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ORIGINAL ARTICLE

Effect of diet and diet combined with chronic aerobic exercise on chemerin plasma concentrations and adipose tissue in obese women

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Abstract

OBJECTIVE: Chemerin concentrations are elevated in obesity and associated with inflammation and insulin resistance. Exercise improves insulin sensitivity, which may be facilitated by changes in chemerin. We explored the effects of 24 –week aerobic exercise program, diet and both combined on serum and abdominal adipose tissue (AT) chemerin, and adiponectin concentrations in obese women.
METHODS: Thirty volunteers participated in a 24-week intervention whereby the women were randomly categorised into three groups: 1) exercise (EX), 2) diet (DI) and 3) exercise and diet group (EXD). Blood samples were taken before and after 12-week and 24-week of the intervention. Adipose Tissue (AT) biopsies were gathered only at baseline and at the end of the experiment.
RESULTS: We found increases in VO_{2max} in EXD (21.8 and 39.5%, $p < 0.05$) and in the EX group (18.1 and 41%, $p < 0.05$) after the 12 and 24-week training, respectively. Chemerin circulating levels were reduced in EXD and DI groups, after 24-week training program ($p < 0.01$). HOMA-R decreased only in the EXD group ($p < 0.05$). However, circulating adiponectin increased in both EXD and DI groups after 24-week training ($p < 0.01$). After 6 months training, AT biopsies showed decreases in chemerin levels in EXD and DI groups ($p < 0.01$). No changes in AT biopsies were observed in EX group. Nevertheless, adiponectin increased ($p < 0.05$) in AT.
CONCLUSION: Aerobic training and diet, whether applied exclusively or combined have an impact on circulating or AT levels of adiponectin and chemerin in to the exclusion of the EX group. Therefore The 24-week training program and diet used in this study was very effective for producing significant benefits to body composition, insulin resistance and lipids profile, as well as lowering chemerin levels in these obese women. Therefore, our data suggests that chemerin serum concentrations are associated with insulin resistance.

INTRODUCTION

Adipose tissue is considered as an active endocrine organ secreting cytokines regulating fat mass, inflammation, and glucose and lipid metabolism (Rabe *et al.* 2008). Chemerin is an atypical adipokine secreted from both visceral and subcutaneous adipose tissue and is related to metabolic syndrome, cardiovascular diseases and type 2 diabetes (Bozaoglu *et al.* 2007, Tan *et al.* 2009)

Currently, chemerin has been shown to play a significant role in adipocyte differentiation, as has glucose homeostasis (Lloyd *et al.* 2015). However, it is uncertain to which extent the chemerin mechanism contributes to cardiovascular and metabolic diseases. Some authors found correlations between chemerin and elevated triglycerides, inflammation, and arterial stiffness (Bozaoglu *et al.* 2007, Weigert *et al.* 2010). Additionally, chemerin brings about a resistance to insulin in adipocytes, hepatocytes and primary human skeletal muscle cell in vitro by impaired glucose uptake (Sell *et al.* 2009, Takahashi *et al.* 2011, Ernst *et al.* 2012). Consequently, this adipokine may have a major role in glucose intolerance (Chakaroun *et al.* 2012).

The relationship between weight reduction and chemerin circulating level was further studied (Simpson & Singh 2008). Chakaroun *et al.* (2012) found that body mass reduction via bariatric surgery, followed by six months of hypocaloric diet and 12 weeks of aerobic exercise consistently reduce serum chemerin levels, as well as chemerin expression in adipose tissue. According to Chu *et al.* (2012) ; and Chakaroun *et al.* (2012), chemerin levels increased with obesity and may activate dyslipidemia and insulin resistance. A reduction in adiposity via exercise or diet would reduce chemerin levels and insulin resistance. However, it is still unclear how a reduction in subcutaneous adipose tissue as a result of diet and/or exercise would impact circulating chemerin levels in association with changes in insulin resistance.

Several studies have documented the effects of bariatric surgery (Sell *et al.* 2009, Chakaroun *et al.* 2012), caloric restriction (Blüher *et al.* 2012), exercise (Saremi *et al.* 2010, Stefanov *et al.* 2014, Venojärvi *et al.* 2013) and combined diet and exercise (Kim *et al.* 2014, Li *et al.* 2014) on adiposity and circulating chemerin). However, none of the exercise-only interventions no dietary restriction regimes' brought about significant weight loss and changes in circulating adipokines. Indeed, the effects of aerobic exercise-combined with dietary restriction inducing fat mass loss and modifications in plasma and adipose tissue adipokines in obese individuals requires further research (Petr *et al.* 2018). Therefore, the aim of the present study was to investigate the effects of aerobic training, or diet restriction, or the combined effects of aerobic training and diet restriction on adiponectin and chemerin plasma levels and in AT and on HOMA-IR in obese participants.

MATERIAL AND METHODS

Participants

Thirty obese healthy females participated in this study (39 years ± 4.37). Obesity was defined as a BMI between 30 and 40 $kg.m^{-2}$. Exclusion criteria were cardiovascular disease, type 2 diabetes, pregnancy, or orthopaedic difficulties causing inability to undertake an exercise program. No subjects received medication that could affect the investigated metabolic markers. Prior to participation, the participants gave a written informed consent. This study was conducted in accordance with the declaration of Helsinki and approved by the local ethics committee. The women gave a written informed consent for the experimental protocol. The 30 obese women were randomized into 3 groups 1) exercise (EX), 2) hypocaloric diet (DI), or 3) hypocaloric diet plus aerobic exercise group (EXD).

Body composition measurements

Height was measured with a standing stadiometer and recorded with a precision of 0.1 cm. Waist circumference was taken as the smallest circumference between the lower costal margin and the pelvic brim measured to the nearest 0.5 cm. Body mass (measured to the nearest 0.1 kg) and percent body fat were measured using bioelectrical impedance analysis (Beurer BF 800, Ulm, Germany). Participants were nude or wearing only underwear for measurements of body mass. Body mass index (BMI) was calculated using the body mass and body height ($kg.m^{-2}$). Their physical characteristics were shown in table 1.

Exercise stress test

Participants performed an incremental exercise test to exhaustion on a calibrated cycle ergometer (Ergometrics 900, Ergo-line, Germany) to determine maximal oxygen consumption (VO_{2max}). The test consisted in a 5 min warm-up followed by increments in power of one minute at 60 rpm until exhaustion. The loads during warm-up and increments were individually adjusted by taking into consideration the age, height and body mass of each subject (Petr *et al.* 2018). The gas analyser was calibrated before the test with gases of known concentration .VO_{2max} was considered to be attained when two of the following criteria were satisfied: 1) an oxygen uptake plateau despite increasing exercise intensity, 2) respiratory exchange ratio ≥ 1.10, 3) maximal heart rate within 10 $beats.min^{-1}$ of the age predicted maximal values (Wasserman *et al.* 1986) and 4) subject exhaustion (Matthews *et al.* 1985).

Exercise intervention

Aerobic training included three sessions per week of walking/running on a treadmill, starting at 55% of maximal heart rate for 30 min for weeks 1-4 for 30 min. Exercise intensity and duration were gradually increased every month until subjects exercised at

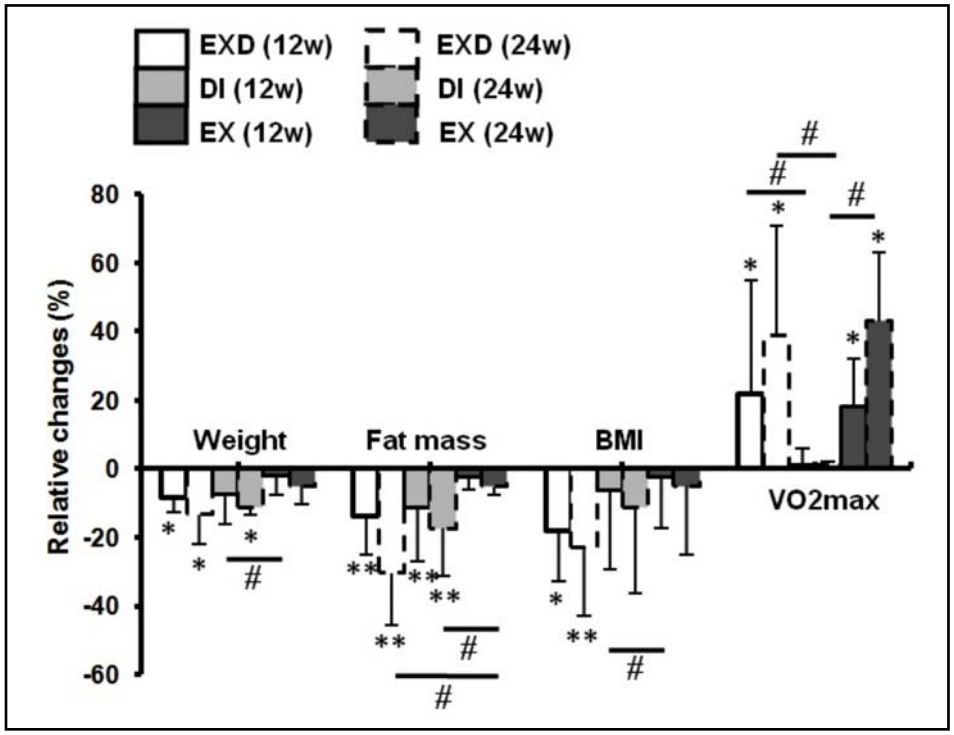

Fig. 1. Changes in weight, fat mass, BMI and maximal oxygen consumption (VO2max) after 12 and 24 weeks in EXD, DI and EX groups. Data are presented as percentage changes in relation to baseline values. EXD: Exercise and diet combined; DI: Diet only; EX: Exercise only. *P<0.05; **P<0.01 *vs* baseline. #P<0.05 changes between groups.

80% of maximum heart rate for 45 min at weeks 20-24 (Lakhdar *et al.* 2013).

Dietary Protocol

A dietician established a balanced and personalised dietary restriction program after an initial dietary assessment in order to define the total amount of calories consumed per day. Subjects in the DI and EXD groups recorded the times and amounts of food and fluid intake for a week before the beginning of the program. The dietary program was set at 2093 kJ per day below the initial dietary records. It was composed of 15% proteins, 55% carbohydrates and 30% lipids (Mathijs *et al.* 2018). The women recorded, in a specifically designed notebook, the quantity of food and the time at which it was eaten (4 times a week). Foods were selected according to the subject's dietary habits. Power Point presentations, videos and role–play scripts were designed for trainers to use during the educational program. Each individual's diet was designed using a Bilnut 4 Software package (SCDA Nutrsoft, Cerelles, France), a computerized database that calculates the food intake and composition from the National Institute of Statistics of Tunis 1978. Body mass was measured every week to assess the immediate effect of the nutritional modifications.

Diet + exercise intervention

The EXD intervention combined the above interventions.

Blood sampling and analysis

Blood samples were collected by venepuncture on 3 occasions: at the beginning of the protocol control value (C), after 12 weeks (S1) and after 24 weeks (S2). Blood samples were collected in the morning at 7 to 8 am after an overnight fast. The plasma was separated and frozen at -80°C for later analysis. Fasting plasma glucose concentrations were measured using an automated device (AU2700, Olympus, France). The interassay coefficient of variability (CV) was 1.7%. An IRMA Insulin kit (Immunotech, France) assayed fasting plasma insulin. The intra-and interassay CV were 3.3-4 % and 3.7-4.8 %, respectively. Insulin resistance was assessed using the homeostatic model assessment for insulin resistance (HOMA-IR). The HOMA-IR has been validated in obese women and was computed as follows: HOMA-IR [insulinemia (μU/ml) x glycemia (mmol/l)]/22.5 (Li *et al.* 2014). Fasting plasma and adipose tissue adiponectin concentrations were measured by immunoradiometric assay (Diagnostic Systems laboratories, USA intra-assay coefficient of variation (CV) was 2.6% and interassay CV was 3.7%) .Fasting plasma and adipose tissue chemerin concentrations were measured by immunoradiometric assay (Diagnostic Systems laboratories, USA intra-assay coefficient of variation (CV) was 5.1 % and interassay CV was 8.3 %).

Adipose tissue biopsies

At baseline and after 24 weeks, the AT biopsies were obtained from the abdominal subcutaneous AT depot

5–10 cm lateral to the umbilicus. The skin was anesthetised with lidocaine (10 mg.ml^{-1}) before a small incision was made, and 200 mg of AT was removed under sterile conditions using a liposuction needle. Immediately after removal, the AT sample was washed in isotonic NaCl, snap-frozen in liquid nitrogen and kept at -80°C.

Statistical analyses

All statistical tests were processed using Statistica software for Windows (version 12.0; Statsoft, Tulsa, OK, USA). The normality of data sets was checked using the Kolmogrov- Smirnov test. Mauchly test was used to test the spherecity and ANOVA two-way (group and time of measurement) with repeated measurements in the second factor was used. Bonferroni test was used as post hoc. Effect sizes were represented by partial eta squared (partial η^2), classified as small (0.01), medium (0.06) and large (0.14) (Nakagawa & Cuthill 2009). Statistical significance was set at ($p < 0.05$). All data were presented as mean and standard deviation.

RESULTS

Changes in anthropometric parameters and VO_{2max}

The anthropometric data of the subjects within each group, at the beginning, after 12 weeks and at the end of the program (after 24 weeks), are shown in table 1.

Subjects in the EXD group obtained a weight loss of 8.18% (7.36 kg, $p < 0.05$) after 12 weeks and of 13.21% (12.36 kg; $p < 0.05$) after 24 weeks. For the DI group, the significant weight decrease of 11.42% is noted only after 24 weeks is (9.1 kg; $p < 0.05$). These groups (EXD and DI) showed also important fat mass losses of 13.74% (5.36 kg; $p < 0.01$) and 11.14% (3.9 kg; $p < 0.05$), respectively, after 12 weeks and of 21.82% (8.51 kg; $p < 0.01$) and 17.42% (6.1 kg; $p < 0.01$), respectively, after 24 weeks. In addition, 12 and 24 weeks provoked a loss in waist circumferences of 2-6 cm ($p < 0.01$) in the EXD group and 2-5 cm in the DI group. In the EXD group, BMI was significantly reduced after 12 ($p < 0.05$) and 24 weeks ($p < 0.01$). There were no changes in the EX group (Fig.1).

Participants in the EXD and EX groups increased their VO_{2max} with 21 and 18% respectively, after 12 weeks ($p < 0.05$) and with 39 and 41% respectively, after 24 weeks ($p < 0.05$). Whereas there was no change in the DI group (Fig.1).

Changes in plasma chemerin and adiponectin levels after the interventions

For plasma HOMA-IR (Table 2), there was only an effect in the time of measurement (17) ($F_{2.52} = 4.82$, $p = 0.012$, partial $\eta^2 = 0.157$, large), with lower values at 24 weeks compared to the initial values ($p = 0.009$). No effect of group ($F_{2.26} = 2.51$, $p = 0.101$, partial $\eta^2 = 0.162$, large) or interaction ($F_{4.52} = 0.80$, $p = 0.534$, partial $\eta^2 = 0.058$, small) were observed.

Plasma chemerin (Table 2) differed between groups ($F_{2.27} = 14.63$, $p < 0.001$, partial $\eta^2 = 0.520$, large) and moment of measurement ($F_{2.54} = 31.04$, $p < 0.001$, partial $\eta^2 = 0.535$, large), but no interaction effect was found ($F_{4.54} = 1.85$, $p = 0.132$, partial $\eta^2 = 0.121$, medium). The exercise plus diet group presented lower chemerin values than diet ($p = 0.006$) or exercise groups ($p < 0.001$). The initial values were higher than those measured after 12 and 24 weeks ($p < 0.001$ for both comparisons).

Plasma adiponectin (Table 2) differed between groups ($F_{2.27} = 103.19$, $p < 0.001$, partial $\eta^2 = 0.884$, large), the moment of measurement ($F_{2.54} = 218.87$, $p < 0.001$,

Tab. 1. The characteristics of the subjects before, after 12 and 24 weeks. Data are presented as means ± SD.

	EXD (n=10)			DI (n=10)			EX (n=10)		
	Before (CS)	**After 12 weeks (S1)**	**After 24 weeks (S2)**	**Before (CS)**	**After 12 weeks (S1)**	**After 24 weeks (S2)**	**Before (CS)**	**After 12 weeks (S1)**	**After 24 weeks (S2)**
Age (Years)		38.90 ± 4.37			38.90 ± 3.94			36.20 ± 5.00	
Height (m)		1.68 ± 0.03			1.60 ± 0.05			1.61 ± 0.05	
Waist circumference (cm)	99 ± 5	97 ± 4	93 ± 5*	98 ± 3	96 ± 2*	93 ± 2**	98 ± 5	98 ± 5	97 ± 5^{c}
Weight (kg)	92.46 ± 5.5	85.10 ± 5.26*	80.30 ± 5.06*	84.60 ± 6.71	79.30 ± 6.18	75.00 ± 6.59*	86.99 ± 7.96	85.00 ± 7.52	82.80 ± 7.52
BMI kg.m^{-2}	32.98 ± 2.17	30.36 ± 2.08*	28.65 ± 2.07**	33.02 ± 1.89	30.94 ± 1.53	29.25 ± 1.55	33.52 ± 3.75	32.76 ± 3.61^{b}	31.92 ± 3.69
Body Fat (%)	42 ± 1.49	39.76 ± 1.28**	38.26 ± 0.84**	41.65 ± 0.97	39.52 ± 0.67**	38.86 ± 0.61**	41.26 ± 1.22	41.24 ± 1.23^{c}	41.12 ± 1.35^{bc}
Body fat mass (kg)	39.26 ± 3.50	33.90 ± 3.08**	30.75 ± 2.47**	35.28 ± 3.44	31.38 ± 2.90*	29.18 ± 2.97**	35.96 ± 4.19	35.13 ± 4.05	34.13 ± 4.10^{bc}
VO2 max (ml.min.kg^{-1})	22.30 ± 2.97	27.10 ± 1.92*^{a}	31.00 ± 2.02*^{a}	20.10 ± 0.94	21.00 ± 1.41	21.00 ± 1.00	21.30 ± 1.42*	25.10 ± 1.22*	29.90 ± 1.14*^{b}

EXD: Exercise and diet combined; DI: Diet only; EX: Exercise only; BMI: Body Mass Index; C: Control; S: Sampling. *P<0.05; **P<0.01 CS *vs* S1 and S2. ^{a}P<0.05 EXD *vs* DI; ^{b}P<0.05 DI *vs* EX; ^{c}P<0.05 EXD *vs* EX.

Tab. 2. Changes in adiponectin, chemerin, HOMA-R and metabolic parameters before, after 12 and 24 weeks. Data are presented as means ± SD.

	EXD (n=10)			DI (n=10)			EX (n=10)		
	Before (CS)	**After 12 weeks (S1)**	**After 24 weeks (S2)**	**Before (CS)**	**After 12 weeks (S1)**	**After 24 weeks (S2)**	**Before (CS)**	**After 12 weeks (S1)**	**After 24 weeks (S2)**
Glucose ($mmol.l^{-1}$)	4.94 ± 0.43	4.83 ± 0.37	4.35 ± 0.40**	4.8 ± 0.40	4.67 ± 0.40	4.48 ± 0.41	4.95 ± 0.44	4.8 ± 0.54	4.62 ± 0.29
Insulin ($\mu U.ml^{-1}$)	15.34 ± 6.37	12.63 ± 7.45	9.53 ± 3.65*	10.33 ± 6.33	10.01 ± 4.52	8.75 ± 5.27	9.48 ± 3.95	9.02 ± 5.31	7.97 ± 4.39
HOMA-R	3.32 ± 1.34	2.69 ± 1.64	1.84 ± 0.68*	2.18 ± 1.32	2.11 ± 1.08	1.72 ± 0.97	2.07 ± 0.86	1.90 ± 1.06	1.95 ± 0.98
Chemerin in plasma (ng/ml)	165.64 ± 28.10	129.18± 18.97*	119.05 ± 12.92**	170.03 ± 40.62	141.96 ± 38.87*	127.46 ± 36.50**	174.81 ± 45.01	150.01 ± 42.23	139.85 ± 43.18
Adiponectin in plasma ($\mu g.ml^{-1}$)	1.72 ± 0.23	2.70 ± 0.32*	3.62 ± 0.31**	4.58 ± 4.41	6.33 ± 5.11	7.88 ± 5.08**	1.71 ± 0.35	1.76 ± 0.26	2.21 ± 0.44
Chemerin in AT ($ng.ml^{-1}$)	184.53 ± 18.27		136.11± 32.42**	182.51 ± 39.10		139.01 ± 40.63**	177.92 ± 40.26		146.87 ± 43.09
Adiponectin in AT ($\mu g.ml^{-1}$)	1.90 ± 0.26		3.39 ± 0.66**	4.55 ± 0.15		6.91 ± 1.11**	1.57 ± 0.30		2.28 ± 0.63*

EXD: Exercise and diet combined; DI: Diet only; EX: Exercise only; HOMA-R: insulin resistance; C: Control; S: Sampling; AT: Adipose tissue.
*P<0.05; **P<0.01 CS *vs* S1 and S2.

partial $\eta^2 = 0.890$, large).An interaction effect was also found ($F_{4.54} = 69.71$, $p < 0.001$, partial $\eta^2 = 0.834$, large). Exercise plus diet group had higher values than the two others ($p < 0.001$ for both comparisons), while the diet group had higher values than the exercise group ($p = 0.024$). Adiponectin increased from the initial moment to 12 weeks and from this point to the 24 weeks ($p < 0.001$ for all comparisons). In addition, we found that first, the initial values did not differ between groups ($p = 1.00$). Second, only the exercise plus diet group had an increase from baseline to 12 weeks and from this time point to the 24 weeks ($p < 0.001$ for all comparisons). The diet group had only an increase from baseline to the 24 weeks and from 12 weeks to 24 weeks ($p < 0.001$ for both comparisons). The exercise group had no change over the period of study ($p = 1.0$ for all comparisons). Finally, the exercise plus diet group presented higher values at the 12-week and 24-week compared to the diet and exercise groups at the same time point ($p < 0.001$ for both comparisons), while the diet group had higher values at the 24-week compared to the exercise group at the same time point ($p < 0.001$).

Changes in chemerin and adiponectin in adipose tissue after the interventions

Chemerin level in Adipose Tissue (Table 2) was influenced by the moment of measurement ($F_{1.27} = 25.62$, $p < 0.001$, partial $\eta^2 = 0.489$, large), and the interaction time x group ($F_{2.27} = 4.44$, $p = 0.022$, partial $\eta^2 = 0.247$, large). However, no main effect of group was found ($F_{2.27} = 3.14$, $p = 0.059$, partial $\eta^2 = 0.189$, large). Chemerin decreased in the 24-week period ($p < 0.001$). Groups did not differ at baseline ($p = 1.0$ for all comparisons). The exercise plus diet ($p = 0.003$) and diet ($p = 0.009$) groups decreased the level of chemerin value across time. Additionally, the exercise plus diet group had lower values at 24-week time-point compared to exercise group ($p = 0.011$).

Adiponectin concentration in Adipose Tissue (Table 2) differed between groups ($F_{2.27} = 191.68$, $p < 0.001$, partial $\eta^2 = 0.934$, large), and moment of the measurement ($F_{1.27} = 593.58$, $p < 0.001$, partial $\eta^2 = 0.957$, large). An interaction between group and moment of measurement was also found ($F_{2.27} = 47.11$, $p < 0.001$, partial $\eta^2 = 0.939$, large). All groups differed between them ($p < 0.001$), with higher values for the exercise plus diet group, followed by the diet group and the exercise group. Adiponectin concentrations increased over time ($p < 0.001$). Additionally, when the interaction effect was considered, groups did not differ at baseline ($p = 1.0$ for all comparisons). All groups increased adiponectin concentrations values across time ($p < 0.001$ for the exercise plus diet and diet groups, and $p = 0.037$ f or the exercise group). However, the final value for the exercise plus diet group was higher than those for the other two groups ($p < 0.001$ for all comparisons), and the diet group had higher final value compared to the exercise group ($p < 0.001$).

DISCUSSION

The present study showed that combined aerobic exercise training and diet restriction program induced greater changes in chemerin and adiponectin plasma

levels or in adipose tissue than diet restriction or aerobic exercise alone in healthy obese women. The combined program was characterized by decreases in chemerin and increases in adiponectin levels.

Our results are similarly like previous research which showed that chronic exercise, often coupled with other lifestyle changes, had a favourable effect on circulating chemerin levels (Stefanov *et al.* 2014, Venojärvi *et al.* 2013, Kim *et al.* 2014, Li *et al.* 2014).

We found that compared with caloric restriction, increased exercise was associated with greater reductions levels of chemerin in serum and adipose tissue, fat mass, insulin resistance, and with increased levels of adiponectin in serum and adipose tissue, despite similar weight loss. Our finding concur with previous studies wherein hypocaloric diets (Chakaroun *et al.* 2012,Blüher *et al.* 2012), combined diet and exercise (Kim *et al.* 2014, Li *et al.* 2014) , and exercise alone (Chakaroun *et al.* 2012,Saremi *et al.* 2010, Stefanov *et al.* 2014, Venojärvi *et al.* 2013) decreased chemerin. However, none of these studies directly compared isocaloric diet and exercise interventions or found greater improvement in adipokines profile and metabolic outcomes with the same degree of exercise-induced weight and fat mass loss.

Therefore, this study provides evidence for the greater benefits of exercise combined to diet compared with diet and exercise only induced fat mass loss on adipokines such as chemerin and adiponectin that were increasingly recognised as novel biomarkers for metabolic outcomes.

Altering circulating adipokines levels can be therapeutic tool to improve insulin resistance and further reduce the risk of metabolic syndrome. The result of this study confirmed that a lifestyle intervention resulting in weight reduction was also associated with decreased circulating chemerin levels. The lifestyle intervention in the current study resulted in significant weight loss and reduction of fat mass, which may contribute to reduction in chemerin levels and HOMA-IR, and an increase in adiponectin levels.

The degree of reduction in circulating chemerin was correlated with the degree of change in HOMA-IR, even after controlling for confounding factors such as age, sex, and changes in adipose tissue and adiponectin levels. The results of the current study showed a close link between circulating chemerin levels and the reduction in HOMA-IR independent of changes in adiponectin levels visceral adipose tissue mass.

The role of chemerin in glucose uptake and insulin sensitivity had previously been tested in humans at baseline and also following weight reduction (Bozaoglu *et al.* 2007, Chakaroun *et al.* 2012, Wasserman *et al.* 1986). Higher circulating chemerin levels were associated with adiposity and metabolic syndrome risk factors including hyperglycaemia (Bozaoglu *et al.* 2007, Petr *et al.* 2018). Moreover, Chakaroun *et al.* (2012), recently demonstrated with both cross-sectional and weight loss intervention studies that mRNA and circulating chemerin levels were correlated with adiposity, HOMA-IR and glucose infusion during euglycemic clamp tests and weight reduction through bariatric surgery, exercise training, and hypocaloric diet significantly decreased circulating chemerin levels. They further demonstrated that an increased glucose infusion rate was a predictor of a reduction in circulating chemerin independent of changes in BMI (Chakaroun *et al.* 2012). Since, Chakaroun *et al.* (2012) measured visceral adipose tissue neither at baseline or in response to body mass reduction, it was unclear whether body mass reduction induced-changes in chemerin levels were independent of visceral adipose tissue.

It has been previously established that adiponectin was associated with weight reduction as well as insulin sensitivity. However, the role of adiponectin in determining the risk of metabolic syndrome in association with changes in chemerin levels still needs to be clarified. The results of the current study add to previous finding that a body mass reduction-induced change in chemerin levels via physical exercise combined with diet was associated with improvement in HOMA-IR independent of changes in visceral adipose tissue and circulating adiponectin levels.

It was still unclear whether insulin resistance contributes to elevated chemerin levels or whether elevated chemerin levels contribute to insulin resistance. Hyperinsulinemia was found to up-regulate circulating chemerin levels, whereas metformin decreases chemerin levels. Six months of metformin treatment reduced circulating chemerin levels (Tan *et al.* 2009, Bauer *et al.* 2012). Further reported that reduced chemerin was the only predictor of changes in HOMA-IR even after controlling for changes in waist-hip ratios, glucose levels and insulin. This suggests that the close link between the metformin-induced reduction in chemerin and HOMA-IR is independent of abdominal adiposity.

Two previous studies shed some light on the role of chemerin in the regulation of glucose and insulin levels (Takahashi *et al.* 2011, Ernst *et al.* 2012). Chemerin in the regulation of glucose and insulin levels (Takahashi *et al.* 2011, Ernst *et al.* 2012) can cause adipose development, glucose intolerance.

Even though, Chemerin messenger RNA (mRNA) expression increases with adipocyte size (Sell *et al.* 2009) and is lower in lean compared with obese and diabetic individuals (Chakaroun *et al.* 2012) while changes in production of other adipokines, particularly adiponectin, may also influence chemerin production (Suzuki *et al.* 2012). They found that baseline chemerin concentration and insulin resistance (HOMA-IR), and reductions in both after fat mass loss, were also significantly correlated, as in previous lifestyle intervention studies (Kim *et al.* 2014). Chakaroun *et al.* (2012) noted that the reduction in chemerin was significantly associated with improvement in insulin sensitivity after

12 weeks of moderate-intensity exercise. Direct links between insulin and chemerin production were demonstrated in human adipose cells where insulin infusion stimulated chemerin synthesis (Tan *et al.* 2009) and in human skeletal muscle cells where administration of chemerin increased insulin resistance (Suzuki *et al.* 2012) and impaired insulin receptor signalling and glycogen synthase kinase 3 phosphorylation. The improvement in insulin sensitivity in addition to being associated with reduction in adiposity my thus also be directly mediated by chemerin reduction as shown by association of reductions in chemerin and HOMA-IR after correction for fat mass loss (Khoo *et al.* 2015).

Additionally, two recent studies observed associations between changes in chemerin and improvements in insulin sensitivity following chronic exercise interventions. Stefanov *et al.* (2014) found decreases in HOMA-IR. Likewise, Li *et al.* (2014) observed a correlation between decreases in serum chemerin and improvements in the insulin sensitivity index following a 12-week lifestyle modification program which also included both aerobic and resistance exercise.

One of the first and most obvious limitations of the current study is the lack of a control group and small sample size. Randomized control trials with bigger sample size are recommended to adequately study the effects of diet and diet combined with exercise on circulating chemerin levels. Furthermore, the chemerin ELISA used in the present study cannot distinguish between pro-chemerin and chemerin. The current study confirmed that lifestyle modifications including exercise and a hypocaloric diet significantly reduced circulating chemerin levels. It also showed a significant association between the degree of change in chemerin level and the degree of change in HOMA-IR level independent of changes in other adipocytokines.

In summary, this investigation showed that the exercise combined with diet significantly reduced subcutaneous adipose tissue and further reduced insulin resistance, and to the circulating chemerin, knowing that the degree of change in chemerin level was significantly associated with the degree of change in HOMA-IR even after adjusting for the change in subcutaneous adipose tissue and adiponectin levels suggests that a reduction in chemerin levels may be an independent contributor to the improvement in insulin resistance.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related to the publication of this article.

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