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ORIGINAL ARTICLE

Lipocalin-2: Response to a Progressive Treadmill Protocol in Obese and Normal-weight Men

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Abstract

***Purpose*: Lipocalin-2 (Lcn2), a newer adipocyte-secreted acute phase protein, was recently reported to be correlated with potential effects on obesity and inflammation. The reaction of this protein to progressive exercise has not been evaluated yet. This study was designed to compare the serum Lcn2 and high-sensitivity C-reactive protein (hs-CRP) levels after participating in an acute bout of treadmill protocol in obese and normal-weight men.**

***Methods*: Nine obese (aged: 43.2±4.6 yrs and body mass index (BMI): 31.4±1.6 kg/m^2) and 9 normal-weight (aged: 42.9±4.4 yrs and BMI: 23.03±1.7 kg/m^2; mean ± SD) sedentary men selected randomly from volunteers performed a single bout of exercise according to the treadmill Bruce protocol.**

***Results*: Before the exercise, Lcn2 level was higher in obese than normal-weight individuals (P<0.05). A significant increase in Lcn2, hs-CRP, white blood cells (WBC) and insulin resistance index was observed after the exercise in both groups (P<0.05). The level of Lcn2, hs-CRP and WBC increase was more significant in obese individuals than normal-weight subjects after the exercise (P<0.05).**

***Conclusions*: It seems that the levels of Lcn2 and other inflammatory markers elevated in obese and normal-weight men after participating in an exhaustive progressive exercise. These changes in obese men were considerable.**

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INTRODUCTION

Participation in a single bout of exercise results in accumulation of inflammatory markers in different tissues[1,2]. Many adipose tissue-derived pro-inflammatory markers containing acute-phase proteins, cytokines, adipokines and chemokines increase in circulation accordingly[1-3]. These pro-inflammatory and inflammatory indexes are secreted predominantly from enlarged adipocytes and activated macrophages in adipose tissue and liver[4-6]. The local inflammatory response is accompanied by a systemic reflex known as the acute phase response[7].

Lipocalin-2 (Lcn2) has recently been recognized as an adipocyte-derived acute phase protein that is positively correlated with potential effects on obesity

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and inflammation[5,8-11]. It has been implicated in apoptosis and innate immunity[5,12,13]. As an adipokine, a more increased circulating Lcn2 level has been reported in obese humans[5] and laboratory animals[12] than lean controls. Besides, the serum level of Lcn2 has been shown to have a significant association with body mass index (BMI), fasting blood glucose, hyperinsulinaemia and insulin resistance[5,8,12,14,15]. Circulating level of this adipokine has also a strong direct correlation with high-sensitivity C-reactive protein (hs-CRP)[16], and can be a marker for acute and chronic inflammation[5,17].

One of the best strategies to prevent obesity and its associated inflammation is participation in regular physical activity[2]. On the other hand, accomplishing hard activities such as a single bout exhaustive exercise may lead to systemic inflammation and acute muscular injuries which is considerable in obese and sedentary participants[16,18]. Local response to inflammation due to obesity and tissue injury involves the production of adipokines released at the site of inflammation[7]. These conditions lead to an increased level of circulatory inflammatory markers such as hs-CRP[18] and Lcn2[5]. It has been demonstrated that cytokine levels are affected by the mood of exercise[2]. The magnitude of the changes in cytokine levels depends also on exercise duration and intensity[2]. As reviewed by different studies, there are large increases in pro-inflammatory cytokines during and after a vigorous prolonged endurance exercise[2,18,19]. Lcn2 may play a role in the inflammation process following acute exhaustive exercises[16]. Although regular physical exercise has been shown to have beneficial effects on obesity and inflammation[8], acute phase response of Lcn2 to exhaustive exercise has not been investigated yet. Choi et al in the only available study have not mentioned any changes in Lcn2 level in obese women after a 12-week moderate exercise training[8]. Hence, this study was performed to evaluate this effect in sedentary obese and normal-weight men after an acute bout of treadmill exercise. Accordingly, we examined the changes of Lcn2 and hs-CRP circulating levels and insulin resistance index before and after an acute bout of exhaustive treadmill exercise in obese and normal-weight middle-aged men.

METHODS AND SUBJECTS

***Subjects*:**

Nine obese and 9 normal-weight sedentary men who did not participate in a regular exercise program[20] volunteered to participate in this study. In tune up session (7 days before exercise), all subjects were asked to complete a personal health and medical history questionnaire which served as a screening tool. The subjects were given both verbal and written instruction outlining the protocol. Written informed consent was obtained before screening. They were familiarized with procedures and walked in different gradients and speeds on treadmill. This protocol was approved by ethics committee of Guilan University. The subjects who smoked cigarettes, were afflicted with cardiovascular diseases, bypass surgery, diabetes, chronic kidney and liver disease, or were taking medication that could affect the laboratory measure results were excluded[21]. Participants were instructed not to do any intensive exercise and not to change their diet till the test day. On the second day (6 days before exercise), VO_2max was measured via indirect calorimetry by an open-circuit gas-analyzer (Cosmed, Quark b^2, Italy) during the graded exercise.

***Experimental protocol and laboratory measurements*:**

On the test day, height, weight and waist circumference were recorded. BMI, fat mass and lean body mass were assessed by bioelectrical impedance analysis using a Body Composition Analyzer (Inbody 3.0®, Biospace Co Ltd, Seoul, Korea). Before that, the subjects had to clean their foot and hands surfaces with a 0.9 percent sodium-chloride liquid. They had not any intensive training for 12 hours and took no drinks or food for 4 hours before the exercise. They also emptied their bladder 30 minutes before the test[22].

Systolic and diastolic blood pressures were obtained with an electronic sphygmomanometer (HESTIA Mannheim, Japan) above the left brachial artery after 30 minutes rest in lying position. After 5 minutes warm up (light treadmill jogging and muscle stretching), the subjects performed a graded-exercise treadmill according to the Bruce protocol[22]. Briefly, the seven stages of this protocol corresponded to progressive greater efforts at treadmill speeds of 2.74, 4.02, 5.47,

6.76, 8.05, 8.85, and 9.65 km/hr respectively. This protocol is valid and suitable for active and sedentary men[22]. The treadmill is set up at the stage 1 (speed 2.74 km/hr), and grade of slope (10% that increases by 2% per stage) and the subjects commence the protocol. While the subjects were running on the treadmill, heart rate was recorded using a telemetric device with monitor (Polar, Kempele, Finland). The exercise was terminated when the subjects were exhausted. Perceived exertion was rated according to Borg's scale by using thumb signs explained previously[23]. The exercise was followed by a cool down period of 5 minutes with low speed and stretching. To control diurnal variation, the measurements were done in two sequential mornings from 8:00 am to 10:00 am[24].

To collect blood samples, all subjects overnight fasted at least for 12 hours and were kept at -20 °C for subsequent assay. Samples were obtained by venipuncture after 45 minutes rest before the exercise. Samples collection was performed immediately after exercise. Serum Lcn2 levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) and an intra-assay CV of 1·0%. A human hs-CRP ELISA kit (Immunodiagnostik, Bensheim, Germany) was used to measure hs-CRP with an intra-assay CV of 1.7%. Glucose level was determined by enzymatic (GOD-PAP, Giucose Oxidase-Amino Antipyrine) colorimetric method (Pars Azmoun, Tehran, Iran) and insulin level was measured by a radioimmunoassay (RIA). Insulin resistance index was calculated regarding the homeostasis model assessment (HOMA-IR) according to the formula[15]: HOMA-IR=[fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5]. WBC count was assessed by a laboratory routine method.

Statistical Analyses:

Values were expressed as mean ± standard deviation (SD). Pre-to-post exercise changes were determined by a paired sample *t*-test. Differences between two groups were analyzed by Mann-Whitney U or student's t tests. P-values of less than 0.05 were considered statistically significant. Data analyses were performed with SPSS program (ver. 13, SPSS, Inc., Chicago, IL).

RESULTS

Anthropometrics and body composition values are presented in Table 1.

Table 1: Mean values (±SD) of characteristics and physiological parameters in obese and normal-weight subjects

Parameters	Normal-weight (n = 9)	Obese (n = 9)	*P* value*
Age (yr)	42.9±4.4	43.2±4.6	0.913
Weight (kg)	69.5±6.2	87.7±10.2	0.002*
Body mass index (kg/m^2)	23.03±1.7	31.4±1.6	0.0001*
Body fat (%)	17.8±3.4	24.01±3.2	0.002*
Fat mass (kg)	12.4±2.8	20.8±4.6	0.001*
Lean body mass (kg)	57.1±5.1	65.9±7	0.014*
Waist circumference (cm)	78±3.7	89.8±3.8	0.0001*
Waist to hip ratio	0.82±0.04	0.93±0.03	0.001*
Running time (min:s)	14.57±2.3	12.48±2.1	0.001*
VO_2max (ml. kg^{-1}. min^{-1})	34.1±1.5	31.6±1	0.002*
Perceived exertion	18.2±1.4	18.4±1.3	0.112
Rest heart rate (b/min)	79±3.4	81±2.3	0.151
Maximal heart rate (b/min)	173±3	174±3.2	0.244
Systolic blood pressure (mm Hg)	120.1±12	126.1±14.6	0.386
Diastolic blood pressure (mm Hg)	79.8±7.7	80.2±8.8	0.906

*Significance of student t-test for independent groups

Table 2: Mean values (±SD) of blood parameters in obese and normal-weight subjects before and after an acute bout of treadmill exercise

		Normal-weight (n = 9)	Obese (n = 9)
Fasting Glucose (mmol/l)	Before	5.4±0.6	5.6±0.6
	After	4.6±0.5†	4.5±0.3†
Insulin (mU/l)	Before	12.1±9.1	11.8±8.9
	After	7.6±5.2†	9.2±5.5†
HOMA-IR	Before	2.8±0.5	2.6±0.9
	After	1.6±0.5†	1.8±0.8†
White Blood Cells (×10cell/L)	Before	8.2±2.3	7.9±2.2
	After	11.4±2.5†	12.6±2.7†
Lipocalin-2 (µg/l)	Before	141±36.5	152.4±29.3*
	After	155.7±37.3†	176.9±30.5*†
hs-CRP (mg/l)	Before	2.9±0.8	3±0.6
	After	5.7±0.5†	6.6±0.4*†
Lactate (mmol/l)	Before	1.9±0.2	1.7±0.2
	After	6.8±0.5*	7.2±0.4*

HOMA-IR: homeostasis model assessment-insulin resistance; hs-CRP: high-sensitivity C-reactive protein.
Significance of student t-test or Mann Whitney U for independent groups: *P<0.05.
Paired significance differences before/after acute bout of treadmill exercise by t-test: †P<0.05.

The changes of measured blood variables before and after the acute bout of treadmill exercise are shown in Table 2. The data showed that baseline Lcn2 level was higher in obese (152.4±29.3 µg/l) than normal-weight (141±36.5 µg/l) subjects (P=0.001). After exercise, Lcn2, hs-CRP and WBC increased significantly in comparison with baseline in obese (176.9±30.5 vs. 152.4±29.3 µg/l; P=0.012), (6.6±0.4 vs. 3±0.6 mg/l; P=0.003), (12.6±2.7 vs. 7.9±2.2 ×10cell/L; P=0.001), and normal-weight (155.7±37.3 vs. 141±36.5 µg/l; P=0.032), (5.7±0.5 vs. 2.9±0.8 mg/l; P=0.04), (11.4±2.5 vs. 8.2±2.3 ×10cell/L; P=0.002) respectively. Additionally, statistically significant increases were observed in Lcn2 (176.9±30.5 vs. 155.7±37.3 µg/l; P=0.034) and hs-CRP (6.6±0.4 vs. 5.7±0.5 mg/l; P=0.042) levels in obese compared with normal-weight subjects. Insulin level decreased in both obese (11.8±8.9 vs. 9.2±5.5 mU/l; P=0.012) and normal-weight (12.1±9.1 vs. 7.6±5.2 mU/l; P=0.002) individuals after exercise in comparison with baseline.

Moreover, a significant decrease was observed in HOMA-IR in obese (2.6±0.9 vs. 1.8±0.8; P<0.001) and normal-weight (2.8±0.5 vs. 1.6±0.5; P<0.001) subjects after the treadmill exercise.

DISCUSSION

In the present study, the effects of one session progressive exercise on the markers linked to inflammation and cardiovascular diseases have been investigated in obese and normal-weight adult men. The major findings of this study were elevated Lcn2, hs-CRP and WBC in circulation. Lcn2 as an adipocyte-derived protein has been identified for more than one decade[5]. Although the effect of longitudinal exercise on Lcn2 has been examined by Choi et al[8], its response to acute exercise training is unknown. They did not report any changes after exercise training. Regarding the effects of acute exercise, the present study was the first one on Lcn2. The results showed that Lcn2 concentration elevated markedly in obese individuals compared with normal-weight subjects with the same hs-CRP and WBC levels. This difference can probably be related to selective augmentation of its expression in adipocytes and hepatocytes[25]. This increase is probably because of metabolism process change, destruction of muscle cells membrane and elevation of other inflammatory markers such as hs-CRP and WBC. Lcn2 expression increases sharply

after inflammatory stimulation. Expression of Lcn2 in adipose tissue can be induced by lipopolysaccharides, suggesting Lcn2 to be an acute phase protein[5]. Lcn2 elicits its adverse effects at least partly by stimulating TNFα, which may in turn magnify the local inflammation and cause impaired energy homeostasis[2].

In accordance with pervious findings[5,8], we found that the Lcn2 level was higher in obese than lean individuals. Furthermore, our finding was in agreement with Choi et al's study[8]. They recently reported that Lcn2 is a risk factor that develops the metabolic syndrome, independent of obesity and insulin resistance. They reported that Lcn2 correlated with HOMA-IRas well[8].

Yan et al have shown that this adipocyte-secreted protein influences systemic metabolism and induces insulin resistance[12]. Recently, Wang et al showed a higher concentration of Lcn2 in obesity and diabetes[5]. Moreover, this adipokine is positively related to BMI, waist circumference and fat percentage. This observation suggests that the increased fat mass might account for the elevated blood levels of that adipokine in obese individuals[5]. A positive relation was observed between Lcn2 level and fat mass, BMI and WC (data are not shown). Choi et al have also demonstrated that Lcn2 can be used by researchers and clinicians as inflammatory index[8]. However, they reported a relationship between Lcn2 and hs-CRP levels. This augment suggests that Lcn2 may not simply be a marker of inflammation. It is likely that there is a complex interconnection between lipocalin protein, obesity-induced metabolic disorders and inflammation.

The results showed that hs-CRP level increased in both obese and normal-weight individuals after the exercise in comparison with baseline. WBC and hs-CRP are related with intensive exercise stresses. An increasing level of these variables has been shown after exhaustive trainings[26,27]. Arazi et al[28] similar to Paczeck et al have demonstrated no significant change in blood level of CRP before and after a single bout of exercise[29]. Whiles Choi et al showed an improvement in hs-CRP without significant change in serum Lcn2 level in obese adult women after 12 weeks of moderate exercise[8]. This contradiction between reports can be a perspective on more investigations into Lcn2 and its relation with other inflammatory markers in acute and chronic exercises. Lcn2 response to acute exercises has not been investigated and needs to be studied. According to previous findings, it seems that the exercise-induced acute phase inflammation results in an increase in Lcn2 and hs-CRP levels in both obese and normal-weight participants. Of course, it is reported that there is a direct relationship between Lcn2 and hs-CRP level[8]. Based on this reason, Lcn2 increase was anticipated. Thus, Lcn2 can be recognized as an inflammatory marker that increases in sedentary individuals after a progressive physiological stress. Previous studies demonstrated that regular exercise protects the organism from diseases and complications linked to chronic low grade systemic inflammation[26]. Indeed, majority of studies suggest that regular exercise has anti-inflammatory effects. However, exhaustive exercises as inflammatory stimuli induce destruction of protein and adipose tissues. The anti-inflammatory effects of an acute bout of exercise (with regular exercise) will protect against chronic systemic low-grade inflammation and offers protection against insulin complications, but a link between the acute effects of exercise and the long-term benefits has not been proven yet[7,29].

The other finding of this study was increase in insulin level after exercise. Insulin is an important component of metabolism process that is involved in insulin resistance. The insulin response to acute and chronic exercise might be different. It has been demonstrated that single bout of exercise increases the glucose disposition by means of insulin in normal subjects and in obese individuals with insulin resistance[1]. These changes may be a reflection of increase in glucose uptake during exercise. A major portion of energy during running exercise is obtained from metabolism of blood glucose. It is clear that this process and glucose intake will be absorbed by insulin effect[1].

Despite the clear benefit of regular exercise to the insulin control, there are situations in which acute exercise does not improve the insulin sensibility and it may even worsen it. The insulin sensibility decreases after the marathon running alike after exhaustive exercise such as running up in a steep street[1]. This

discussion is in contrast to our finding. To draw a robust conclusion about the relation between Lcn2 and insulin more studies with larger sample sizes should be performed.

CONCLUSION

The response of circulatory level of Lcn2 to an exhaustive exercise in sedentary obese men has been shown in the present study for the first time. The results provide novel insights into Lcn2 as an inflammatory marker and its association with metabolic complications in a single bout acute exercise. It seems that participation in an exhaustive aerobic exercise can lead to Lcn2 and hs-CRP increase in sedentary obese men. However, these results need to be investigated more thoroughly in future. The findings of the present study are profitable for individuals who are doing only 1 session of progressive aerobic exercise. If this study was performed on a group of obese women and also with more subjects, the results could have been more beneficial. Our recommendation is a new study with high intense exercise on similar subjects.

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Conflict of interests: None

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