Acute long-distance trail running increases serum IL-6, IL-15, and Hsp72 levels

Journal:	<i>Applied Physiology, Nutrition, and Metabolism</i>
Manuscript ID	apnm-2018-0520.R1
Manuscript Type:	Article
Date Submitted by the Author:	02-Oct-2018
Complete List of Authors:	Yargic, Melda; Hacettepe Universitesi Dahili Tip Bilimleri Bolumu, Department of Sports Medicine Torgutalp, Seyma; Hacettepe Universitesi Dahili Tip Bilimleri Bolumu Akin, Senay; Hacettepe Universitesi, Division of Exercise and Sport Physiology, Department of Exercise and Sport Sciences, Faculty of Sport Sciences Babayeva, Naila; Hacettepe Universitesi Dahili Tip Bilimleri Bolumu, Department of Sports Medicine Torgutalp, Murat; Ankara Universitesi Tip Fakultesi Ibni Sina Hastanesi, 3- Division of Rheumatology, Department of Internal Medicine Demirel, Ali; Hacettepe Universitesi, Division of Exercise and Sport Physiology, Department of Exercise and Sport Sciences, Faculty of Sport Sciences; Hacettepe Universitesi Dahili Tip Bilimleri Bolumu, Department of Sports Medicine
Keyword:	running < sports, endurance training < exercise, exercise metabolism < exercise, exercise endocrinology < exercise, cytokines < muscle, stress < exercise
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

SCHOLARONE™
Manuscripts

Acute long-distance trail running increases serum IL-6, IL-15, and Hsp72 levels

Melda Pelin YARGIC¹, Seyma TORGUTALP¹, Senay AKIN², Naila BABAYEVA¹, Murat TORGUTALP³, Ali Haydar DEMIREL^{1, 2}

- 1- Department of Sports Medicine, Faculty of Medicine, Hacettepe University, Ankara, Turkey.
- 2- Division of Exercise and Sport Physiology, Department of Exercise and Sport Sciences, Faculty of Sport Sciences, Hacettepe University, Ankara, Turkey.
- 3- Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Ankara University, Ankara, Turkey.

Corresponding Author: Melda Pelin YARGIC

meldapelin@gmail.com,

Phone Number: 905076069586

Fax Number: 903322236181

Present Address of Corresponding Author: Necmettin Erbakan University, Meram Faculty of Medicine, Sports Medicine Department, Beysehir cad. 281, 42080, Konya Turkey

Email addresses of the other authors:

Seyma Torgutalp: seymatorgutalp@gmail.com

Senay Akin: akin.senay@gmail.com

Naila Babayeva: naila.babaeva@gmail.com

Murat Torgutalp: mtorgutalp@gmail.com

Ali Haydar Demirel: haydar.demirel@gmail.com

Abstract

IL-6, Hsp72, and IL-15 are molecules that have significant metabolic effects on glucose and fat metabolisms and cell's stress response. The aim of this study is to determine their serum levels after a long-distance trail run. Serum IL-15 levels after such endurance events have not been investigated yet. Blood samples were collected from 37 athletes (11 female, 26 male) before and after a 35-km trail run, with a total climb of 940m. Serum was obtained from the samples and IL-6, IL-15, and Hsp72 levels were measured from serum by sandwich ELISA method. The athletes completed the race in 308.3 ± 37.4 minutes on average. After the race, the mean serum IL-6, IL-15, and Hsp72 concentrations increased 13.2 fold, 2.22 fold and 1.6 fold respectively ($p < 0.001$, $p < 0.001$ and $p < 0.039$). This is the first study to demonstrate the increase in serum IL-15 levels following an acute endurance exercise. In addition to IL-15, we report that IL-6 and sHsp72 levels also increase significantly following a 35-km trail run. Since these molecules involve in regulating effects on glucose and fat metabolism, significant increases of IL-6, IL-15, and sHsp72 may have health benefits that may be associated with long-distance trails which are becoming more popular worldwide.

Keywords: running, endurance training, exercise metabolism, exercise endocrinology, cytokines, stress

Introduction

Myokines are peptides that are released by skeletal muscle, which act on remote tissues in addition to their autocrine-paracrine effects. They are known to play regulatory roles in glucose, fat and bone metabolisms (Schnyder and Handschin 2015). Because of its ability to secrete myokines, muscle tissue is now defined as an endocrine organ with effects on metabolism (Pedersen 2013).

Exercise is known to promote glucose uptake independent of insulin, increase insulin sensitivity, accelerate fat metabolism, prevent obesity and development of the metabolic syndrome. Exercise-induced upregulation of various molecules has been linked to exercise-related health benefits of physical activity and exercise. Exercise-related increases in the plasma concentration of IL-6 (Interleukin-6), IL-15 (Interleukin-15) and heat shock proteins are of special interest.

Especially IL-6, has a regulatory role in glucose metabolism, increasing glucose uptake in muscle tissue and stimulating glycogenolysis in the liver (Gleeson 2000). IL-15, on the other hand, demonstrates its metabolic effects on fat metabolism and white fat tissue volume (Carbó et al. 2001). IL-6 and IL-15 have prominent effects on immunity as well, namely, muscle tissue derived IL-6 suppresses TNF- α (Tumor Necrosis Factor – α) and increases IL1Ra, IL-10, and cortisol; whereas IL-15 promotes NK cell development and survival (Lutz and Quinn 2012; Starkie et al. 2003; Steensberg et al. 2003). Extracellular or soluble Hsp72 (sHsp72), which is an inducible form of 70kDa heat shock proteins, is effective on the immune system and it has been implicated acting as a powerful cytokine which elicits a potent pro-inflammatory immune response in human monocytes (A. A. A. Asea et al. 2000).

Although it is known that IL-6, IL-15 and Hsp72 levels increase following exercise, there are contradictory results concerning whether this increase can be found after any exercise bout in any population and the magnitude of the increase. For instance, IL-15 elevation is found to be prominent following concentric and eccentric exercise in sedentary people, but only eccentric exercise elevated serum IL-15 levels in athletes (Bazgir et al. 2015a). The most prominent increase in the prototype myokine IL-6 is observed after running exercise involving several large muscle groups (Febbraio and Pedersen 2002). IL-6 levels can elevate up to 100 fold following a marathon run (Ostrowski et al. 1998). When different durations and intensities of running exercise are compared, early, pronounced increases of sHsp72 are detected immediately after all types of exercise with highest levels after a competitive marathon. Exhaustive exercise induces significantly higher levels of sHsp72 compared with moderate exercise of the same duration. Long-lasting, competitive endurance exercise induces a more significant response of sHsp compared with more intensive but shorter exercise (E. Fehrenbach et al. 2005). Research has also shown that body temperature also plays a role in the adrenal secretion of Hsp72; exercise only results in elevation of adrenal Hsp72 when accompanied by hyperthermia (Akin et al. 2017).

Long-distance runs are gaining more popularity each year and becoming widespread all over the world. A large number of elite and recreational athletes from all age groups are involved in such events (Cejka et al. 2014). A trail run typically consists of up-/downhill running and is more demanding compared to a same distance city run. Therefore the physiological effects of such compelling long-distance trail runs arouse curiosity, considering the increasing popularity of these events.

Although IL-6 and Hsp72 levels are expected to elevate in serum following an acute long-distance trail run, IL-15 levels following such endurance events haven't been investigated yet (Elvira Fehrenbach et al. 2000; Niemelä et al. 2016). This study covers serum levels of three molecules that all together have effects on glucose and fat metabolism and cellular stress response following an acute long-distance trail run.

Materials and Methods

Ethics approval was obtained for this study from Hacettepe University Ethics Committee for Non-Interventional Clinical Investigations. (GO 15/590-17) Informed consent was obtained from all individual participants included in the study.

The runners who attended Cappadocia Ultra-Trail® had to complete the 35-km course in less than 6 hours, during which the total climb was 940 m. There were two stations on the race course on the 10.6th and 22.8th km's, with a 2 hour limit to pass each station. Participants were provided with fruits and beverages at stations and were allowed to consume ad libitum. The temperature on the race day was between 13-18 °C, with heavy rain. The air humidity on the race day was 68-93% and the wind speed was 13km/h on average. The race started at 10:00 in the morning.

On the day before the race, 3-5 mL of blood was collected from the athletes, total blood count was analyzed immediately, and rest of the samples were kept at room temperature for half an hour, then centrifuged at 1500 rpm for 10 minutes to obtain serum. The same procedure was repeated within the first 15 minutes after the end of the race. The obtained serum samples were stored in ice, transferred to the laboratory on the same day and stored at -80 ° C until the day of analysis.

Serum IL-6, IL-15 and Hsp72 levels were measured by sandwich ELISA method with commercially available kits (Elabscience, China; Elabscience, China; STRESSMARQ Bioscience, Victoria, BC, Canada) according to the manufacturer's instructions. The concentration of each protein was determined using a standard curve according to the kit instructions. The minimum protein concentration that the kits could detect was 4.69 pg/mL for IL-6 and 9.38 pg/mL for IL-15. All serum samples were tested in triplicate. IL-6 levels were measured in all participants (n=37), but due to low serum volume, IL-15 and Hsp72 were assayed only in 26 and 17 subjects, respectively.

The change in plasma volume was calculated according to the hematocrit values in the blood samples taken before and after the run. Corrections were made for each athlete to eliminate the effect of hemoconcentration on the serum IL-6, IL-15, and Hsp 72 levels.

Statistical analyzes were performed using IBM SPSS Statistics (Version 21, IBM SPSS, Chicago, IL, USA). Data were tested for normal distribution visually (histogram and probability graphs) and by analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk tests). Wilcoxon test was performed when comparing pre- and postrace values of IL-6 and Hsp72 and t-test of dependent groups was performed for comparing pre- and postrace values of IL-15. The Pearson test was used for the correlation between IL-15 levels and parameters that show normal distribution, and the Spearman test was used for all other correlation analyzes. Correlation is regarded as “very low” in the range of 0-0.3, “weak” in 0.31-0.5, “medium” in 0.51-0.7 and “high” in 0.71-1. Significance was accepted at $p < 0.05$.

Results

A total of 37 athletes participated in the study ($n = 37$). Eleven of the athletes were female (29.72%) and 26 were male (70.27%). Mean age of the athletes was 38.97 ± 10.14 . Mean body mass index was 23.22 ± 2.21 . Athletes completed the race in 308.3 ± 37.4 minutes on average.

Baseline values of IL-6 were found to be below the minimum detectable value of 4.69 pg/mL in thirty athletes. For this reason, no numerical data could be obtained. In order for the statistical analysis to be possible, baseline IL-6 levels of these athletes were accepted as 4.68 pg/mL.

As demonstrated in Figure 1, median IL-6 basal level increased from 4.68pg/mL to 61.82pg/mL following the race. After the race, the mean IL-6 concentration increased 13.2 fold with a conservative approach. ($p < 0.001$) Increase in IL-15 is shown in Figure 2; mean IL-15 increased from 3312.95pg/mL to 7357.25pg/mL following the race. Mean IL-15 concentration showed a 2.22 fold increase. ($p < 0.001$). It can be seen in Figure 3 that mean Hsp72 levels in serum increased from 1.67ng/mL to 2.81ng/mL. Serum concentration of Hsp72 increased 1.6 fold over the baseline. ($p < 0.039$)

The race completion time displayed a weak negative correlation between weekly training distance, weekly running distance and number of years participants have been a regular runner ($r = -0.372$, $p = 0.023$;

$r=-0.418$, $p=0.01$; $r=-0.484$, $p=0.002$, respectively), while showing weak positive correlation with BMI ($r=0.384$, $p=0.019$).

On the other hand, age, weekly running distance, weekly training time, running age and race completion time did not affect the amount of increase in IL-6, IL-15 or Hsp72.

Discussion

Exercise has a critical role in maintaining health; IL-6, IL-15, and Hsp72 have many beneficial effects through this process. Our study demonstrates that IL-6, IL-15, and sHsp72 all increase significantly in serum following a long-distance trail run. This study was carried out in a real race environment. The trail was very compelling with a 35 km racecourse and a total climb of 940 m; in addition there were stations in every 10 km's and a time limit to enter these stations; also there was a 6-hour limit to finish the race. Although we do not know the VO₂ values of the athletes, the participants were regularly trained athletes and were able to finish the race in average 308.3 ± 37.4 minutes, about one hour before the time limit.

IL-6 and IL-15 are myokines with significant metabolic effects. Therefore their increase after a 35-km trail run is important in understanding health effects of prolonged strenuous exercise. IL-6 is the most investigated, prototype myokine. IL-6 has been shown to increase muscle tissue glucose uptake and fatty acid oxidation by inducing AMPK and/or phosphatidyl inositol 3 kinase (PI3K) activation in the skeletal muscle (Carey et al. 2006). Similarly, increased AMPK activation due to IL-6 signaling may also occur in the fat tissue (Kelly et al. 2004). In addition, IL-6 stimulates glycogenolysis and glucose release in the liver and stimulates secretion of GLP-1 (glucagon-like peptide 1) leading to increased insulin release from pancreatic α and intestinal L cells (Ellingsgaard et al. 2011; Gleeson 2000).

The increase in IL-6 level is affected by the duration of exercise, the intensity, the size of the muscle mass involved, and the muscular glycogen stores. The most prominent increase in IL-6 was observed after running exercise involving several large muscle groups. It has been reported that following exercise, the basal plasma IL-6 concentration may increase up to 100 fold, but often studies demonstrate more modest increases (Pedersen et al. 2001). The increase in IL-6 following exercise happens rapidly, peaks at the end of the exercise and decreases rapidly as well (Nehlsen-Cannarella et al. 1997).

In our study serum IL-6 concentration after the completion of the race was approximately 13 times higher. The lowest protein concentration that our kit could detect (4.69pg/ml) is approximately 4-8 times higher than the baseline IL-6 levels expected in endurance athletes. With this in mind, it can be deduced that this increase is actually higher than 13 fold. As a matter of fact, IL-6 levels were found to be 0.51 ± 0.26 pg/mL in blood samples taken from twenty elite rowers (Jürimäe et al. 2017). Similarly, the mean basal IL-6 level in 23 male marathoners was lower than 1 pg/mL and at the end of the marathon an increase of approximately 40 times was observed (Santos et al. 2016). In other studies basal IL-6 levels were found to be 1.1 ± 0.2 pg/mL in 16 half marathoners (Nielsen et al. 2016) and 0.54 ± 0.07 pg/mL in 9 male ultramarathon runners (Krzemiński et al. 2016). The participants of our study are not elite athletes, but the difficulty of the race course and the average race completion time of our participants imply that they are well-trained recreational runners. Therefore it would be more reasonable to interpret our results as “change in the concentration of IL-6 in the athletes following a 35-km trail run is at least 13 fold”.

On the other hand, IL-15 is a myokine mostly known for its effects on adipose tissue. Administration of IL-15 has been shown to reduce the volume of white adipose tissue in animal experiments (Carbó et al. 2001). In humans, when IL-15 is perfused on adipose tissue by a microdialysis probe, this effect is observed in the lean subjects but not in the obese (Pierce et al. 2015). There are few studies measuring the serum IL-15 concentration after an acute exercise bout. IL-15 is elevated after resistance exercise or running for half an hour, while basal levels have been shown to decrease with regular physical activity. It is demonstrated by Bazgir et al. that in sedentary people both concentric and

eccentric exercise elevates serum IL-15 levels, whereas in athletes only eccentric exercise was shown to have this effect (Bazgir et al. 2015b). The IL-15 response to ultra-endurance activities has not yet been investigated. Studies on IL-15 are predominantly focused on cancer immunotherapy and although it is thought to be a promising agent in the treatment of obesity, and type 2 diabetes mellitus, its role in exercise, obesity and fat metabolism is not fully established. To our knowledge this is the first study to test the IL-15 levels following an endurance exercise, reporting a 2.2 fold increase in IL-15 serum concentration.

Heat shock proteins are synthesized to chaperone misfolded and denatured proteins (Whitham and Fortes 2008). The inducible isoform of the 70Kda heat shock protein family, Hsp72 can be detected extracellularly and the release of sHsp72 can occur in response to exercise, since exercise induces many cellular stressors, such as heat, oxidative stress, glucose deprivation, hypoxia, lowered pH and increased Ca concentrations (Powers et al. 2001) Numerous studies show that Hsp72 levels increase following acute bouts of exercise both in the muscle and in serum. Walsh RC et al were first to demonstrate that an acute bout of treadmill running for 60 minutes increased mRNA levels in skeletal muscle tissue samples and protein levels in serum both in male and female participants (Walsh et al. 2001). In our study, we observed a 1,6 fold increase in Hsp72 serum concentration after the race. It is known that an increase in temperature is the main factor for exercise-related increases in Hsp72 levels (Akin et al. 2017). Even though we did not measure the core temperature of the athletes during running, it would be assumed that the long duration of the race could cause a rise in body temperature, which results in a prominent induction of Hsp72. Although increased sHsp levels are associated with disease state, exposure to acute physiological and/or psychological stressors resulted in rapid increases in sHsp72 concentration in blood. Active and passive release of sHsp have been proposed. For example, Hsp's can be secreted from a variety of cells and also can leak passively from necrotic cells when membranes are disrupted (Mambula and Calderwood 2006). Especially in response to various stresses including heat or inflammatory mediators, active release of Hsp has been described as acting as a danger signal (A.A.A. Asea 2007). Although liver and brain have

been shown to contribute to elevated levels of sHsp72 during exercise, our study does not specify the source of circulating Hsp72 (Febbraio et al. 2002; Lancaster et al. 2004). sHsps may have potent cytokine-related properties necessary for immune response. The effects of heat shock proteins on the immune system seem to be bivalent and depend upon the particular tissue milieu. Hsp72 is a strong inflammatory agent when tissues are undergoing necrosis (Calderwood et al. 2016). On the other hand, Hsp72 can directly stimulate anti-inflammatory cytokines while inhibiting release of a variety of inflammatory cytokines (Wieten et al. 2007). Nevertheless, sHsp 72 is believed to improve immune responses and facilitate host defense to pathogenic challenges and increased levels of sHsp72 following long-distance trail running may have potential beneficial effect on inflammation and metabolism (Fleshner and Johnson 2005).

This is the first study to our knowledge to show increasing serum IL-15 levels following an acute endurance exercise. In addition to IL-15, we report that IL-6 and sHsp72 levels also increase significantly following a 35-km trail run. Since these molecules are involved in regulating effects on glucose and fat metabolism, significant increases of IL-6, IL-15 and sHsp72 may have health benefits that may be associated with long-distance trails which are becoming more popular worldwide.

Acknowledgements

We thank the Cappadocia Ultra-Trail® organisers and the runners who participated in our study.

Conflict of Interest

Authors decline no conflict of interest.

This study was funded by Hacettepe University Scientific Research Projects Coordination Unit (Grant Number 2017-13130).

References:

- Akin, S., Naito, H., Ogura, Y., Ichinoseki-Sekine, N., Kurosaka, M., Kakigi, R., et al. 2017. Short-term treadmill exercise in a cold environment does not induce adrenal Hsp72 and Hsp25 expression. *J. Physiol. Sci.* 67(3), 407–413. <https://doi.org/10.1007/s12576-016-0473-0>
- Asea, A. A. A. 2007. Heat Shock Proteins: Potent Mediators of Inflammation and Immunity. (A. A. A. Asea & S. K. Calderwood, Eds.), Springer (Vol. Volume 1). Springer.
<https://doi.org/10.1097/00045413-199411000-00009>
- Asea, A., Kraeft, S.-K., Kurt-Jones, E. A., Stevenson, M. A., Chen, L. B., Finberg, R. W., et al. 2000. HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat. Med.* 6(4), 435–442. <https://doi.org/10.1038/74697>
- Bazgir, B., Salesi, M., Koushki, M., and Amirghofran, Z. 2015a. Effects of Eccentric and Concentric

Emphasized Resistance Exercise on IL-15 Serum Levels and Its Relation to Inflammatory Markers in Athletes and Non-Athletes. *Asian J. Sports Med.* 6(3), e27980.

<https://doi.org/10.5812/asjasm.27980>

Bazgir, B., Salesi, M., Koushki, M., and Amirghofran, Z. 2015b. Effects of Eccentric and Concentric Emphasized Resistance Exercise on IL-15 Serum Levels and Its Relation to Inflammatory Markers in Athletes and Non-Athletes. *Asian J. Sports Med.* 6(3), e27980.

<https://doi.org/10.5812/asjasm.27980>

Calderwood, S. K., Gong, J., and Murshid, A. 2016. Extracellular HSPs: The Complicated Roles of Extracellular HSPs in Immunity. *Front. Immunol.* 7, 159. <https://doi.org/10.3389/fimmu.2016.00159>

Carbó, N., López-Soriano, J., Costelli, P., Alvarez, B., Busquets, S., Baccino, F. M., et al. 2001.

Interleukin-15 mediates reciprocal regulation of adipose and muscle mass: a potential role in body weight control. *Biochim. Biophys. Acta*, 1526(1), 17–24.

Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G., et al. 2006.

Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes*, 55(10), 2688–2697.

<https://doi.org/10.2337/db05-1404>

Cejka, N., Rüst, C. A., Lepers, R., Onywera, V., Rosemann, T., and Knechtle, B. 2014. Participation and performance trends in 100-km ultra-marathons worldwide. *J. Sports Sci.* 32(4), 354–366.

<https://doi.org/10.1080/02640414.2013.825729>

Ellingsgaard, H., Hauselmann, I., Schuler, B., Habib, A. M., Baggio, L. L., Meier, D. T., et al. 2011.

Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat. Med.* 17(11), 1481–1489. <https://doi.org/10.1038/nm.2513>

Febbraio, M. A., Ott, P., Nielsen, H. B., Steensberg, A., Keller, C., Krstrup, P., et al. 2002. Exercise

- induces hepatosplanchnic release of heat shock protein 72 in humans. *J. Physiol.* 544(Pt 3), 957–962.
- Febbraio, M. A., and Pedersen, B. K. 2002. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB J.* 16(11), 1335–1347. <https://doi.org/10.1096/fj.01-0876rev>
- Fehrenbach, E., Niess, A. M., Schlotz, E., Passek, F., Dickhuth, H.-H., and Northoff, H. 2000. Transcriptional and translational regulation of heat shock proteins in leukocytes of endurance runners. *J. Appl. Physiol.* 89(2), 704–710. <https://doi.org/10.1152/jappl.2000.89.2.704>
- Fehrenbach, E., Niess, A. M., Voelker, K., Northoff, H., and Mooren, F. C. 2005. Exercise Intensity and Duration Affect Blood Soluble HSP72. *Int. J. Sports Med.* 26(7), 552–557. <https://doi.org/10.1055/s-2004-830334>
- Fleshner, M., Johnson, J. D. 2005. Endogenous extra-cellular heat shock protein 72: releasing signal(s) and function. *Int. J. Hyperthermia*, 21(5), 457–471. <https://doi.org/10.1080/02656730500088211>
- Gleeson, M. 2000. Interleukins and exercise. *J. Physiol.* 529 Pt 1, 1.
- Jürimäe, J., Tillmann, V., Purge, P., and Jürimäe, T. 2017. Body composition, maximal aerobic performance and inflammatory biomarkers in endurance-trained athletes. *Clin. Physiol. Funct. Imaging*, 37(3), 288–292. <https://doi.org/10.1111/cpf.12299>
- Kelly, M., Keller, C., Avilucea, P. R., Keller, P., Luo, Z., Xiang, X., et al. 2004. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. *Biochem. Biophys. Res. Commun.* 320(2), 449–454. <https://doi.org/10.1016/j.bbrc.2004.05.188>
- Krzemiński, K., Buraczewska, M., Miśkiewicz, Z., Dąbrowski, J., Steczkowska, M., Kozacz, A., et al. 2016. Effect of ultra-endurance exercise on left ventricular performance and plasma cytokines in healthy trained men. *Biol. Sport*, 33(1), 63–69. <https://doi.org/10.5604/20831862.1189767>
- Lancaster, G. I., Møller, K., Nielsen, B., Secher, N. H., Febbraio, M. A., and Nybo, L. 2004. Exercise induces the release of heat shock protein 72 from the human brain in vivo. *Cell Stress Chaperones*,

- 9(3), 276–280. <https://doi.org/10.1379/CSC-18R.1>
- Lutz, C. T., and Quinn, L. S. 2012. Sarcopenia, obesity, and natural killer cell immune senescence in aging: altered cytokine levels as a common mechanism. *Aging*, 4(8), 535–546.
<https://doi.org/10.18632/aging.100482>
- Mambula, S. S., and Calderwood, S. K. 2006. Heat induced release of Hsp70 from prostate carcinoma cells involves both active secretion and passive release from necrotic cells. *Int. J. Hyperthermia*, 22(7), 575–585. <https://doi.org/10.1080/02656730600976042>
- Nehlsen-Cannarella, S. L., Fagoaga, O. R., Nieman, D. C., Henson, D. A., Butterworth, D. E., Schmitt, R. L., et al. 1997. Carbohydrate and the cytokine response to 2.5 h of running. *J. Appl. Physiol.* 82(5), 1662–1667. <https://doi.org/10.1152/jappl.1997.82.5.1662>
- Nielsen, H. G., Øktedalen, O., Opstad, P.-K., and Lyberg, T. 2016. Plasma Cytokine Profiles in Long-Term Strenuous Exercise. *J. Sports Med.* (Hindawi Publ Corp). 2016, 7186137.
<https://doi.org/10.1155/2016/7186137>
- Niemelä, M., Kangastupa, P., Niemelä, O., Bloigu, R., and Juvonen, T. 2016. Acute Changes in Inflammatory Biomarker Levels in Recreational Runners Participating in a Marathon or Half-Marathon. *Sports Med. Open*, 2(1), 21. <https://doi.org/10.1186/s40798-016-0045-0>
- Ostrowski, K., Rohde, T., Zacho, M., Asp, S., and Pedersen, B. K. 1998. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J. Physiol.* 508 (Pt 3)(Pt 3), 949–953.
<https://doi.org/10.1111/J.1469-7793.1998.949BP.X>
- Pedersen, B. K. 2013. Muscle as a secretory organ. *Compr. Physiol.* 3(3), 1337–1362.
<https://doi.org/10.1002/cphy.c120033>
- Pedersen, B. K., Steensberg, A., Fischer, C., Keller, C., Ostrowski, K., and Schjerling, P. 2001. Exercise and cytokines with particular focus on muscle-derived IL-6. *Exerc. Immunol. Rev.* 7, 18–31.

- Pierce, J. R., Maples, J. M., and Hickner, R. C. 2015. IL-15 concentrations in skeletal muscle and subcutaneous adipose tissue in lean and obese humans: local effects of IL-15 on adipose tissue lipolysis. *Am. J. Physiol. Endocrinol. Metab.* 308(12), E1131-9.
<https://doi.org/10.1152/ajpendo.00575.2014>
- Powers, S. K., Locke, and M., Demirel, H. A. 2001. Exercise, heat shock proteins, and myocardial protection from I-R injury. *Med. Sci. Sports Exerc.* 33(3), 386–392.
- Santos, V. C., Sierra, A. P. R., Oliveira, R., Caçula, K. G., Momesso, C. M., Sato, F. T., et al. 2016. Marathon Race Affects Neutrophil Surface Molecules: Role of Inflammatory Mediators. *PloS One*, 11(12), e0166687. <https://doi.org/10.1371/journal.pone.0166687>
- Schnyder, S., and Handschin, C. 2015. Skeletal muscle as an endocrine organ: PGC-1 α , myokines and exercise. *Bone*, 80, 115–125. <https://doi.org/10.1016/j.bone.2015.02.008>
- Starkie, R., Ostrowski, S. R., Jauffred, S., Febbraio, M., and Pedersen, B. K. 2003. Exercise and IL-6 infusion inhibit endotoxin-induced TNF- α production in humans. *FASEB J.* 17(8), 884–886.
<https://doi.org/10.1096/fj.02-0670fje>
- Steensberg, A., Fischer, C. P., Keller, C., Møller, K., and Pedersen, B. K. 2003. IL-6 enhances plasma IL-1 α , IL-10, and cortisol in humans. *Am. J. Physiol. Endocrinol. Metab.* 285(2), E433-7.
<https://doi.org/10.1152/ajpendo.00074.2003>
- Walsh, R. C., Koukoulas, I., Garnham, A., Moseley, P. L., Hargreaves, and M., Febbraio, M. A. 2001. Exercise increases serum Hsp72 in humans. *Cell Stress Chaperones*, 6(4), 386–393.
- Whitham, M., and Fortes, M. B. 2008. Heat shock protein 72: release and biological significance during exercise. *Front. Biosci.* 13, 1328–1339.
- Wieten, L., Broere, F., van der Zee, R., Koerkamp, E. K., Wagenaar, J., and van Eden, W. 2007. Cell stress induced HSP are targets of regulatory T cells: A role for HSP inducing compounds as anti-

inflammatory immuno-modulators? FEBS Lett. 581(19), 3716–3722.

<https://doi.org/10.1016/j.febslet.2007.04.082>

Draft

Figure Captions:

Fig.1 Serum IL-6 levels before and after the race, * $p < 0.001$

Fig.2 Serum IL-15 levels before and after the race, * $p < 0.001$

Fig.3 Serum Hsp72 levels before and after the race, * $p < 0.039$

Draft

Fig.1 Serum IL-6 levels before and after the race, * $p < 0.001$

135x111mm (300 x 300 DPI)

Fig.2 Serum IL-15 levels before and after the race, *p<0.001

148x117mm (300 x 300 DPI)

Fig.3 Serum Hsp72 levels before and after the race, * $p < 0.039$

147x120mm (300 x 300 DPI)