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ORIGINAL ARTICLE

Plasma chemerin in young untrained men: association with cardio-metabolic traits and physical performance, and response to intensive interval training

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Abstract

OBJECTIVES: Chemerin is an adipose tissue-derived adipokine thought to decrease insulin sensitivity and increase cardiometabolic risk. This study aimed to assess the association of chemerin with cardiometabolic risk and physical performance and examine its response to high-intensity interval training (HIIT).
METHODS: Eighteen young men have been applied a HIIT program during 8 weeks. Plasma chemerin together with several cardiometabolic factors and physical performance indices were determined before and after the training program. Plasma chemerin and insulin were assessed using immunoenzymatic methods. The homeostasis model assessment (HOMA-IR) index was calculated as an estimate of insulin resistance.
RESULTS: Basal plasma chemerin was positively correlated with body mass index (r=0.782, $p<0.001$), body fat (r=0.767, $p<0.001$), total (r=0.686, $p=0.002$) and LDL (r=0.587, $p=0.010$) cholesterol, triglycerides (r=0.775, $p<0.001$), HOMA-IR (r=0.673, $p=0.002$) and C-reactive protein (r=0.765, $p<0.001$). With regards to physical performance, chemerin was negatively correlated with maximal oxygen uptake (r=–0.572, $p=0.013$) and squat jump (r=–0.627, $p=0.005$), but positively related to 10-m sprint (r=0.716, $p=0.001$) and 30-m sprint (r=0.667, $p=0.002$) times. HIIT program resulted in significant improvements in body composition, plasma lipids and insulin sensitivity. However, no significant change was detected for plasma chemerin in response to HIIT (134±50.7 ng/mL vs. 137±51.9 ng/mL, $p=0.750$).
CONCLUSIONS: Basal plasma chemerin is associated with cardiometabolic health and physical performance in young men. Following HIIT, cardiometabolic health and physical performance had improved, but no significant change had occurred for plasma chemerin.

Abbreviations:
CMJ - counter movement jump
CRP - C-reactive protein
FJT - five jump test
HRmax - maximal heart rate
HIIT - high-intensity interval training
HOMA-IR - homeostasis model assessment index for insulin resistance
MAV - maximal aerobic velocity
SJ - squat Jump
VO2max - maximal oxygen uptake.

INTRODUCTION

Adipose tissue is an active endocrine organ that produces a variety of bioactive mediators called adipokines. These factors signal to liver, skeletal muscle, brain, immune system, and adipose tissue itself, modulating lipid and glucose homeostasis, blood pressure and inflammatory response (Ouchi *et al.* 2011; Ohashi *et al.* 2014). Chemerin is a newly discovered adipokine that modulates adipocyte differentiation and activates macrophages infiltration into the adipose tissue (Goralski *et al.* 2007; Bozaoglu *et al.* 2007). Increased circulating chemerin was associated with adiposity, insulin resistance, inflammation, metabolic syndrome and cardiovascular diseases (Bozaoglu *et al.* 2007; Sell *et al.* 2009; Weigert *et al.* 2010; Yan *et al.* 2012; Sledzinski *et al.* 2013).

Physical exercise has been proven to promote cardiovascular fitness (Blair & Morris, 2009; Sakurai *et al.* 2013) and improve circulating adipokines pattern (Saremi *et al.* 2010; Kadoglou *et al.* 2012a,b; Faramarzi *et al.* 2016). Several recent reports documented significant decrease in circulating chemerin following aerobic and/or resistance exercise (Saremi *et al.* 2010; Chakaroun *et al.* 2012; Aghapour & Farzanegi, 2013; Venojärvi *et al.* 2013; Malin *et al.* 2014; Kim *et al.* 2014; Stefanov *et al.* 2014; Faramarzi *et al.* 2016). However, no study has investigated chemerin response to interval training. This gap in the literature needs to be addressed given the increasing interest in this exercise modality in recent years. Current literature highlights greater improvements in cardiometabolic health with interval vs. continuous exercise, or if similar, providing the advantage of less time (Tjønna *et al.* 2009; Kessler *et al.* 2012). Studies that addressed the topic have been conducted in persons at high cardio metabolic risk. This makes unclear whether the effect is attributable to exercise or to cardiometabolic improvement. Also, some of the prior work included dietary restriction, thereby limiting interpretation of exercise vs. exercise+diet effect. This study sought at examining the relationship of chemerin with cardiometabolic profile and physical performance and its response to high-intensity interval training (HIIT) in individuals with low to moderate cardiometabolic risk. We hypothesized that chemerin is related to cardiometabolic status and physical fitness, and will decrease following interval exercise.

MATERIAL AND METHODS

Study design and participants

A pre-post test study was conducted among twenty young male aged 17 to 20 years, randomly selected among pre-terminal and terminal classes' students in high secondary schools (Dahmani, Tunisia). Participants were recreationally active subjects at low to moderate grade. Individuals with acute or chronic disease (except obesity) were excluded. All participants beneficed of a medical exam prior to inclusion that revealed no cons-indication for physical exercise. They were divided based on body mass index (BMI) in normal-weight group (NWG; BMI<25 kg/m^2; age, 18.1±0.93 years; n=10) and overweight/obese group (OG; BMI>25 kg/m^2; age, 18.3±1.22 years; n=10). The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Scientific and Ethics Committee of High Institute of Sports and Physical Education of Kef. Participants gave their written informed consent to participate in the study. During the study, one participant had difficulty adapting to the intense exercise and decided to leave the program. Another participant had been absent several times and was subsequently excluded from the study. The remaining eighteen participants attended all sessions of program and no one was injured. No standard diet or supplements were provided, nor was dietary restriction recommended. Dietary intake was not recorded, but participants were requested to maintain their normal eating habits during the program.

Experimental protocol

The study was conducted from February to April 2014. Temperature ranged from 17°C to 23°C and humidity from 70% to 75%. Body composition, physical performance and biological profile were assessed before and after the training program.

Anthropometric measurements

Weight and height were measured with subjects barefooted and lightly clothed, allow the calculation of BMI (kg/m^2)=weight/hight^2. Biceps, triceps, sub-scapular and supra-iliac skinfolds thickness were measured using Harperden's skinfold calipers (Baty International, West Sussex, England). Percentage of body fat was assessed based on skinfold thickness at the four sites according to Durnin & Wormersley, 1974.

Training program

The training program consisted of 3 sessions weekly during 8 weeks. The session started with 15-min warm-up consisting of 10-min continuous jogging at moderate intensity (50% of Maximal aerobic velocity (MAV), followed by 5-min dynamic stretching exercises and 5 short bursts of 20-m accelerations. The main stage consisted of two series of 30-s run at 100%–110% of MAV interspersed by periods of active recovery of 30-s

run at 50% of MAV. Training progression was accomplished by increasing the number of repetitions from 8 to 10 repetitions from the third week, and increasing the intensity of work from the fifth week (5% increase of the MAV every two weeks) (Table 1). Finally, participants were cooled down by running at low intensity and performing static stretching during 10 min.

Tab. 1. Eight weeks of high-intensity interval training (HIIT) program.

Week of training	1–2	3–4	5–6	7–8
Number of series	2	2	2	2
Number of races per series	8	10	10	10
Run/active recuperation time, second	30/30	30/30	30/30	30/30
Percent of MAV (run/active recuperation)	(100/50)	(100/50)	(105/50)	(110/50)
Passive recovery time, min	5	5	5	5
Training load, ATU	600	750	775	800

MAV, maximal aerobic velocity; ATU, arbitrary training units
Example: [2 × (8 × 30s/30s); 100%/50% MAV; passive recovery time = 5 min]. It means that the subject had to run 2 series of 8 times 30s: composed of 30s running at 100% of MAV and 30s active recovery at 50% of MAV. The subject recovers passively 5 min between each two series. Each session is repeated 3 times a week. Example of training load calculation for training sessions during the first week: [(100 + 50)/2] × 4 × 2 = 600 ATU.

Physical performance tests

Maximal aerobic velocity (MAV) and maximal oxygen uptake (VO2max) were measured via a continuous incremental test called Vameval Test (Cazorla, 1990). The test was performed on a 400-m outdoor running track. It starts at a running speed of 8 km/h and increases by 0.5 km/h every minute until exhaustion. Participants adjusted their running speed to the cones placed at 20-m intervals. The test ended when the subjects could no longer maintain the required running speed dictated by the audio beep for 2 consecutive occasions. Heart rate was recorded during the test, using a Polar heart rate monitor (*Polar^{TM} S810, Kempele, Finland*). The highest value recorded during the Vameval Test was considered to be the maximal heart rate (HRmax). Accuracy of MAV measurement was verified by ensuring that HRmax is within the interval theoretical HRmax±10.

Squat-jump (SJ) and countermovement jump (CMJ) were carried out as described by Bosco *et al.* (1983) using an Optojump system (Globus; Microgate Ltd., Italy). The two tests differ by the starting position, which is standing position for CMJ and 90° of flexion of the knees joints for SJ. Participants are instructed to jump as high as possible with keeping their hand on the hips. SJ and CMJ performance was expressed in flight height (cm). Participants performed three trials with 1 min of recovery in-between for each test. The best performance was chosen.

Five jump test (FJT) was carried out as described by Chamari *et al.* (2008). FJT is composed of five successive horizontal jumps. The subject begins the test with joined feet and ends in the same position. Starting at right station, the subject performs five strides. He jumps on one leg (right or left) into raising the knee and the arms in front. During the fifth stride the subject brings back both legs together to go back to the same starting position. The performance in FJT was expressed into total distance (m).

Sprint tests were carried out as described by Chamari *et al.* (2004). Each subject was asked to run a distance of 30-m in a straight line as fast as possible with a free standing start. Three pairs of photocells were disposed in a straight line: the first on the starting line, the second at 10-m and the third on the finish line (30-m). The sprint time has been registered with photoelectric cells (Microgate SARL, Italy) placed at a height of 1 m above the ground. Each subject performed three trials with 3-min of recovery between efforts. The best performance was selected.

Blood sampling and methods of analysis

Blood was sampled from an antecubital vein into EDTA-containing tubes after 12 hours of fasting. Blood was drawn after a 48 hours exercise-free period, one day before the start and 2 days after the completion of the training program. Blood samples were centrifuged at 2000×*g* for 25 min and the plasma was frozen at –80 °C until analysis (within 3 months). Plasma chemerin was assessed using a commercially enzyme-linked immunosorbent assay (ELISA) kit (Biovendor-Laboratorni medicina a. s., Karasek, Czech Republic) according to the manufacturer's protocol. Plasma insulin was measured by a chemiluminescence immunoassay method using a Liaison analyzer and the respective reagents kit (DiaSorin Inc., Stillwater, MN). Plasma glucose, total cholesterol, HDL cholesterol and triglyceride were assessed by enzymatic colorimetric methods, and C-reactive protein (CRP) by immunoturbidimetric method using an Architect C8000 analyzer and the respective reagents kits (Abbott Laboratories, Abbott Park, IL). LDL cholesterol was calculated using the Friedwald formula (Friedewald *et al.* 1972). The insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) index as follows: HOMA-IR=[fasting insulin (mU/L)*fasting glucose (mmol/L)/22.5] (Matthews *et al.* 1985).

Statistical Analysis

The Kolmogorov-Smirnov test was used to attest normal distribution for continuous variables. Independent-samples t-test was used to compare basal variables

between OG and NWG and paired-samples t-test was used to compare pre-HIIT and post-HIIT variables in each group (OG or NWG). Between group changes in variables resulting from the training program were assessed by two-way (time * group) repeated measures analysis of variance. Correlations between continuous variables at baseline were tested using Pearson's correlation. A p-value <0.05 based on two-sided calculation was considered significant. All analyses were performed using software package SPSS 18.0 for Windows® (SPSS Inc., Chicago, IL, USA).

RESULTS

Association of baseline chemerin with cardio metabolic traits and physical performance

Basal plasma chemerin concentrations were significantly higher in OG than NWG (172±40 *vs.* 103±38 ng/mL; p=0.002). In a combined group of normal-weight and overweight/obese participants, basal plasma chemerin was positively correlated with weight (r=0.790, p<0.001), BMI (r=0.782, p<0.001), body fat (r=0.767, p<0.001), total cholesterol (r=0.686, p=0.002), triglycerides (r=0.775, p<0.001), LDL cholesterol (r=0.587, p=0.010), glucose (r=0.497, p=0.036), insulin (r=0.567, p=0.014), HOMA-IR (r=0.673, p=0.002) and CRP (r=0.765, p<0.001) (Figure 1). At baseline, plasma chemerin was inversely correlated with MAV (r=–0.573, p=0.013), VO2max (r=–0.572, p=0.013), FJT (r=–0.498, p=0.035), SJ (r=–0.627, p=0.005) and CMJ (r=–0.550, p=0.018), but positively correlated with 10-m sprint (r=0.716, p=0.001) and 30-m sprint (r=0.667, p=0.002) times (Figure 2). There was a trend to significant positive correlation of VO2max, SJ, FJT and CMJ, and a trend to significant negative correlation of 30-m sprint time with plasma chemerin. VO2max, SJ and 30-m sprint time were significantly different between the first and the third tertiles of plasma chemerin (Figure 3).

Response to high-intensity interval training program

Compared to pre-HIIT, post-HIIT BMI, body fat, plasma triglycerides, total and LDL cholesterol, and HOMA-IR were significantly lower in OG, while plasma CRP did not changed in both groups. In parallel, indices of physical performance had improved in both groups following HIIT. However, no significant change was observed between pre-HIIT and post-HIIT plasma chemerin concentrations in both groups. Repeated measures detected a significant difference in change of BMI between OG and NWG, but no significant differences in changes for the remaining variables (Table 2).

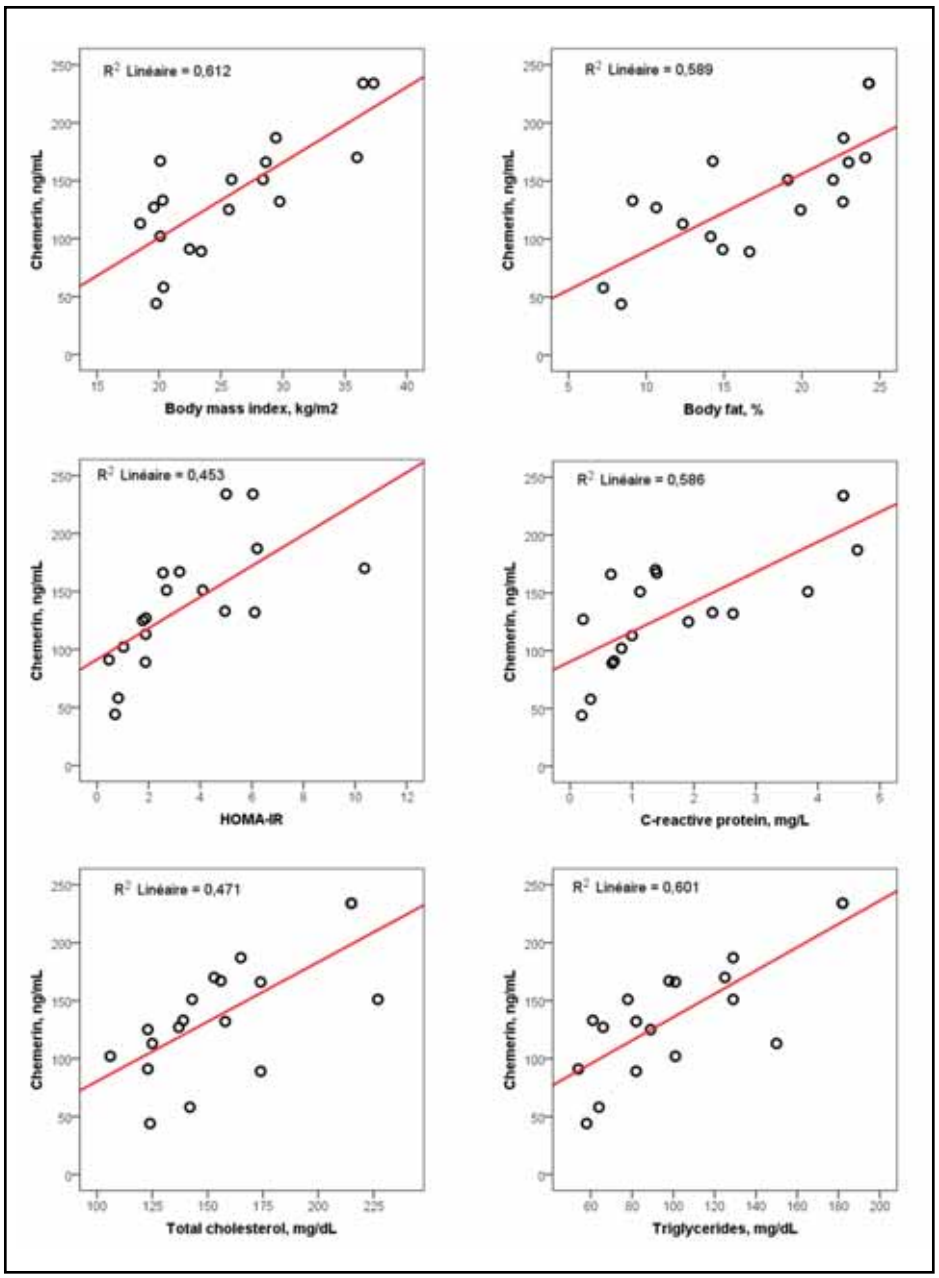

Fig. 1. Correlation of basal plasma chemerin with selected cardiometabolic traits.

DISCUSSION

This study showed higher plasma chemerin concentrations in overweight/obese compared to normal-weight participants. Plasma chemerin was tightly associated with plasma lipids and markers of insulin resistance, inflammation and adiposity. Positive correlations of chemerin with cardiometabolic traits including BMI, body fat, waist circumference, plasma lipids, glucose, insulin, CRP and HOMA-IR have been largely reported (Bozaoglu *et al.* 2007; Sell *et al.* 2009; Weigert *et al.* 2010; Ernst & Sinal, 2010; Yan *et al.* 2012; Sledzinski *et al.* 2013; Malin *et al.* 2014; Stefanov *et al.* 2014). All such data corroborate the assumed role of chemerin as a cardiometabolic risk factor.

Another finding of the study was negative correlations of plasma chemerin with indices of aerobic (MAV, VO2max) and anaerobic physical performance (FJT, SJ, CMJ, 10-m and 30-m sprints). In agreement, Saremi *et al.* (2010) observed negative correlation of chemerin with VO2max suggesting an inverse association with aerobic performance. However, other studies did not observe such an association (Malin *et al.* 2014; Stefanov *et al.* 2014). This report is the first that describes an inverse association between chemerin and anaerobic physical performance. The relationship of chemerin with physical performance is somewhat understandable, being likely explained by a confounding effect of adiposity. This condition is generally associated with both higher chemerin levels and lower physical performance. However, a potential role of chemerin on skeletal muscle function or other factors that modulate physical performance couldn't be excluded (Eckardt *et al.* 2009). Further research is required to clarify the underlying mechanisms.

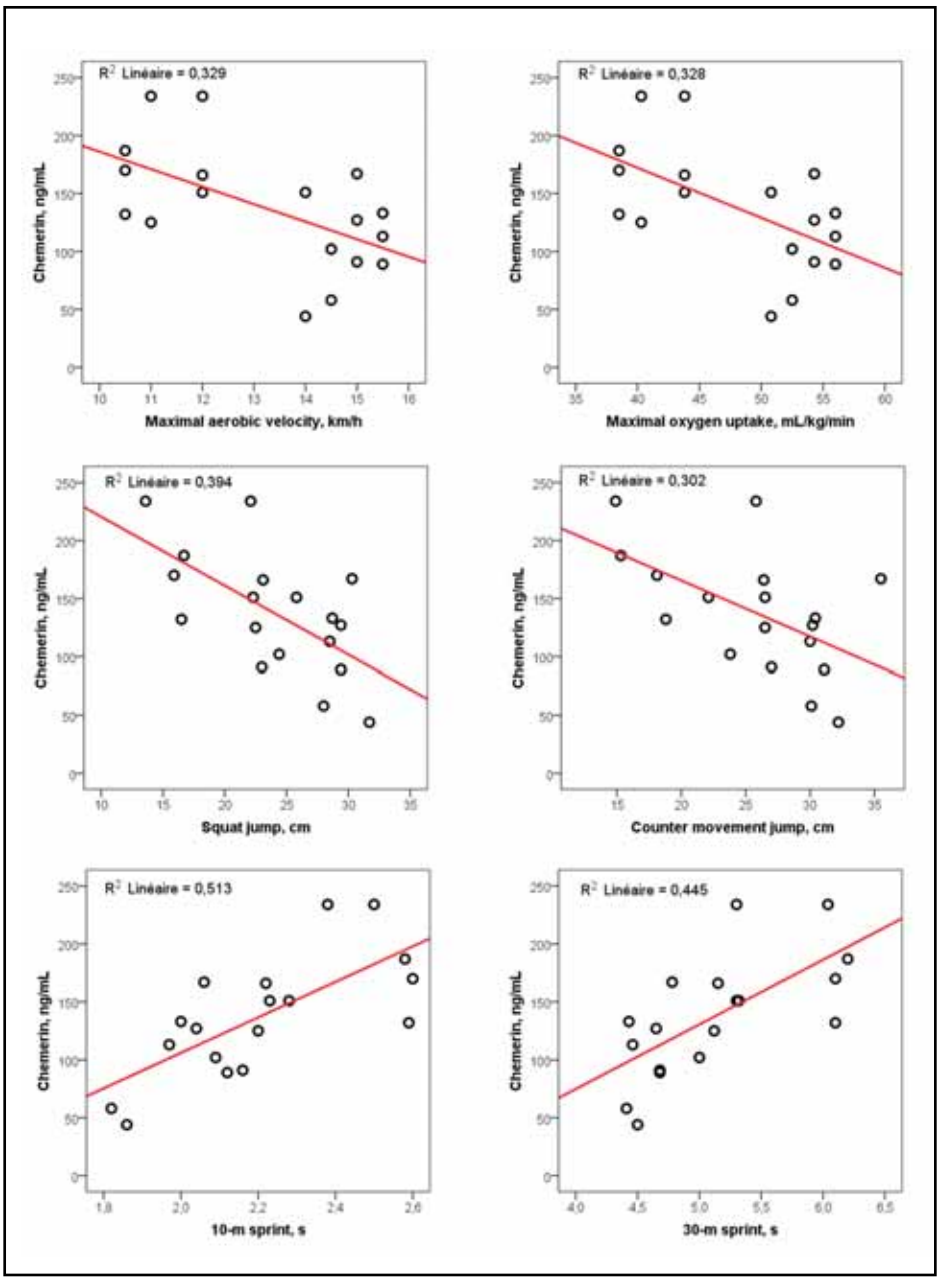

Fig. 2. Correlation of basal plasma chemerin with selected physical performance indices.

The study demonstrated a significant improvement in body composition, HOMA-IR and plasma lipids after the HIIT program in overweight/obese participants. These observations are in accordance with literature data (Tjønna *et al.* 2009; Ziemann *et al.* 2011; Kessler *et al.* 2012; Weston *et al.* 2014) and corroborate a beneficial role of HIIT in cardiometabolic health.

In disagreement with previous studies that showed consistent decrease in chemerin levels following physical training (Saremi *et al.* 2010; Chakaroun *et al.* 2012; Aghapour & Farzanegi, 2013; Venojärvi *et al.* 2013; Malin *et al.* 2014; Kim *et al.* 2014; Stefanov *et al.* 2014; Faramarzi *et al.* 2016), the present study failed to detect any change in plasma chemerin after the HIIT program. Lack of such a change in our series may be due to differences in the type, intensity, frequency and duration of training compared to the preceding

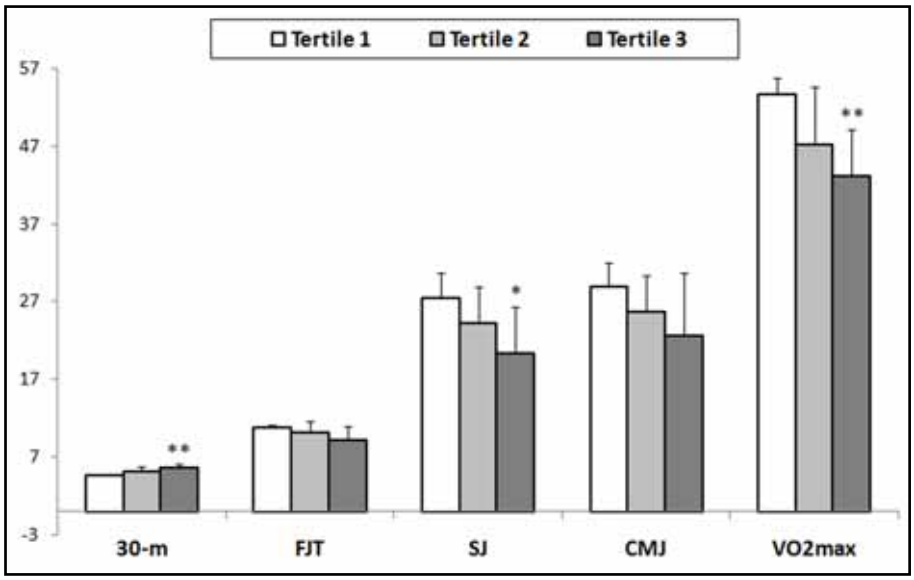

Fig. 3. Selected physical performance indices according to the tertiles of basal plasma chemerin concentrations. 30-m, 30-m sprint time; FJT, five jump test; SJ, squat jump; CMJ, counter movement jump; VO2max, maximal oxygen uptake; *p<0.05; **p<0.01

Tab. 2. Anthropometric, physical performance and biochemical parameters at baseline (Pre-HIIT) and after high-intensity interval training (Post-HIIT) in normal-weight group and overweight/obese group.

	Normal-weight group (n=9)		Overweight/obese group (n=9)		Interaction (time * group)[a]	
	Pre-HIIT	Post-HIIT	Pre-HIIT	Post-HIIT	F	*p*-value
Body mass index, kg/m^2	20.5±1.51	20.5±1.67	30.8±4.56***	30.3±4.25†	6.24	0.024
Body fat, %	12.0±3.28	11.9±3.10	22.5±1.87***	22.1±1.82†	2.17	0.160
MAV, km/h	14.9±0.53	15.4±0.74††	11.5±1.15***	12.1±0.96††	0.64	0.434
VO2max, ml/kg/min	54.1±1.84	55.6±2.58††	42.0±4.03***	44.2±3.37††	0.58	0.459
30-m sprint time, s	4.62±0.19	4.59±0.20†	5.63±0.47***	5.58±0.43†	0.03	0.859
Squat jump, cm	28.2±2.77	30.0±2.71††	19.8±4.18***	20.8±4.21††	1.13	0.304
Counter movement jump, cm	30.0±3.24	32.0±3.43††	21.6±4.92***	23.0±5.11††	0.30	0.591
Five jump test, m	11.1±0.53	11.4±0.69††	8.99±1.18***	9.30±1.15††	2.31	0.148
Cholesterol, mg/dL	136±20.3	127±20.2	175±26.0**	150±15.8†	0.74	0.404
Triglycerides, mg/dL	82.5±31.1	68.4±16.5	122±39.0*	90.0±21.2†	0.01	0.910
LDL cholesterol, mg/dL	85.2±19.2	79.2±15.4	113±30.0*	96.2±13.1†	2.39	0.142
HDL cholesterol, mg/dL	36.3±6.15	36.4±7.23	37.0±2.12	37.2±3.21	1.25	0.280
Fasting glucose, mg/dL	89.8±10.5	91.1±9.98	100±13.9	93.9±7.83	1.62	0.221
HOMA-IR	1.87±1.43	1.26±0.51	4.99±2.62**	3.12±1.47†	0.09	0.764
C-reactive protein, mg/L	0.85±0.67	1.26±1.42	2.78±1.58**	2.97±1.78	1.10	0.309
Chemerin, ng/mL	103±37.9	106±41.9	172±39.6**	162±43.5	1.19	0.292

Data are expressed as mean±SD; HIIT, high-intensity interval training; MAV, maximal aerobic velocity; VO2max, maximal oxygen uptake; HOMA-IR, homoeostasis model assessment index for insulin resistance; *, p<0.05; **, p<0.01; ***, p<0.001 (compared to baseline in normal-weight group); †, p<0.05; ††, p<0.01 (compared to baseline in the same group); [a], comparison using two-way repeated measures ANOVA.

studies. chemerin decrease was observed following regular aerobic exercise (Saremi *et al.* 2010; Chakaroun *et al.* 2012; Aghapour & Farzanegi, 2013; Malin *et al.* 2014), resistance training or Nordic walking (Venojärvi *et al.* 2013) or combined endurance and resistance exercise training (Stefanov *et al.* 2014; Faramarzi *et al.* 2016), whereas this study has applied intermittent exercises. To the best of our knowledge, this is the first study that investigated the effect of HIIT on circulating chemerin. Lack of change may be due to the low frequency and duration of the training program applied here (three sessions a week for eight weeks). Most training programs that resulted in change in chemerin have applied greater work charge (three to six

sessions weekly for at least 12 weeks). Even if a significant decrease in chemerin was detected after a six-week training program (Aghapour & Farzanegi, 2013), such a decrease has occurred after aerobic exercise program and among high cardio-metabolically exposed subjects (post-menopausal hypertensive women). Hence, characteristics of participants could also be of importance. This study included healthy young males with low cardiometabolic risk, while most others studies were carried out among middle/advanced-aged, overweight/obese or diabetic subjects who are exposed to a high cardiometabolic risk. Further studies are necessary to clarify the impact of training type, frequency and duration, and participants characteristics on chemerin level.

In this pre-post test study, the baseline could be seen as a reference; changes observed may be attributed principally to training program. Some limitations have to be mentioned. Due to the reduced number of subjects, the study may be underpowered to detect significant changes in some variables, especially for chemerin and CRP. Also, the study did not control for dietary intake and energy expenditure, which could affect adipose tissue metabolism. However, no participant declared having changed his eating habits while participating to the training program. Therefore, it is improbable that the diet had influenced the results.

In conclusion, basal chemerin is related to cardiometabolic health and physical performance. Eight-week HIIT program has been effective to improve body composition, circulating lipids, insulin sensitivity and physical performance in obese/overweighed in young males. However, the HIIT program has resulted in no significant change in circulating chemerin.

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Conflict of Interest:

The authors declare that there are no conflicts of interest.

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