 hr in heat conditions.

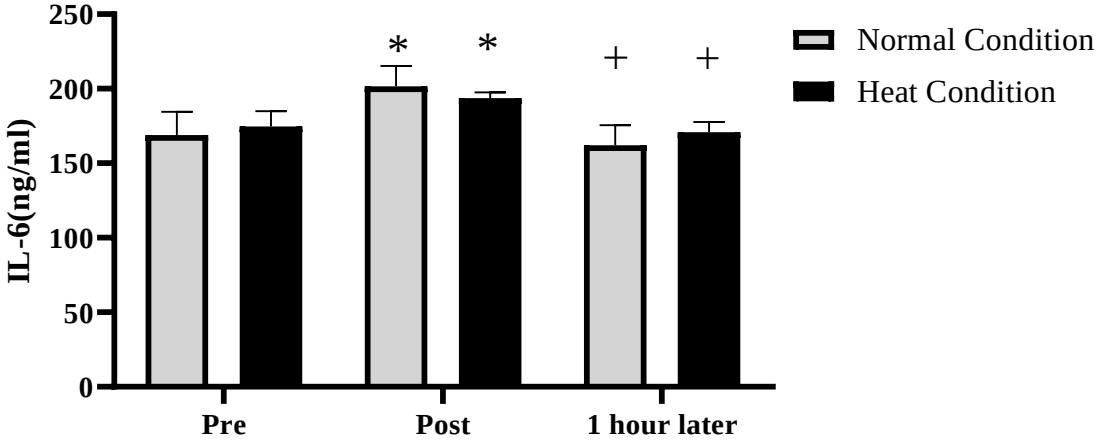
Table 1. Mean of stimulated IL-6, TNF-α prior to (Baseline), immediately following (Post), and 60 min into recovery (1 hr later) from 55 min of resistance exercise at 25 °C (NORMAL) or 35 °C (HEAT)

	Normal Condition(*n=6*)			**Heat Condition(*n=6*)**		
	Pre	**Post**	**1hr later**	**Pre**	**Post**	**1hr later**
IL-6 (pg/ml)	169.0±15.4	201.5±13.7*	162.12±13.5 **	174.77 ±10.1	193.60 ±3.9*	170.73 ±7.0**
TNF-α (pg/ml)	543.9±289.9	502.4±343.2	481.4±315.1	754.8±412.7	508.8±229.1*	599.4±217.6

*Note: * Denote different from pre, P<0.05; ** Denote different from post, P<0.05;*

In addition, the changes of LPS-IL-6 was significant (F= 9.44, P=0.005, η coefficient=0.65) in normal condition, LPS-IL-6 significantly increased immediately after exercise compared with pre-exercise (P=0.048). Besides, the amount of LPS-IL-6 significantly decreased 1 hr post-exercise compared with immediately after exercise (P=0.033). LPS-IL-6 between heat and normal conditions did not alter at various time points (F= 1.35, P=0.28, η coefficient=0.12) (Fig. 2, top).

Upon stimulation with LPS, the concentration of TNF-α was significant changed in heat condition (F= 7.32, P=0.01, η coefficient=0.60). it reduced immediately after exercise in heat conditions(P=0.029). Still, heat conditions did not affect LPS-TNF-α 1 hr post-exercise compared with pre and post-exercise (P<0.005). Moreover, LPS-TNF-α in various time points did not change in normal conditions (F= 0.16, P=0.86, η coefficient=0.03). TNF-α production also did not change in all the time points between the two conditions (F= 1.22, P=0.31, η coefficient=0.11) (Fig. 2, bottom).



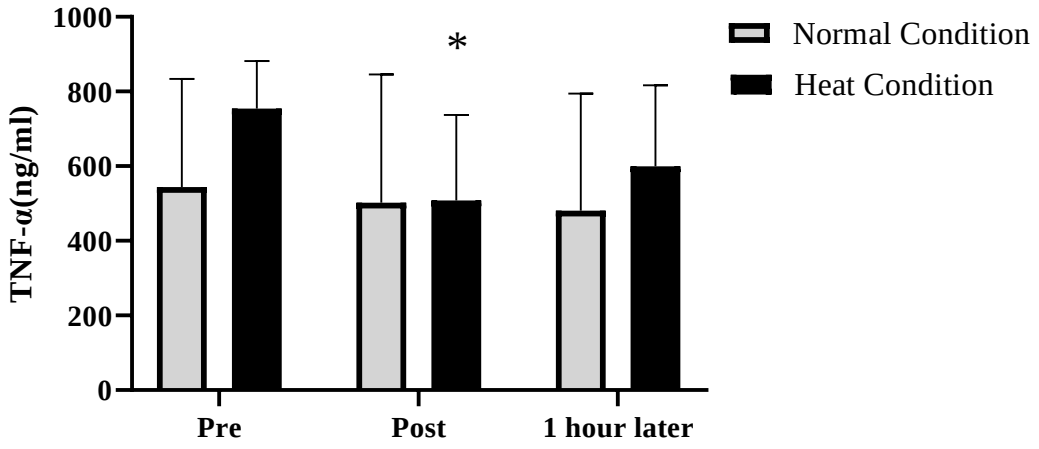


Figure 2. IL-6 (top) and TNF-α (bottom) concentration stimulated by LPS prior to (baseline), immediately following (post), and 60 min into recovery (1 hr later) from 60 min of resistance exercise at 25°C (NORMAL) or 35°C (HEAT). Dates are presented as mean±SD (n= 6). * Denotes different from pre, ($p < 0.05$). + Denotes different from post, ($p < 0.05$).

3.2. Plasma Levels of Cytokines and Cortisol

Changes in plasma concentration variables are presented in Table 2. The level of IL-6 in normal conditions did not change significantly in pre, post, and 1 hr later (F= 0.1, P=0.91, η coefficient=0.02). In contrast, changes of IL-6 in heat condition was significant (F= 7.32, P=0.036, η coefficient=0.59). The heat condition elevated the circulating concentrations of this cytokine immediately (P=0.027) and 1 hr later (P=0.026) exercise compared with pre-exercise. The concentration of plasma IL-6 had a significant difference in heat conditions compared with normal conditions (F= 5.57, P=0.01, η coefficient=0.36) in 1 hr later (t= -2.25, P=0.048) and post-exercise (t=- 4.81, P=0.007), (Fig. 3, top).

Table 2. Plasma IL-6 and cortisol prior to (Baseline), immediately following (Post), and 60 min into recovery (1hr post) from 54 min of resistance exercise at 25 °C (NORMAL) or 35 °C (HEAT)

	Normal Condition(*n=6*)			**Heat Condition(*n=6*)**		
	Pre	**Post**	**1hr later**	**Pre**	**Post**	**1hr later**
IL-6 (pg/ml)	78.37±22.4	74.53±12.6	76.53±5.56	67.03±13.5	98.7±23.1*+	117.2±23.2*+
Cortisol (µg/ dl)	25.82±4.6	29.06±1.3	23.79±2.9	28.95±5.8	31.01±4.7	29.45±5.0

*Note: Dates are presented as Mean±SD. * Denote different from pre, P<0.05; + Denote different from NORMAL, P<0.05.*

Furthermore, plasma cortisol levels were not affected by either time or conditions (F= 0.17, P=0.84, η coefficient=0.02) (Table 2). In addition, there were no differences in the cortisol levels between various time points during the heat trial (F= 1.35, P=0.3, η coefficient=0.21) or normal conditions (F= 3.16, P=0.09, η coefficient=0.39) (Figure 3).

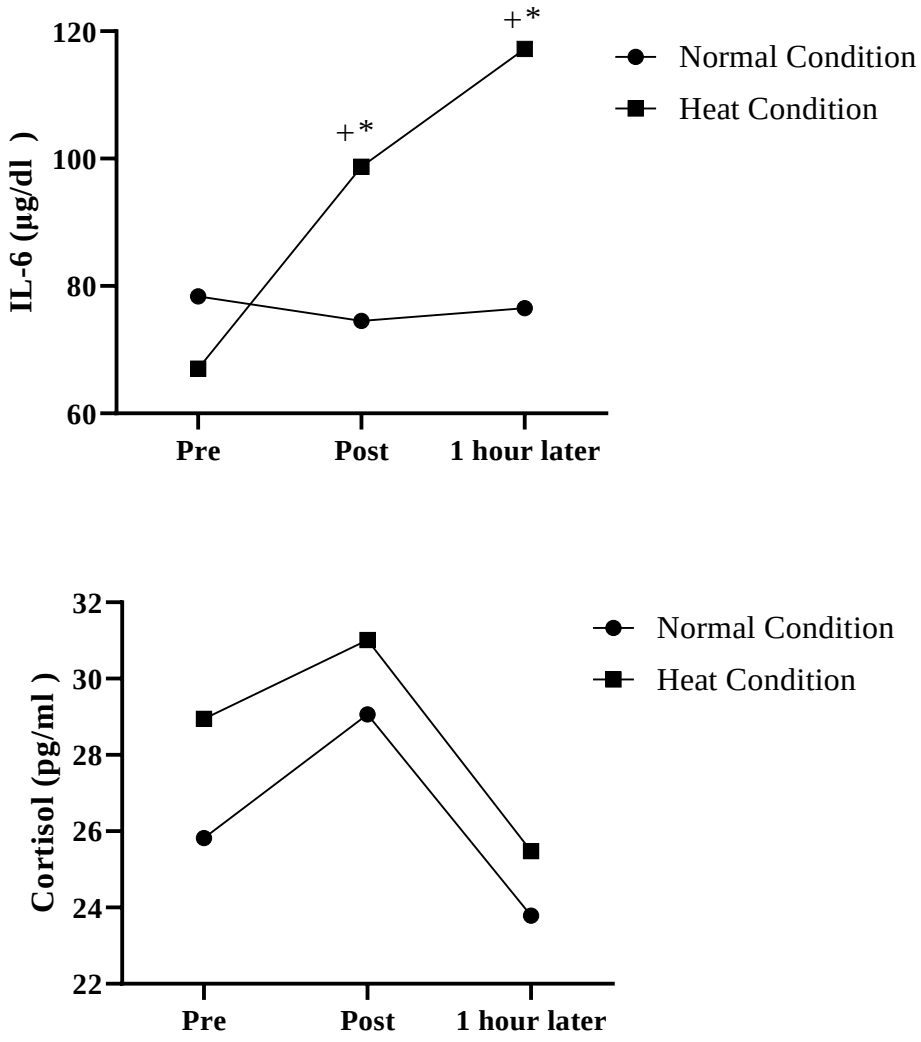


Figure 3. Plasma concentration of IL-6 (top) cortisol (bottom) prior to (baseline), immediately following (post), and 60 min into recovery (1 hr later) from 60 min of resistance exercise at 25°C (NORMAL) or 35°C (HEAT). Dates are presented as mean±SD (n= 6). * Denotes different from Pre, (P<0.05). + Denotes different from NORMAL, (P<0.05).

4. DISCUSSION

The main findings of the present study were that IL-6 plasma concentration in heat conditions increased in post- and 1 hr post-exercise compared with normal conditions. The concentration of

cultured IL-6 from cells stimulated by LPS increased immediately and returned to near baseline within 1 hr after exercise in both groups. In contrast, TNF-α production decreased after exercise in heat conditions, but cortisol did not change. Furthermore, 1 hr after exercise, LPS-IL-6 decreased significantly in heat and normal groups, whereas plasma IL-6 remained high. Our findings were consistent with the results of the studies that indicated an exercise-induced increase in IL-6 production (Costello et al., 2018; Eskandari et al., 2020; Heled et al., 2013; Phillips et al., 2008; Presbitero et al., 2021).

On the other hand, some previous studies demonstrated a reduction in LPS-IL-6 post-exercise (Starkie et al., 2005; Starkie et al., 2001). Starkie's studies examined endurance-trained men and found that LPS-IL-6 was reduced immediately and 2 hrs post-exercise. However, they noticed an increase in plasma IL-6 post-exercise but not in 2 hrs post-exercise. In addition, they discovered that cortisol concentration arose consistently during this period in a different environment (heat and normal conditions). This discrepancy was likely due to increased stress hormones such as cortisol, which have an inhibitory effect on cytokine production. The present study indicated that the cortisol level, as a stress hormone, did not change. This finding replicated the results of some studies (Fatouros et al., 2010), whereas some investigations demonstrated an increase (Kraemer & Ratamess, 2005; Starkie et al., 2001).

In detail, the exercise protocol, which consisted of high volume (>3 sets), moderate to high intensity (65–75% 1RM), and short rest (≤1 min), extracted the greatest cortisol compared with protocols of higher intensity but lower total volume and more extended rest. However, in the present study, the exercise protocol was to serve low work volume, which might be due to less production of cortisol. Thus, it showed cortisol was not responsible for IL-6 release changes in resistance exercise with this volume. Moreover, trained subjects might have had low metabolic stress in response to strength exercise (Cadore et al., 2008). Regularly trained subjects indicated lower cortisol levels after exercise than untrained ones (Gröpel, Urner, Pruessner & Quirin, 2018).

Although the findings suggest that IL-6 is a pro-inflammatory cytokine, it is more reasonable to call it an inflammation-responding cytokine. This latter calling is because it causes stimulation of B cells differentiation and acute phase proteins responses and inhibits IL-1b and TNF-α secretion, suggesting that IL-6 has an anti-inflammatory action (Scheller et al., 2011). These findings indicated that the HPA axis did not stimulate during low-volume resistance exercise due to mild inflammation responses.

The LPS-IL-6 increase occurred after exercising in the heat and normal environments. Furthermore, despite a 10-degree temperature increase, enhancement of LPS-IL-6 was not exacerbated. While LPS-IL-6 did not intensify in heat conditions, cortisol levels did not change. Thus, lack of cortisol induction could be the main reason for the IL-6 release increase from mononuclear cells stimulated by LPS in both normal and heat conditions. Transient LPS-IL-6 increase during exercise could respond to the induced resistance exercise inflammation for maintaining homeostasis since the study realized diminished LPS-IL-6 levels 1 hr post-exercise.

Surprisingly, TNF-α production was suppressed in heat conditions or had a none-statistically significant decrease in normal conditions. Previous studies indicated that resistance exercise, heat stress, and their combination caused a decrease in spontaneous and LPS-induced release of TNF-α (Dimitrov et al., 2017; Phillips et al., 2008; Weinstock et al., 1997). There is no evidence that LPS-TNF-α increased either in heat or normal conditions after exercise, but some studies indicated an increase in plasma TNF-α. The liver may play the leading role in TNF-α increasing during exercise. Moreover, cortisol is another culprit for this rise (Docherty et al., 2022; Starkie et al., 2005).

Heat shock protein 70 (HSP70) serum exposed to heat shock (35 °C) increases significantly after resistance exercise (Eskandari et al., 2020). In addition, the TNF-α release and synthesis are diminished by heat shock proteins (HSP) (Ferat-Osorio et al., 2014), preparing a possible mechanism for suppressing LPS-TNF-α by heat in our study.

Furthermore, Derjick et al. (1997) showed that TNF-α was much more sensitive to suppression by cortisol than IL-6 (DeRijk et al., 1997), but our data demonstrated plasma cortisol level was unaffected. Plasma IL-6 (as an anti-inflammatory cytokine) may increase the suppressed TNF-α (as an inflammatory cytokine) in heat. This finding follows the previous findings that IL-6 inhibited TNF-α production after exercise (Scheller et al., 2011).

Besides, we had an increase in LPS-IL-6 in heat conditions immediately after exercise, which could show its importance in reducing the release of TNF-α. In this study, despite the lack of increase in plasma IL-6 in normal conditions, LPS-TNF-α also had a mild decrease. However, this decrease was not significant in this group after exercise, in which IL-6 increased production from monocytes was the main responsible factor as an inflammation-responding cytokine. It is worth noting that the post-exercise IL-6-LPS was enhanced in normal conditions. In conclusion, the current study suggests that IL-6 production from monocytes has a more significant role in reducing LPS-TNF-α than plasma IL-6.

One of the most important findings of this study was higher plasma levels of systemic IL-6 in the heat than at normal temperature. In contrast, IL-6 production capacity on mononuclear cells was enhanced post-exercise in normal and heat conditions. These results propose that blood monocytes do not only manage the IL-6 body levels. It is known that IL-6 is also produced by muscle contraction (myokine) (Eskandari et al., 2020).

In addition, increased concentration of IL-6 in heat conditions can be caused by increasing glycogen consumption during exercise, suggesting heat is affected by muscle and liver metabolism by IL-6 secretion (Obi et al., 2017). Furthermore, the LPS-induced IL-6 decreased significantly after 1 hr in normal and heat conditions. Moreover, it approximately reached the basal levels while plasma IL-6 remained high, proving that the plasma IL-6 increase is not necessarily associated with LPS-induced IL-6 production. This finding may also be due to the continued glycogen decrease regarding the increased energy expenditure and excess post-exercise oxygen consumption associated with resistance exercise.

5. LIMITATIONS

The present study had several limitations. Inflammatory and anti-inflammatory cytokines beyond IL-6 or TNF-α, which can be influential, couldn't be evaluated. In addition, it was better to assess plasma changes which can contribute to analyzing plasma factors. There are some other approaches for interested researchers to execute similar studies. Moreover, long-term adaptations and performing exercise in higher temperatures might induce more stress, bringing more data to investigate.

6. CONCLUSIONS

Findings of this study indicated that resistance exercise in heat conditions (35 °C) increased plasma IL-6, which might contribute to more hypertrophy or excessive exercise metabolism. Furthermore, the findings depicted that exercise in heat conditions might not have any side effects on the immune system. These findings note that people working in harsh and hot environments do not need to worry about their health since, in recovery, the amount of pro-inflammatory cytokines returns to baseline. However, long-term studies are required to investigate adaptations and chronic effects.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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