Endocrinology & Metabolic Syndrome
ISSN: 2161-1017

Aghamohammadi et al., Endocrinol Metab Syndr
2018, 7:6
DOI: 10.4172/2161-1017.1000295

Research Article Open Access

Whey Supplementation Combined with Energy-Restricted Diet Alleviates 2-Arachidonoylglycerol, Adipocytokines, Inflammatory Factors and Body Composition in Obese Women with Metabolic Syndrome: A Randomized Controlled Trial

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Received date: December 04, 2018; **Accepted date:** December 26, 2018; **Published date:** December 31, 2018

Abstract

This study is designed to determine the effect of whey protein on 2-AG, some biochemical indices and body composition in women with metabolic syndrome under a weight loss diet. Each subject was assigned randomly to one of two groups: whey group (n=12) received whey supplementation for 8 weeks (30 gr daily) + weight loss diet, and control group (n=12) received only weight loss diet. For weight loss intervention, all volunteers consumed -800 kcal/day under estimated energy requirements. The results of ANCOVA test showed that changes of weight, BMI did not differ significantly between whey and control groups after the intervention, but values of percent changes of WC (-3.84%), body fat (-7.46%), fat free mass percent (0.37%), 2-AG (-41.1%), TNF-α (-19.07%), IL-6(-15.28) and adiponectin (45.93%) were significant difference between two groups. For the first time, the effects of simultaneous weight loss diet and whey protein supplementation on 2-AG was demonstrated in this study, and the other benefits of whey protein supplementation on health were reported. It was concluded that using ways other than weight loss could reduce endocannabinoids and other metabolic risk factors.

Keywords: Whey protein; Weight loss diet; 2-Arachidonoylglycerol (2-Ag); Glycemic indices; Adipocytokines; Inflammatory factors; Body composition

Introduction

Contrary to initial assumptions, adipose tissue is not just a fatty tissue, but also has a great role in regulating the metabolism of the body. At least part of this effect is related to the secretion of the proteins that are called Adipokines, but next to them, cytokines, chemokines and various growth factors are also secreted by adipose tissue. Hence, today, fat tissue is considered as an endocrine tissue [1].

Differentiated adipocytes express CB1R (cannabinoid 1 receptor) and the enzymes involved in endocannabinoids (ECs) biosynthesis and degradation, thus, cellular ECs levels are comparable with those in the brain [2,3]. CB1R stimulation induces the activation of lipoprotein lipase and decreased expression of adiponectin, also, contributes to lipid accumulation by providing fatty acids for re-esterification into triglycerides and by reducing fatty acid oxidation [4,5]. Endocannabinoid system dysregulation in obesity is accompanied by increased generation of visceral adipose tissue TNF-α which, in turn, stimulates EC system activation *in vitro*, thereby generating a potential vicious circle for atherogenic inflammation [6]. Arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), endogenous ligands of CB1R, are the two most widely studied ECs that Generated "on demand" via enzymatic cleavage from membrane phospholipid precursors [7]. Overweight/obese and type 2 diabetes patients exhibit increased plasma AEA and 2-AG levels [6]. Endocannabinoids system is an established player in CNS control of food intake and systemic macronutrient metabolism between gastrointestinal tract, liver, muscle, and adipose [8-10].

Although plasma levels of endocannabinoids are too low to produce hormone-like activation of CB1 receptors, the increased plasma levels of endocannabinoids detected in obese subjects could also reflect increased endocannabinoid activity in this group [4,5-11]. Changes in levels of endocannabinoids in obesity are different by gender, amount of visceral adipose tissue and insulin levels. Studies have shown that increased plasma 2-AG levels correlate with abdominal adipose, not subcutaneous adipose of obese subjects [2,12]. The deregulation of 2-AG levels is not only a result of obesity, an increase in 2-AG itself is one of the main causes of visceral adiposity and insulin resistance [5,12].

Apart from the effect of PUFA (polyunsaturated fatty acid) and other fatty acids on endocannabinoids levels, the impact of other nutrients on these variables is unclear [13,14].

Contrary to the role of endocannabinoids in the increase of metabolic changes related to obesity, the effects of whey protein and other dairy products on these endogenous lipids are not considered. Therefore, this study is designed to determine the effect of whey

protein on 2-AG, leptin, adiponectin, TNF-alpha, Il-6 and body composition in obese women with metabolic syndrome under a weight loss diet.

Materials and Methods

This clinical trial study was approved by the Ethics Committee of Ahvaz University of Medical Sciences and it was registered on the Iranian Registry of Clinical Trials website (IRCT2017021410181N8). Twenty-four obese women with metabolic syndrome who met inclusion criteria were included in the study. Inclusion criteria consisted of aged 18 years and older, BMI range of 30 to 40 kg/m^2 and voluntary to participation. Subjects with Metabolic Syndrome were diagnosed according to the international criteria, i.e., meeting at least three of the following criteria: fasting blood sugar (FBS) >100 mg/dl, triglyceride (TG) ≥ 150 mg/dl, blood pressure (BP) ≥ 130/ 85 mmHg, HDL-C (high density lipoprotein cholesterol) <40 mg/dl (men) or <50 mg/dl (women), and waist circumference (WC) >95 cm (both men and women). WC cutoff value for abdominal obesity has been defined by the Iranian National Committee of Obesity's report, for Iranian population. Subjects were excluded with following characteristics: menopause, lactation, pregnancy, food allergies, having cancer, hepatic and renal failure, thyroid, endocrine and gastrointestinal disorders, surgery for weight loss, weight loss over the past 6 months, taking herbs and vitamin-mineral supplements and a drug that reduce appetite and weight.

After obtaining informed consent, each subject was assigned randomly to one of two groups: whey group (n=12) received whey supplementation for 8 weeks (30 g daily) + weight loss diet, and control group (n=12) received only weight loss diet. Whey protein powders were supplied by Karen Pharma & food supplement Co., Tehran, Iran. Each of the sachet comprised 116 kcal, 0.5 g of lipid, 0.4 g of carbohydrate, and 27.5 g of protein. The sample size was estimated based on the studies that had changes in endocannabinoids level, in response to weight loss [15-17]. At baseline, each participant was interviewed to acquire demographic information. At baseline and end of study the International Physical Activity Questionnaire (IPAQ) was applied to assess the physical activity [18]. For weight loss intervention, all volunteers consumed -800 kcal/day under estimated energy requirements. Distribution of macronutrients was as follows: carbohydrate 55%, fat 30% and 15% protein [19]. Energy requirements were determined by Mifflin Jeor St equation [20]. Participants were also interviewed about their dietary intake using 24-hour dietary recalls (2- week days and 1- weekend day) at the baseline and end of the study. In order to analyze 24-hour dietary recalls Nutritionist IV software (the Hearst Corporation, San Bruno, CA) was applied. In order to evaluate compliance, intervention group subjects were contacted every three days by a dietitian. Any volunteer that failed to consume less than 90% prescribed whey sachets, removed from the study for noncompliance to the protocol. All participants were recommended to avoid changes in their habitual diet or physical activity levels during the intervention.

At baseline and end of study, weight and height were measured to the nearest 0.5 kg and 0.1 cm, respectively. BMI was calculated as weight (kg) divided by height squared (m^2). Waist circumference (WC) was measured in the standing position at above the iliac crest, just below the lowest rib margin at the end normal expiration. TANITA BC-418 body composition analyzer was applied to measure total body fat and fat free mass percent.

At the beginning and 8th week of the trial, 10 ml blood samples were collected after overnight fasting. For measurement of plasma 2-AG, the blood in EDTA coated tubes were centrifuged at 1500 g at 4°C for 15 min and were stored at -80°C. ELISA kits (HANGZHOU EASTBIOPHARM CO., LTD.) were used to determine levels of 2-Ag, leptin, adiponectin, IL-6 and TNF- alpha. Blood glucose and insulin levels were determined by the enzymatic method with kits from Pars-Azmoon (Tehran, Iran) and chemiluminescent immunoassay (LIAISON analyzer (310360) Diasorin S.P.A, Vercelli, Italy), respectively. Homeostasis model assessment – insulin resistance (HOMA-IR) was estimated by the following formula: fasting glucose (mg/dl) × fasting insulin (μu/ml)/405.

Statistical Analysis and Covariates

The data were analyzed using IBM SPSS Statistics software version 24 (IBM SPSS Statistics, Armonk, USA). A p value less than 0.05 was considered to be statistically significant. The normality of variables was determined using Kolmogorov- Smirnov test. For comparison of categorical data between treatment groups at baseline, chi-square test was used. Independent sample t-test and the paired sample t test were used for comparing parametric continuous data between and within the groups, respectively. Mann-Whitney test and Wilcoxon test were used to test the differences in asymmetric variables between and within the groups, respectively. To control confounding variables, analysis of covariance (ANCOVA) test were applied to test the differences between the two groups post intervention (adjusting for changes in intake of energy, percent of carbohydrate, protein, total fat, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and other fat and baseline values). All categorical and numeric variables were expressed as number (percentage) and mean ± standard deviation, respectively.

Results

Baseline characteristics of the participant in the study groups are shown in Table 1. The initial characteristics were similar in the intervention and control groups. Results of 24-h recalls revealed no significant differences in the intake of energy, macronutrients, SFA, MUFA, PUFA and other fats between the two groups at baseline and at the end of the intervention period (Table 2).

Variable	Intervention group (n=12)	Control group (n=12)	p[c]
Age(yrs.)a	31.6 (5.33)	32.16 (6.46)	0.836
Weight(kg)a	96.37 (9.11)	92.38 (7.85)	0.251
Height(m)a	1.64 (0.03)	1.63 (0.03)	0.365

BMI (kg/m^2)a	35.68 (3.20)	34.63 (2.28)	0.364
WC (cm)a	105.00 (8.78)	103.83 (8.43)	0.743
TG (mg/dl)a	156.41 (34.67)	152.16 (55.3)	0.824
HDL-c (mg/dl)a	42.08 (3.31)	41.16 (4.91)	0.598
FBS (mg/dl)a	87.33 (10.03)	87.25 (9.30)	0.983
SBP (mmHg)a	129.67 (7.45)	129.08(8.72)	0.862
DBP (mmHg)a	87.50 (7.39)	85.33(7.22)	0.475
Physical activityb			1.000
Low	10 (83.3%)	10 (83.3%)	
Moderate	2 (16.7%)	2 (16.7%)	
High	0(0%)	0(0%)	

Note: BMI: body mass index, WC: waist circumference, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, FBS: fasting blood sugar, SBP: systolic blood pressure, DBP: diastolic blood pressure.

[a]Mean (SD).

[b]Number (%).

[c]Independent t-test for numeric variables and Pearson Chi-Square test for categorical variables.

Table 1: Baseline characteristics of study participants.

Variable	Intervention group (n=12)	Control group (n=12)	p
Energy (kcal/d)			
Before	2667.99 (179.15)	2593.53 (182.49)	0.324[a]
After	1699.88 (178.10)	1620.74 (178.23)	0.288[b]
P[c]	0.000	0.000	
Carbohydrate (%energy)			
Before	66.08 (6.96)	64.16 (8.12)	0.541[a]
After	54.59 (0.52)	54.83 (0.57)	0.296[b]
P[c]	0.000	0.003	
Protein (%energy)			
Before	12.25 (2.89)	13.8 (2.48)	0.164[a]
After	15.07 (0.52)	14.83 (0.71)	0.357[b]
P[c]	0.006	0.047	
Total Fat (% energy)			
Before	21.66 (5.61)	22.00 (8.25)	0.909[a]
After	30.33 (0.77)	30.33 (0.77)	1.000[b]
P[c]	0.000	0.007	
SFA (% energy)			
Before	6.16 (2.4)	6.91 (2.96)	0.504[a]

After	7.32 (2.38)	7.91 (2.97)	0.592[b]
P[c]	0.000	0.000	
MUFA (% energy)			
Before	4.75 (2.09)	6.16 (3.56)	0.248[a]
After	4.16 (2.12)	5.25 (3.67)	0. 384[b]
P[c]	0.025	0.000	
PUFA (% energy)			
Before	4.75 (2.56)	4.92 (3.80)	0.901[a]
After	13.84 (4.46)	13.33 (5.69)	0.810[b]
P[c]	0.000	0.005	
Other fat (% energy)			
Before	5.66 (3.48)	4.83 (2.33)	0.497[a]
After	5.01 (3.16)	3.83 (2.33)	0.311[b]
P[c]			

Note: SFA: saturated fatty acid; MUFA: mono unsaturated fatty acid; PUFA: poly unsaturated fatty acid.

Results are expressed as mean (SD). P-values of statistical significance ($p < 0.05$) are presented in bold.

[a]Independent t-test for intake of energy, carbohydrate, protein and total fat and Mann-Whitney U for SFA, MUFA, PUFA and other fat.

[b]Analysis of covariance (adjusted for changes in intake of energy, percent of carbohydrate, protein, total fat, SFA, MUFA, PUFA and other fat and baseline values).

[c]Paired t-test intake of energy, carbohydrate, protein and total fat and Wilcoxon for SFA, MUFA, PUFA and other fat.

Table 2: Daily dietary intakes of the study participants at baseline and 8weeks after the intervention.

As shown in Table 3, at baseline entry, there were no significant differences in anthropometric measurements between the study groups. After whey supplementation in subjects in the intervention group, the all anthropometric variables were decreased significantly except fat free mass, that its change did not quite reach statistical significance. In the control group, over the eight weeks, the all anthropometric measurements decreased significantly. The results of ANCOVA test (adjusted for changes in intake of energy, percent of carbohydrate, protein, total fat, SFA, MUFA, PUFA and other fat and baseline values), showed that changes of weight, BMI did not differ significantly between whey and control groups after the intervention, but values of percent changes of WC (-3.84%), body fat (-7.46%) and fat free mass percent (0.37%) were significant difference between two groups.

Variable	Intervention group (n=12)	Control group (n=12)	p
Body weight(kg)			
Before	96.37 (9.11)	92.28 (7.85)	0.251[a]
After	92.29 (9.00)	88.67 (8.07)	0.464[b]
P[c]	0.000	0.000	
BMI (kg/m^2)			
Before	35.68(3.19	34.63 (2.28)	0.364[a]
After	34.19(3.17)	33.27 (2.35)	0.694[b]
P[c]	0.000	0.000	
WC (cm)			
Before	105.00 (8.78)	103.83 (8.43)	0.742[a]

After	100.96 (8.39)	101.91 (8.60)	0.000[b]
P[c]	0.000	0.000	
Body fat (%)			
Before	45.36 (2.43)	44.32 (2.54)	0.339[a]
After	41.93(2.32)	42.2(2.47)	0.001[b]
P[c]	0.000	0.000	
Body fat free mass (%)			
Before	24.15(1.46)	24.36(1.08)	0.695[a]
After	24.22(1.18)	22.88(1.13)	0.001[b]
P[c]	0.691	0.000	

Note: BMI: Body Mass Index; WC: Waist Circumference

Results are expressed as mean (SD). P-values of statistical significance ($p < 0.05$) are presented in bold.

[a]Independent t-test.

[b]Analysis of covariance (adjusted for changes in intake of energy, percent of carbohydrate, protein, total fat, SFA, MUFA, PUFA and other fat and baseline values).

[c]Paired t-test.

Table 3: Anthropometric indices of study groups at baseline and 8weeks after the intervention.

No significant between-group differences were detected in biochemical indices, at baseline. At the end of study, the levels of 2-AG, leptin, TNF-α, IL-6 decreased and serum adiponectin increased significantly in both groups. Serum FBS, insulin and HOMA-IR reduced in intervention group, but in the control group, these variables changes were not statistically significant (Table 4). Results of analysis of covariance revealed that whey supplementation in the intervention group, induced significantly reduction in 2-AG (-41.1%), TNF-α (-19.07%), IL-6(-15.28) and increase in adiponectin levels (45.93%) compared to control group (Table 5).

Variable	Intervention group (n=12)	Control group (n=12)	p
2-AG (ng/ml)			
Before	17.72 (16.49)	13.16 (9.43)	0.413[a]
After	9.88 (9.58)	11.14 (7.62)	0.043[b]
P[c]	0.013	0.024	
FBS (mg/dl)			
Before	87.33 (10.04)	87.25 (9.30)	0.983[a]
After	82.08 (10.68)	83.58 (7.22)	0.536[b]
P[c]	0.003	0.075	
Insulin (µlU/mL)			
Before	8.46 (4.68)	10.01 (6.32)	0.501[a]
After	5.43 (2.86)	7.59 (4.03)	0.090[b]
P[c]	0.050	0.090	
HOMA-IR			
Before	1.81 (1.02)	2.19 (1.58)	0.163[a]
After	1.10 (0.65)	1.56 (0.87)	0.075[b]
P[c]	0.029	0.053	

TNF-α (ng/l)			
Before	12.59 (7.28)	10.25 (4.56)	0.357[a]
After	8.59 (2.07)	9.79 (4.75)	0.045[b]
P[c]	0.038	0.038	
IL-6 (ng/l)			
Before	3.90 (0.61)	3.57 (0.29)	0.117[a]
After	3.25 (0.31)	3.43 (0.25)	0.020[b]
P[c]	0.001	0.001	
Adiponectin (mg/l)			
Before	13.70 (8.39)	16.07(9.16)	0.516[a]
After	19.09 (10.65)	18.25(9.73)	0.003[b]
P[c]	0.001	0.008	
Leptin (ng/ml)			
Before	83.54 (21.87)	86.62 (15.20)	0.693[a]
After	69.36 (19.95)	78.45 (18.35)	0.403[b]
P[c]	0.014	0.012	

Note: 2-AG:2-arachidonoylglyceride; FBS: fasting blood sugar; HOMA-IR: homeostasis model assessment for insulin resistance; TNF-α: Tumor Necrosis factor-α; IL-6: Interleukin- 6.

Results are expressed as mean (SD). P-values of statistical significance (p<0.05) are presented in bold.

[a]Independent t-test for FBS and Mann-Whitney U for 2-AG, insulin and HOMA-IR, TNF-α, IL-6, Adiponectin and leptin.

[b]Analysis of covariance (adjusted for changes in intake of energy, percent of carbohydrate, protein, total fat, SFA, MUFA, PUFA and other fat and baseline values).

[c]Paired t-test for FBS and Wilcoxon for 2-AG, insulin and HOMA-IR, TNF-α, IL-6, Adiponectin and leptin.

Table 4: Biochemical parameters values of the study groups at baseline and 8weeks after the intervention.

Variable	Intervention group (n=12)	Control group (n=12)	P[a]	P[b]
Weight	-4.26 (1.16)	-3.95 (1.08)	0.527	0.949
BMI	-4.19 (0.99)	-3.95 (1.08)	0.575	0.983
WC	-3.84 (0.41)	-1.86 (0.60)	0.000	0.000
Body fat	-7.43 (1.25)	-4.79 (0.88)	0.000	0.000
Body for free mass	0.38 (2.60)	-6.02 (4.14)	0.000	0.005
FBS	-6.00 (5.43)	-3.72 (7.83)	0.415	0.520
Insulin	-15.69 (57.04)	-13.71 (47.41)	0.921	0.279
HOMA-IR	-21.49 (54.52)	-18.69 (37.81)	0.885	0.252
2-AG	-41.10 (20.33)	-15.82 (10.84)	0.001	0.017
TNF-α	-19.07 (27.03)	-5.26 (7.74)	0.103	0.043
IL-6	-15.28 (11.50)	-3.85 (2.94)	0.003	0.020
Adiponectin	45.93 (34.1)	20.39 (26.65)	0.042	0.050
Leptin	-16.37 (19.96)	-10.05 (11.53)	0.352	0.474

Note: BMI: Body Mass Index; WC: Waist Circumference; 2-AG: 2-arachidonoylglyceride; FBS: fasting blood sugar; HOMA-IR: homeostasis model assessment for insulin resistance; TNF-α: Tumor Necrosis factor-α; IL-6: Interleukin-6.

Results are expressed as mean (SD). P-values of statistical significance ($p<0.05$) are presented in bold.

P^a: Mann-Whitney U

P^b: Analysis of covariance (adjusted for changes in intake of energy, percent of carbohydrate, protein, total fat, SFA, MUFA, PUFA and other fat and baseline values).

Table 5: Percentage changes of anthropometric and biochemical variables in intervention and control groups during study.

Multiple stepwise linear regression analysis using plasma 2-AG as a dependent variable and all other clinical and laboratory parameters as independent variables indicated that intake of SFA (β=0.843, P=0.000), other fat (β=0.829, P=0.001), levels of HDL-c (β=-0.480, P=0.000), TNF-α (β=1.976, p=0.000) and adiponectin (β=-0.288, P=0.006) were independently associated with 2-AG concentration. Also, at the end of study there was a significant positively correlation between decreases in plasma 2-AG concentrations and decrease in TNF-α (β=0.883, P=0.001) and IL-6(β=0.449, P=0.030), decrease in 2-AG inversely correlated with decrease in leptin levels (β=-0.962, P=0.004).

Discussion

This study showed that 30gr supplementation with whey protein for 8 weeks in obese women with metabolic syndrome under caloric restriction led to significant decrease in plasma 2-AG, serum levels of TNF-α, IL-6, WC, body fat percent and increase in adiponectin, and retention of fat free mass.

2-AG is formed on demand from arachidonic acid of membrane phospholipid in the sn-2 position, significantly increases in obese subjects [4]. Endocannabinoids can be found in the circulation and their evaluation in blood samples is a simple strategy used for the study of the endocannabinoids system [2,21]. Adipose tissue is the main source of endocannabinoids in the circulation and their tones is proportional to the rate of visceral fat [4,13]. Therefore, it is possible that the reduction of plasma levels of 2-AG in this study would be due to a significant reduction in body fat percent and waist circumference. Also, decrease of inflammatory factors and increase of adiponectin levels by whey supplementation in this study, moreover the presence of ACE inhibitor in whey protein, may reduce inflammation and increase the sensitivity of leptin. Leptin blocks the depolarization-induced suppression of inhibition by inhibiting voltage-gated calcium channels, resulting in decreased calcium influx and consequently decreased ECB synthesis [22].

Recent studies have shown the significant role of whey protein supplementation in blood glucose regulation, possibly through delayed gastric emptying and stimulation of incretins secretion [23]. Of course, in the obese women with metabolic syndrome, insulin resistance is associated positively with the plasma levels of the branched-chain amino acids, but the weight loss eliminates this correlation [24]. This may explain why studies on the whey supplementation along with weight loss, hyperglycemia does not appear by whey supplementation. In other studies, inconsistent with the present study, was seen a reduction in serum glucose, but most of these studies had considered non-obese subjects [25,26].

In vivo and invitro studies, have confirmed the anti-inflammatory effects of whey protein compounds (alpha-lactalbumin, beta-lactoglobulin and lactoferrin) [27,28]. Of course, more studies are needed to determine the substance responsible for these effects. However, some studies have linked the whey anti-inflammatory properties to synergistic effects of its compounds and others to lactoferrin [29]. In the Pal and et al study 54 grams whey supplementation for 12 weeks in 70 obese individuals, compared to casein and glucose, no significant changes in serum levels of inflammatory factors were observed [30]. In a meta-analysis study, it has been shown that ≥ 20 gr/day whey supplementation in high CRP subjects can significantly decrease CRP in adult humans [31]. In the Bohl and Zembel study in consistent with our study, increase of adiponectin and decrease of inflammatory factors was observed [32,33].

Many dietary guidelines recommend the milk and dairy products as an important part of a healthy and balanced diet. Milk proteins, especially whey protein, is associated with improved lipid profiles, blood pressure and insulin sensitivity, and whey has been shown to have a positive effect on the body composition. Whey protein has beneficial effects on the metabolic syndrome through fast digestion, increase of amino acids concentration and inhibition of ACE [34]. Also, whey protein can reduce body fat by stimulating the hormones that affect the body metabolism [35]. In Baer and colleagues' study, 56 g whey supplementation reduced the uptake of the T3 hormone [36]. In another investigation, rats fed high-fat diet were supplemented with various types of proteins (WPI, alpha-lactalbumin, beta-lactoglobulin, and lactoferrin) and low-calorie diet (for 50 days), those who had received alfa-lactalbumin (45.9 grams) showed a higher reduction in total body fat mass and visceral fat during the study [37]. In a study by Machado 16 weeks of supplementation with 30 g of whey and soy protein in 68 Crohn's patients produced significant changes in body composition, skinfold thickness, and body fat percent, but no significant difference was observed between the two groups [38].

One of the important findings of present study is that the whey supplementation without a significant reduction in weight and body mass index than the control group could significantly reduce the body fat, WC, TG, inflammatory factors and 2-AG, increase adiponectin levels and retain the muscle mass. For the first time, another whey benefits to improve metabolic risk factors in obesity was demonstrated, that it could decrease endocannabinoid by ways other than weight loss.

Contradictory results about whey supplementation return in the dose, type supplements (WC, WI or WH), differences in blood sampling time (fasting or postprandial), time consuming supplements (immediately before a meal or within two hours or more), the sample size, study population, supplementation with a weight loss diet or without it. The strengths of present study were the use of a lower dose of whey protein in the study, regular follow-up of subjects for dietary and supplements intake, and physical activity. A limitation of our study is that we didn't measure the expression of the metabolizing 2-AG enzymes.

In conclusion, for the first time, the effects of simultaneous weight loss diet and whey protein supplementation on 2-AG was

demonstrated in this study. It was concluded that using ways other than weight loss could reduce endocannabinoids and other metabolic risk factors.

Conflicts of Interest

The authors state that there are no personal conflicts of interest in the present study.

Acknowledgements

This paper was a part of degree PhD dissertation fulfilled by Vahideh Aghamohammadi. This study was financially supported by Nutrition and Metabolic Disease Research Center of Ahvaz Jundishapur University of Medical Sciences (Grant number: NRC-9513). We hereby express our gratitude to the study participants.

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