

# Effect of Lifestyle Modification Combined with Metformin on Serum Chemerin Concentration in Metabolic Syndrome Subjects

Brama Ihsan Sazli<sup>1</sup>, Dharma Lindarto<sup>1\*</sup>, Dian Anindita Lubis<sup>1</sup>, Melati Silvani Nasution<sup>1</sup>

<sup>1</sup>Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara  
H. Adam Malik Hospital, Jalan Bunga Lau No 17, Medan 20136, Indonesia

**Keywords:** Lifestyle modification, Chemerin, Metabolic Syndrome.

**Abstract:** Chemerin is an adipokine that plays an important role in inflammation development and insulin resistance by accommodating macrophage infiltration into adipose tissue. This study aimed to determine the effect of lifestyle modification with and without metformin on chemerin in metabolic syndrome. Forty-five metabolic syndrome subjects (IDF-2005) were randomly assigned to one of the two groups: placebo group ( $n=22$ ) and metformin group ( $n=23$ ). Both groups underwent a 12-week lifestyle modification (diet and moderate aerobic-exercise). Only 40 participants (placebo group  $n=20$  and metformin group  $n=20$ ) completed the survey whereas 5 participants dropped out of the study. After their lifestyle was modified, body weight, body mass index, waist circumference, and chemerin decreased significantly ( $P<0.001$ ) in both groups. Moreover, there was a significant difference between both groups in body weight, BMI, and WC ( $P<0.05$ ) but not for chemerin. Thus, lifestyle modification with metformin improved BW, BMI, and WC on metabolic syndrome, and there were no significant differences in reduced chemerin between placebo and metformin groups. Further investigations should be done to establish the effect of lifestyle modification combined with metformin on chemerin after an extended follow-up period.

## 1 INTRODUCTION

Metabolic syndrome represents a combination of cardiometabolic risk factors including glucose intolerance, insulin resistance, central adiposity, hypertension, non-alcoholic fatty liver disease, and dyslipidemia. The prevalence of metabolic syndrome increases rapidly worldwide as a result of the continuous obesity epidemic. This will also increase global risk in the incidence of cardiovascular disease and type 2 diabetes mellitus (T2DM) (Bruce and Byrne, 2009). Adiposity has been known as an important endocrine organ that does not only store energy but also regulates metabolism and energy homeostasis (Rosen, 2006)

Chemerin, an adipokine that was recently found, increased its expression in obesity state (Goralski *et al.*, 2007; Bozaoglu *et al.*, 2007). Several specific functions of chemerin are regulation of specific immune cell migration (Zabel, Silverio and Butcher, 2007), anti-inflammatory effects on macrophages (Cash *et al.*, 2008), and regulation of adipogenesis (Zabel, Silverio, and Butcher, 2007). Previously, a significant association has been identified between characteristics of the metabolic

syndrome and circulating chemerin levels in a relatively small sample of human subjects from Mauritius (Bozaoglu *et al.*, 2007). Bozaoglu *et al.* (2009) evaluated plasma chemerin concentration in human subjects and found that plasma chemerin concentrations were highly associated with body mass index (BMI), blood pressure, and plasma triglycerides. And in women with polycystic ovary syndrome, treatment with metformin decreases serum chemerin levels. (Tan *et al.*, 2009).

Physical inactivity is well known as the risk factor for T2DM (Venables and Jeukendrup, 2009) and aerobic training in obese adults has been shown to reduce adiposity and insulin resistance (O'Leary *et al.*, 2006). There has been no previous report about lifestyle modification induces alteration in chemerin concentrations in metabolic syndrome, which may serve a connection between obesity and insulin resistance. Modification of lifestyle against overweight, physical inactivity, and atherogenic diet has been recommended as a primary in the management of metabolic syndrome (Eckel, Grundy and Zimmet, 2005). However, lifestyle modifications alone often cannot achieve clinically

meaningful weight loss (Miler, Kojeca and Hamilton, 1997).

Metformin, a biguanide insulin sensitizer agent has been shown to decrease body weight, hyperinsulinemia, and hyperglycemia in adult patients with T2DM (UKPDS 34, 1998). Metformin has recently also been suggested to increase the effect of insulin sensitivity from exercise (Sharoff *et al.*, 2010), inhibit platelet aggregation, as an antioxidant activity, reduce weight, and gives effect on lipid parameters like total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and arterial hypertension [Glueck *et al.*, 2001; Wulffele *et al.*, 2004]. Metformin does not induce hypoglycemia so it can be given safely to euglycemic patients (Pasquali *et al.*, 2000).

Therefore, the aim of this study was to evaluate the effect of lifestyle modification combined with metformin on chemerin in metabolic syndrome.

## 2 MATERIALS AND METHODS

The research subjects were recruited in Haji Adam Malik Hospital, Medan, Indonesia. From 184 obese nurses (BMI  $\geq 25$ ), only 45 subjects were diagnosed with metabolic syndrome (IDF-2005) and agreed to involve in lifestyle modification for 12 weeks. Diagnosis of metabolic syndrome by IDF-2005 guidelines were used, namely central obesity with a Waist Circumference (WC) of  $\geq 90$  cm for men and  $\geq 80$  cm for women (Asian population), and any two of the following four factors: (1) triglyceride  $\geq 150$  mg/dl or specific treatment for lipid abnormality; (2) HDL-C (men  $< 40$  mg/dl and women  $< 50$  mg/dl) or specific treatment for lipid abnormality; (3) hypertension  $\geq 130/85$  mmHg or history of treatment previously diagnosed hypertension; and (4) FBS  $\geq 100$  mg/dl or previously diagnosed T2DM (WHO, 2004; Grundy *et al.*, 2005). They were divided randomly to either the placebo group ( $n=22$ ) or metformin group ( $n=23$ ).

All subjects gave their fully informed consent before participated in the study. The Ethics Committee of Medical Faculty of Universitas Sumatera Utara has approved the study. We excluded subjects who had cardiovascular diseases or any other major illnesses, smokers, or were taking medications that could affect the laboratory test results. Before and during the study, trained health nurses and participants discussed the lifestyle

modification programs including diet and exercise. To facilitate changes in behavior, each participant receives leaflets and diaries to record their behavioral performance, diet, physical activity, WC, and body weight (BW) monitored by phone

. Participants attended a follow-up meeting every week to confirm how the participants had complied with the targeted behaviors and checked whether the participants had experienced any health and safety problems related to behavioral changes including the side effect of drugs.

### 2.1 Anthropometric Measurements

Weight in kilograms (kg) and heights in meters (m) were measured, and the weight in kilograms divided by the square of the height in meters to calculated BMI as For the Asian population, BMI  $< 18.5$  is classified as underweight, BMI 18.5-22.9 is classified as normal, BMI 23-24.9 is classified as overweight, BMI 25-29.9 is classified as obese I and BMI  $\geq 30.0$  is classified as obese II. The WC was measured midway between the uppermost border of the iliac crest and the lower border of the coastal margin (rib cage), and using Asian values (male  $\geq 90$  cm; female  $\geq 80$  cm) (WHO, 2004). The exercise program consists of moderate aerobic exercise at least 3 times per week (30 minutes each) (Misra, Misra and Wijesuriya, 2006). The aerobic exercise was supervised by a physiotherapist at each training session. The exercise group performed a warm-up exercise for 5 min, followed by the main exercise for 20 min, and relaxation exercise for 5 min at the end of the exercise period (PERKENI, 2015).

### 2.2 Diet

Between 0 and 12-week period throughout the study, all subjects followed a standard weight maintenance diet (55–60% carbohydrate, 15–20% protein, and 20–25% fat) (PERKENI, 2015). All subjects were free to consume and choose the food according to their dietary habits and from the list of food replacement.

### 2.3 Blood Pressure and Blood Sample Analysis

Blood pressure was measured by an average of twice measurement after a 10-minute break with a mercury sphygmomanometer. After overnight fasting and collection, blood samples were centrifuged for 15 min while plasma and serum containing tubes were stored at  $-20^{\circ}\text{C}$  until analysis. Blood glucose levels

were measured by photometer autoanalyzer Modular P 800, plasma HDL-C and LDL-C were measured by the Architect Ci 8200 (Abbott, USA), triglyceride was measured by GPO-PAP methods of Architect, hs-CRP was measured by sensitive immunoassay (Siemens Medical Solution Inc, IL, USA) of Immulite 1000, HbA1c was measured by high-performance liquid chromatographic (HPLC) of the Bio-Rad D 10, and chemerin was measured by Mediagnost ELISA E-102 (Sandwich-Assay).

## 2.4 Statistical Analysis

Data were presented as mean  $\pm$  SD. The normality assumption of the placebo group and metformin group data were evaluated and confirmed using Shapiro-Wilk in each group. Differences between and within each data of the placebo group and metformin group were tested using an independent sample t-test and dependent t-test. However, the abnormal data were tested using the Mann-Whitney U test and Wilcoxon test. Two-sided *P*-values of

$<0.05$  were considered as statistically significant. The data were analyzed using SPSS 22 software.

## 3 RESULTS

Of the 45 participants at the baseline, 40 participants (placebo group,  $n=20$ ; metformin group,  $n=20$ ) completed in the 12-week survey, whereas 5 participants (2 participants from the placebo group and 3 participants from the metformin group) dropped out of the study. In Table 1, there was no significant difference in the baseline characteristics of the two groups.

In Table 2, after 12 weeks of lifestyle modification, there was a significant decrease in BW, BMI, WC, SBP, and chemerin in placebo and metformin group. But there were no statistically significant differences in reduced chemerin between the two groups.

Table 1: Clinical Characteristics of Placebo and Metformin Groups on Metabolic Syndrome Subjects

Variable	Placebo group	Metformin group	<i>P</i> <sup>a</sup>
	=22	=23	
<i>n</i> (F/M)	17/5	20/3	
Age (yr)	40.41 $\pm$ 5.61	42.91 $\pm$ 5.62	0.142
BW (kg)	76.98 $\pm$ 11.64	80.53 $\pm$ 14.72	0.374
BMI (kg/m <sup>2</sup> )	32.11 $\pm$ 4.09	34.03 $\pm$ 5.76	0.206
WC (cm)	95.40 $\pm$ 7.41	97.23 $\pm$ 10.95	0.927
SBP (mmHg)	123.18 $\pm$ 11.29	125.21 $\pm$ 19.74	0.567
DBP (mmHg)	82.00 $\pm$ 9.99	80.65 $\pm$ 10.69	0.247
HDL-C (mg/dl)	45.72 $\pm$ 8.48	48.34 $\pm$ 15.47	0.918
TG (mg/dl)	150.40 $\pm$ 51.05	152.00 $\pm$ 63.87	0.974
FBG (mg/dl)	85.27 $\pm$ 11.98	86.69 $\pm$ 10.06	0.351
PPG (mg/dl)	115.72 $\pm$ 11.98	104.21 $\pm$ 21.40	0.401
hs-CRP (mg/dl)	3.45 $\pm$ 2.47	3.83 $\pm$ 2.34	0.467
Chemerin (ng/mL)	344.09 $\pm$ 104.58	345.15 $\pm$ 83.90	0.970

BW: body weight; BMI: Body Mass Index; CRP: C-reactive protein; DBP: Diastolic Blood Pressure; FBS: fasting Blood Sugar; PPG: Postprandial Glucose; SBP: Systolic Blood Pressure; TG: Triglyceride; WC: Waist Circumference; PPG: Postprandial glucose.

Table 2: Clinical Characteristics of Placebo and Metformin Groups after 12-Week Follow-Up for both Groups of Metabolic Syndrome Subjects

Variable	Placebo group (n=20)				Metformin group (n=20)				P <sup>d</sup>
	Baseline	At 12-week	Difference	P <sup>b</sup>	Baseline	At 12-week	Difference	P <sup>c</sup>	
Weight (kg)	77.6 ± 11.0	75.2±10.8	-2.3	0.001**	81.4±14.6	77.4±14.5	-3.9	0.001**	0.001*
BMI (kg/m <sup>2</sup> )	32.1 ± 4.1	30.9±4.1	-1.1	0.001**	34.2±5.6	32.4±5.6	-1.8	0.001**	0.002*
WC (cm)	95.7 ± 7.3	89.9±7.5	-5.8	0.001**	97.9±11.5	91.8±10.7	-6.2	0.001**	0.047*
SBP (mmHg)	123.5±11.4	114.0±8.2	-9.5	0.007**	127.0±20.3	112.8±8.5	-14.3	0.001**	0.231
DBP (mmHg)	82.2±10.5	69.0±5.5	-31.8	0.001*	80.6±11.0	67.5±7.2	-13.3	0.001**	0.300
HDL-C (mg/dl)	46.4±8.5	45.3±10.0	-1.1	0.628	48.9±16.4	46.5±9.8	-2.4	0.653	0.103
TG (mg/dl)	147.5±50.5	153.3±67.9	5.8	0.634	152.3±66.9	149.0±102.4	-3.3	0.147	0.285
FBS (mg/dl)	83.4±10.6	91.7±20.64	8.3	0.001*	84.9±8.9	87.7±10.7	2.8	0.305	0.039*
PPG (mg/dl)	114.9±35.4	112.3±37.7	-2.5	0.717	105.1±22.4	102.3±19.3	-2.8	0.491	0.142
CRP (mg/dl)	3.6±2.5	3.0±2.2	-0.6	0.048*	3.9±2.4	3.5±1.9	-0.6	0.327	0.482
Chemerin (ng/mL)	339.5±106.6	225.5±43.7	-113.9	0.001**	339.9±82.9	226.5±46.1	-113.9	0.001**	0.430

BMI: Body Mass Index; CRP: C-reactive protein; DBP: Diastolic Blood Pressure; FBS: Fasting Blood Sugar; PPG; SBP: Systolic Blood Pressure; TG: Triglyceride; WC: Waist Circumference; 2h-PPG: 2 hour-Postprandial glucose.

<sup>a</sup> Difference between the baseline and 12-week follow-up surveys

<sup>b</sup> Difference between the baseline and 12-week follow-up surveys in the Placebo group, based on a dependent *t*-test.

<sup>c</sup> Difference between the baseline and 12-week follow-up surveys in the Metformin group, based on a dependent *t*-test.

<sup>d</sup> Δ Difference between the Placebo group and Metformin group after the 12-week follow-up surveys, based on an independent *t*-test.

\**P* < 0.05, \*\**P* < 0.01

## 4 DISCUSSIONS

Adipose tissue is an active organ secreting many metabolically important proteins known as adipokines (Lau *et al.*, 2006). Some of these adipokines have important functions in insulin resistance and cardiovascular complications with

central or visceral obesity (Murakami *et al.*, 2006). The latest systematic review of the literature promotes the dose-response effect of aerobic exercise on visceral adiposity, but the ability of exercise to reduce visceral adipose tissue is less significant in those who have metabolic disorders (Kelley and Kelley, 2007). Thus, it remains unclear whether a dose-response of exercise on central

adiposity is also consistent in metabolic syndrome. Nevertheless, regular exercise plays an important role in abdominal fat loss during weight maintenance and can avoid weight gain in those who have successfully reduce body weight. (Wing and Hill, 2001). Chemerin may play an important role in the metabolic syndrome and may be an independent promising adipokine marker.

In these studies, lifestyle modification decreased BW, BMI, WC, diastolic BP, and chemerin significantly in the placebo group and metformin group, whereas FBS and CRP only decreased significantly in the placebo group.

Saremi *et al* in 2010 showed that chemerin levels decreased significantly after body weight reduction (particularly visceral fat) in overweight and obese males after 12-week of aerobic training. The recent results of a large-scale epidemiological study from Mauritius also indicate the same results (Bozaouglu *et al.*, 2007).

Plasma CRP levels are well known to be an important part of systemic inflammation. Some previous studies have reported the effect of exercise training on plasma CRP concentrations. Mattusch *et al.* found a significant reduction in CRP levels after nine months of marathon training in 12 athletes. Furthermore, Smith *et al.* also reported lower CRP levels in 43 volunteers after six months of exercise training. Based on these studies, chemerin decreased more significantly than CRP, and the future chemerin might replace the position of CRP as the key index of systemic inflammation.

Thus, lifestyle modification with metformin improved BW, BMI, WC, and chemerin on metabolic syndrome. But there were no significant differences in reduced chemerin between placebo and metformin groups. Esteghamati *et al* in 2014 found 3 months monotherapy with metformin was associated with a significant reduction in chemerin in type 2 diabetes patient, so we need further evaluation of using metformin to reduce chemerin in a nondiabetic patient like in this study.

There are some limitations to our study. Some detailed exercise records by the participant were not obtained, which can attenuate the outcome of some adipokines. In addition, we did not evaluate whether the beneficial effects on b-cell function, insulin sensitivity, glycemic control, other inflammatory parameters support this result. We evaluated only a limited number of inflammation biomarkers. Longer and larger sample size studies are needed to evaluate the positive effects lifestyle and metformin on chemerin level, as to prevent cardiovascular event related to metabolic syndrome.

## 5 CONCLUSIONS

Lifestyle modification with metformin improved BW, BMI, WC on metabolic syndrome, and there was no significant decrease of chemerin between placebo and metformin groups. Further investigations should be done to confirm the effects of lifestyle modification combined with metformin on chemerin after an extended follow-up period.

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