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Investigation the effect of oleoylethanolamide supplementation on the abundance of *Akkermansia muciniphila* bacterium and the dietary intakes in people with obesity: A randomized clinical trial

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ABSTRACT

Akkermansia muciniphila bacterium is one of the inhabitant gut microbiota involving in the energy homeostasis and inhibition of the inflammations. The present study was designed to evaluate the effects of Oleoylethanolamide (OEA) supplementation on the abundance of *A. muciniphila* and the dietary intakes in obese people. In this randomized, double-blind, controlled clinical trial, 60 eligible obese people were selected and divided randomly into two groups including OEA group (received two capsules containing 125 mg of OEA daily) and placebo group (received two capsules containing 125 mg of starch daily). The treatment lasted for 8 weeks. Dietary intakes were evaluated according to the three -day food record and, were analyzed by the Nutritionist 4 software. In order to evaluate the changes in the abundance of A. *muciniphila* bacterium, faeces samples were collected at baseline and at the end of study. The targeting of the 16S rRNA gene in *A. muciniphila* was measured by the quantitative real-time PCR analysis.

For OEA group, the energy and carbohydrate intakes decreased significantly after adjusting for baseline values and confounder factors; ($p = 0.035$), the amount of carbohydrate was reported as 422.25 (SD = 103.11) gr and 368.44 (SD = 99.08) gr; ($p = 0.042$)), before and after the treatment, respectively. The abundance of *A. muciniphila* bacterium increased significantly in OEA group compared to placebo group ($p < 0.001$). Considering the accumulating evidence identified OEA as a novel, safe, and efficacious pharmaceutical agent increasing the abundance of *A. muciniphila* bacterium and modifying the energy balance, therefore it is suggested to use its supplement for treatment of the obese people. However, future studies are needed to confirm the positive results obtained in this study.

1. Introduction

Obesity is a growing public health problem imposing a high economic burden on the societies worldwide (Payahoo, Khajebishak, & Ostadrahimi, 2019c). Globally, the prevalence of obesity has been doubled during the time period of 1980–2015 (Francisco, Carl, & Xuemei, 2017). In Iran, the prevalence of overweight and obesity has been estimated approximately by 37% and 17%, respectively, in 2009 (Saadatifar et al., 2018). Obesity is a robust risk factor for the incidence of numerous metabolic disorders such as diabetes, poly cystic ovarian syndrome, cardiovascular diseases, cancers, insulin resistance and non-alcoholic fatty acids (Arnold, Renehan, & Colditz, 2017; Cani, Everard, & Duparc, 2013). Due to the detrimental consequences of the obesity, etiological assessments should be regarded as a priority in health-care systems (Glickman, Parker, Sim, Del Valle Cook, & Miller, 2012).

Besides the genetic and environmental factors such as high-calorie food intake and physical inactivity, dysbiosis of the gut microbiota is accounted for as another crucial risk factor (Singh et al., 2017). Gut

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microbiota involve in the biological functions such as maintaining the energy homeostasis, regulating immune system, mucin degrading, providing nutrients for growth of the other microbiota and vitamin synthesis for the host (Round et al., 2009; Tremaroli & Bäckhed, 2012). Changes in the gut microbiota composition would result in development of the various metabolic abnormalities and a low-grade inflammation (Moran, 2008; Marchesi et al., 2016).

Nearly 1% of gut epithelium consists of the neuroendocrine cells having the gut hormones secretory properties. Induction of G protein-coupled receptors (GPCRs) in L cells by ligands results in the production of the peptides and hormones such as Glucagon-like peptide-1 with optimal host metabolism properties including appetite control, involvement in the glucose and fat metabolism, intestinal barrier function, gastric empty and intestine motility (Brown et al., 2003; Cani et al., 2013; Parker et al., 2012). GPCRs are a large group of protein receptors in L cells act as responder to the internal signal transduction pathways (Trzaskowski et al., 2012, pp. 1090–1109). GPR41 and GPR43 are two main receptors influenced by short chain fatty acids produced by the fermentation of indigestible carbohydrates (de Almeida, Abranches, & de Luces Fortes Ferreira, 2015; Schrezenmeir & de Vrese, 2001). In addition to SCFAs, other factors such as bile acids and inhabitant gut microbiota have been identified to induce the GPCRs (Samuel et al., 2008; Scott Karen. et al., 2013). *Akkermansia muciniphila* bacterium is one of the inhabitant gut microbiota with the induction potential for the GRPs receptors (van Passel et al., 2011).

A. muciniphila as a mucin degrading bacterium belongs to *verrucomicrobia* phylum accounting for about 3–5% of the total microbiota population in the human gut (Derrien, Collado, Ben-Amor, Salminen, & de Vos, 2008; Payahoo et al., 2019). *A. muciniphila* bacterium ferments the indigestible carbohydrates and, produces the SCFAs such as acetate and propionate (van Passel et al., 2011). The produced SCFAs act as ligands of GPR 41 and GPR43, which in turn induce the secretion of the GLP-1 (Anhê et al., 2015; Shin et al., 2014). *A. muciniphila* also provides an enriched environment for growth of the other inhabitant beneficial microbiota (Kim & Ho, 2010), strengthening the mucus layers in the epithelium and decreasing the inflammation through reducing the gut epithelium permeability (Belzer & DeVos, 2012; Everard et al., 2013).

There is a negative correlation between the abundance of *A. muciniphila* and factors such as aging, BMI, inflammatory cytokines, gut permeability, insulin resistance and waist to hip ratio, adiposity markers, cardiovascular indices and the fasting glucose (Dao et al., 2016; Everard et al., 2013). Previous studies showed that the abundance of *A. muciniphila* bacterium is in the low level in obese people than healthy people (Derrien, Belzer, & de Vos, 2017; Santacruz et al., 2010). Diets with high saturated fatty acids (but not unsaturated fatty acid such as fish oil) lead to a decrease in the abundance of *A. muciniphila* (Collado MC et al., 2007; Schneeberger et al., 2015).

The abundance of *A. muciniphila* is significantly and positively associated with the parameters of fatty acid oxidation and fat burner factors, the gut secretory capacity and the number of L cells in the epithelium (Everard et al., 2011; Schneeberger et al., 2015). Previous studies showed that the increase in the abundance of *A. muciniphila* prevents the development of the metabolic abnormalities caused by high BMI and, improves the glucose abnormality (Everard et al., 2013; Plovier et al., 2017; Shin et al., 2014). Various manipulations would modify the abundance of this bacterium in obese people (Delzenne, Neyrinck, Bäckhed, & Cani, 2011). In addition to the probiotics and prebiotics, resistance starch and SCFAs are able to modify the composition of gut microbiota. Acetate, propionate and butyrate involve in the many biological functions through induction of gene expression, as a result they contribute in the energy homeostasis, glucose and lipid metabolism (Heimann, Nyman, Pålbrink, Lindkvist-Petersson, & Degerman, 2016).

GRP119 is another GPR receptor in L cells. GPR119 is the main ligand of the endocannabinoid-like lipids specially 2-Oleoyl glycerol (2-OG) and OEA (Hansen et al., 2011; Syed et al., 2012). OEA as a bioactive monounsaturated lipid mediator belongs to the acylglysrol and N-acylethanolamine family of endocannabinoids, mainly expressed in the adipose tissues, and in the neurons and astrocytes (Herrera, Kölliker-Frers, Barreto, Blanco, & Capani, 2016; Hu & Mackie, 2015; Lauffer, Iakoubov, & Brubaker, 2009). The concentration of OEA increases in the feeding status and, it decreases in the fasting status (Fu et al., 2007). Cocoa powder, nuts and oatmeal are the food sources containing low amounts of OEA (up to 2 μg/gr). Dietary oleic acid is the main precursor of OEA (Schwartz et al., 2008). High amounts of oleic acid are needed to produce enough OEA for induction of the targeted receptors (Fu et al., 2003; Premkumar et al., 2014; Schwartz et al., 2008).

The biological functions of OEA include decreasing the pro-inflammatory cytokines, improving awareness and memory, and declining the stress and depression (Antón et al., 2017; vanKooten, Veldhuizen, de Araujo, O'Malley, & Small Dana, 2016). Anti-obesity property is considered as another main function of OEA. Numerous clinical and experimental studies have confirmed that, OEA causes the treatment for various abnormalities in obese people (Barbaro, Menasci, Baldini, Pasquini, & Lapi, 2011; Oveisi, Gaetani, Eng, TP, & Piomelli, 2004; Payahoo et al., 2018a, b, c). OEA exerts its biological functions as ligands of various receptors such as peroxisome proliferator-activated receptor-α (PPAR-α), GPR119 and transient receptor potential vanilloid member 1 (TRPV1) (Payahoo et al., 2018a, b, c). The main outcomes regarding the targeted receptors activation include decreasing the appetite and food intake, weight loss, increasing the feeling of fullness and enhancing the GLP-1 levels (Brown et al., 2018; Payahoo et al., 2018a, b, c). Some evidence suggested that there is a relationship between endocannabinoid-like lipids and *A. muciniphila* bacterium. Everard et al., demonstrated that *A. muciniphila* supplementation increased the content of 2-OG as one of the endocannabinoids-like lipids in the ileum (Everard et al., 2013). Various dietary or pharmaceutical compounds such as prebiotics (Andersson et al., 2013; Zhong, Nyman, & Fåk, 2015), unsaturated fatty acids (Chaplin, Parra, Serra, & Palou, 2015), probiotics (Alard et al., 2016) and antibiotics (Nobel et al., 2015; Vrieze et al., 2014) influence the growth of *A. muciniphila* bacterium. In this study, it was supposed that OEA can also influence the abundance of *A. muciniphila*, modulating the dietary intake in obese people. Thus, this study was designed to evaluate the effects of OEA supplementation on the abundance of *A. muciniphila* and the dietary intakes in obese people.

2. Materials and methods

Sixty-seven healthy obese people were voluntarily recruited among people referred to the healthcare clinics affiliated to Tabriz University of Medical Science to participate in this randomized, double-blind, controlled clinical trial. All subjects participated in this study through being informed by the posters installed in the healthcare clinics affiliated to Tabriz University of Medical Science. At baseline, eligible participants completed a written consent form. The whole protocol of study was approved by the regional ethics committee of the Tabriz University of Medical Science and was allocated the number code IR.TB MED. REC.1395.618. As well as, the study was registered in the Iranian Registry of Clinical trials center with number IRCT201607132017N30, and with URL: www.IRCT.IR.

Inclusion criteria included having the age between 18 and 59 years old, and BMI between 30 and 40 kg/m^2. Individuals with kidney diseases, liver and heart failure, gastrointestinal and rheumatic disorders were excluded from the study. In addition to cigarette smokers, pregnant, breastfeeding and menopause women, people who take antibiotics, probiotics and prebiotics supplements, weight loss drugs, omega 3 supplements, and multivitamin and mineral supplements during last month were not allowed to participate in the study.

According to the inclusion criteria, eligible subjects were selected and (n = 60) divided randomly into two groups. Intervention group who received the supplementation in the form of two capsules

containing 125 mg of OEA daily before lunch and dinner meals (synthesized at the Nutrition Research Center, Tabriz University of Medical Science, Iran) (Payahoo et al., 2018a, b, c), and the placebo group who received two capsules containing 125 mg of starch daily similar to the intervention group. All capsules were similar in terms of shape and color and, all participants were blinded regarding the difference between the capsules, except a third person who labeled and allocated them between two groups until the end of the assays. The treatment lasted for 8 weeks. During the follow-up period, all participants were monitored weekly through making phone calls to ensure that they regularly consume the capsules. Subjects who took more than 90% of the capsules were selected for the statistical analysis. In total, 56 obese people completed the study. All participants were advised to hold on the usual intake and physical activity during the intervention period. The sample size was obtained according to the body mass variable in a previous study (Mangine et al., 2012). The minimum sample size was calculated to be 26 healthy obese people in each group (by considering the CI 95%, power 90%, and taking into account the 0.9% changes), by considering losses were possible in the follow-up period, 30 individuals were included in each group. We used per protocol approach to analyze our data. Overall, four participants discontinued the study for reasons irrelevant to our study protocol. Fig. 1 depicted the whole protocol of study until the end of study.

Participants completed a demographic questionnaire at baseline. Anthropometric indices including weight, height and BMI were measured according to the standard methods at baseline. In order to measure the dietary intakes, three-day food records were completed at baseline and at the end of intervention period. Calorie and macronutrient intakes were calculated by analyzing food data using the Nutritionist IV software.

To assess the changes in the abundance of *A. muciniphila* bacterium, faeces samples were collected at baseline and at the end of study and, were stored at −80 C for bacterium analysis. The DNA isolation of bacterium was performed using the modified protocol used in the study by Atashpaz et al., (Atashpaz et al., 2010). The quality and quantity of the extracted DNA was assessed using NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, United States). To detect the undesired contaminations, the A_{260}/A_{280} absorbance ratio was used. QRT-PCR was used to assess the abundance of *A. muciniphila* bacterium. The primers were designed based on regions of the 16S rRNA gene sequences of this bacterium from NCBI GenBank using OLIGO software: Akkermansia muciniphila; F, CAACGCTTGAGACCTC TGTATT and R, CCTGTCATGTGGGAGCAAATTA and β-actin; F, TCCC TGGAGAAGAGCTACG and R, GTAGTTTCGTGGATGCCACA as standard gene. PCR amplification and detection were quantified using real-time PCR (Mic − qPCR, Australia). For each sample, the Cycle Threshold (CT) was compared with the housekeeping gen (β-actin). Date was expressed as logarithm (Log10 CFU)/100 mg of fecal samples.

3. Statistical analysis

The SPSS software (version 20; SPSS Inc., Chicago, IL) was used to analysis of data. The normal distribution of variables was assessed by the Kolmogorov-Smirnov test. To present numerical data as mean (Standard deviation) was used and categorical data was presented as frequency (percentage). The baseline characteristics were compared by independent sample t-tests and the chi-squared test (for quantitative variables and qualitative variables, respectively). The within-group changes of dietary intakes were assessed by paired sample *t*-test. To detect difference between the intervention and placebo groups after adjusting for baseline measurements and confounder factors including age, sex, occupational and educational status, we used analysis of covariance (ANCOVA) test. The difference between samples of both groups in terms of the abundance of *A. muciniphila* bacterium was

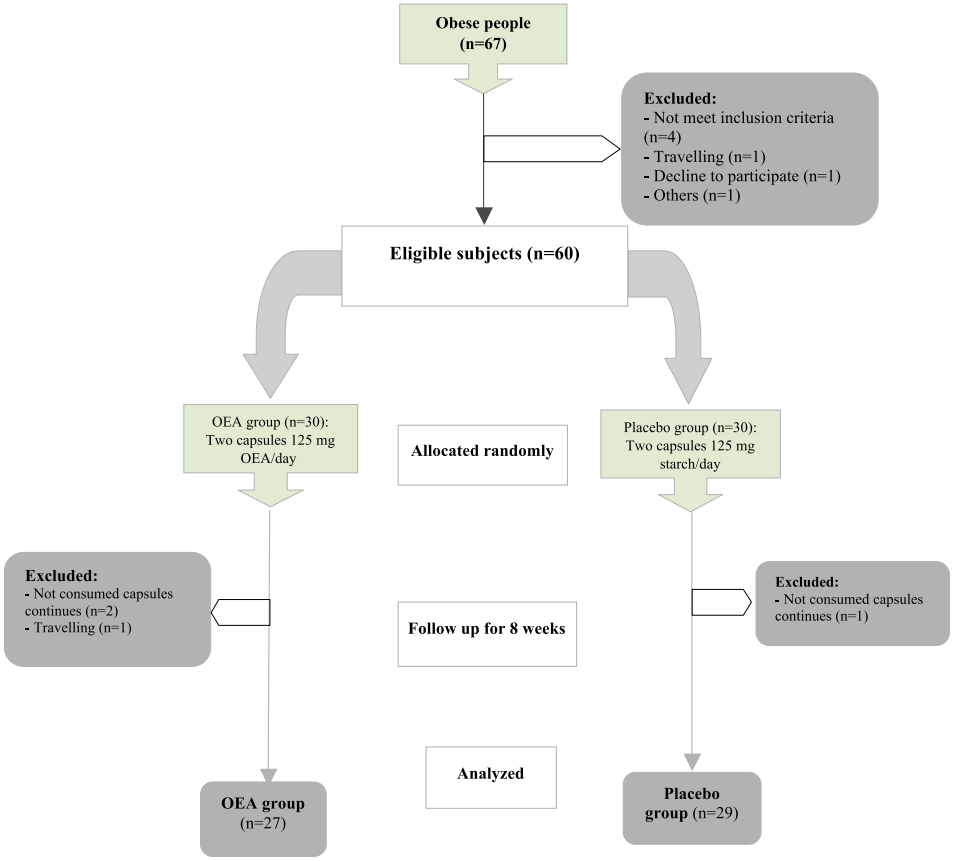

Fig. 1. The whole protocol of study, the number of the obese people who were recruited and completed the study until the end.

Table 1
The demographic characteristics of obese people in the onset of study (n = 56).

Variables	Intervention group (n = 27)	Placebo group (n = 29)	p[a]
Age (year)[b]	37.37 (8.74)	38.13 (9.28)	0.572
Gender[d]			
Female	15 (55.6)	19 (65.5)	0.109
Male	12 (44.4)	10 (34.5)	
Education level [b]			
Illiterate	13 (48.1)	15 (51.7)	< 0.001
Diploma	3 (11.1)	11 (37.9)	
Bachelor degree	8 (29.6)	3 (10.3)	
Master degree	3 (11.1)	0 (0)	
Occupation [b]			
Clerk	7 (25.9)	4 (13.8)	< 0.001
Retired	1 (3.7)	0 (0)	
Housewife	14 (51.9)	19 (65.5)	
Worker	5 (18.5)	6 (20.7)	
Weight (kg) [b]	93.68 (17.25)	91.74 (15.96)	0.272
Height (cm) [b]	159.62 (11.69)	160.96 (8.91)	0.062
BMI (kg/m^2) [b]	34.84 (4.19)	35.27 (4.03)	0.838
Energy (kcal)	2714.30 (423.06)	2535.92 (461.77)	0.149
Carbohydrate (g)	422.25 (103.11)	394.05 (93.69)	0.302
Fat (g)	80.77 (31.94)	70.47 (30.68)	0.236
Protein (g)	87.23 (20.94)	80.21 (17.00)	0.186
Fiber (g)	25.73 (6.62)	23.34 (3.90)	0.114

[a] Independent sample *t*-Test/Chi^2 test.
[b] Presented as mean (SD).
[d] Presented as frequency (percent).

assessed by the Independent Samples T-tests. P values less than 0.05 were considered as statistically significant.

4. Results

Table 1 represented the demographic characteristics of participants at baseline in both groups. The mean (SD) age of obese people was 37.37 years old (8.74) in the intervention group and, it was 38.13 years old (9.28) ($p > 0.05$) in placebo group. About 60% and 65% of subjects were female, respectively in the intervention and placebo groups ($p > 0.05$). The body mass index was reported as 34.84 (4.19) kg/m^2 and 35.27 (4.03) kg/m^2, respectively in the intervention and placebo groups.

A significant change was observed in the energy intake for participants in the intervention group. Indeed, in the OEA group, energy intake was equal to 2714.30 (423.06) kcal at baseline and, this amount decreased significantly to 2379.07 (476.46) ($p < 0.001$). The results were confirmed by the ANCOVA test after adjusting for baseline values and confounding factors ($p = 0.035$). For OEA group, the intake of carbohydrate decreased significantly ($p < 0.001$), and this result was confirmed by the ANCOVA test after adjusting for baseline values and confounder factors ($p = 0.042$). In spite of a significant decrease in the protein and fat intakes compared to baseline for the intervention group, the results of the ANCOVA test did not confirm this reduction ($P > 0.05$). Undesirable changes were observed in the dietary intakes for placebo group. Table 2 shows the results of the OEA and placebo supplementation on the dietary intakes of obese people.

The results showed that, after eight weeks of OEA supplementation, the abundance of *A. muciniphila* bacterium increased significantly for OEA group compared to placebo group ($p < 0.001$). The mean (SD) of *A. muciniphila* bacterium abundance in OEA and placebo groups were changed from 1.22 (0.14) to 2.20 (0.17) and 0.86 (0.23) to 1.40 (0.31) (Log10 CFU)/100 mg), respectively. Fig. 2 depicted the results of OEA supplementation on the abundance of *A. muciniphila* bacterium in the gut microbiota of obese people.

5. Discussion

The high prevalence of obesity highlighted the efforts of healthcare providers and pharmaceutical companies to search for the new, efficacious, and safe treatments to counter this problem (Overton et al., 2006). In this study, the abundance of *A. muciniphila* increased significantly as a result of OEA supplementation. According to the literature, there is no clinical trial assessed the effect of OEA supplementation on the abundance of *A. muciniphila*; however, various components have been recognized to modify the abundance of this bacterium. Table 3 shows the various compounds used to enhance the abundance of *A. muciniphila* bacteria.

Decreasing the calorie intake and anthropometric measurements such as BMI would modify the gut dysbiosis (Brown, DeCoffe, Molcan, & Gibson, 2012). In this study, OEA supplementation caused a significant decrease in the energy and carbohydrate intakes. It was shown that, the abundance of *A. muciniphila* increases during weight loss in obese people. In a study by Remely et al. 33 obese people were recruited to participate in a weight loss program for 4 weeks. The results showed that, the abundance of *A. muciniphila* and *Archaea* increased significantly after weight loss intervention. In addition to anthropometric improvement, the calorie intake decreased significantly at the end of study ($p < 0.01$) (Remely et al., 2015). The effect of OEA supplementation on the anthropometric measurements was assessed in a clinical trial. In the study by Payahoo et al., OEA supplementation at the dose of 250 mg for 60 days caused a significant decrease in weight, body mass index, waist circumference, and fat percentage in obese people, as well as a significant increase in PPAR-α gene expression at the end of the study. A significant improvement was also observed in the feeling of fullness for OEA group (Payahoo et al., 2018a, b, c).

Regarding the control of obesity, there are some mechanisms attributed to the role of *A. muciniphila* including improving the thickness of the mucus layer, decreasing the macrophage infiltration and, enhancing the gut barrier function (Everard et al., 2013). *A. muciniphila* also reduces the inflammation through decreasing the systemic circulatory levels of endotoxins TLR2 and TLR4 (Wen, Peng, Li, & Wong, 2004). Acetate and propionate produced by *A. muciniphila* act as the nutrient for commensal microbial, regulating the metabolic processes and energy homeostasis through induction of GPR43 and GPR41 receptors in L cells (Delzenne et al., 2011). GLP-1 produced by L-cells, modulates the appetite by the central and peripheral receptors (Shah & Vella, 2014). Due to the beneficial effects of *A. muciniphila* in modifying the numerous abnormalities in the obesity, it has drawn more interest since (Payahoo et al., 2019).

As mentioned above, the complementary interventions to maintain the energy balance, decreasing the appetite, reversing the obesity-related metabolic abnormalities and causing weight loss would be considered as interested strategies in the obesity management (Khajebishak, Payahoo, Alivand, & Alipour, 2018; Mobasseri et al., 2014; Payahoo et al., 2014a, b). OEA can be considered as a supplement for treatment of the obesity.

Two potential mechanisms are involved in elevation of the *A. muciniphila* abundance by the OEA supplementation. First, OEA is a ligand for GPR119 receptors stimulating the releases of GLP-1 in the L-cells. GLP-1, as an incretin hormone modulates the appetite and food intake, and regulates the glucose tolerance and insulin sensitivity as well as liver functions (Claus et al., 2008; Thomas et al., 2009). GLP-1 decreases the gastric motility and gastric emptying and, it also decreases the transit time as well as increasing the energy expenditure and thermogenesis. The second mechanism is related to the roles of OEA in the induction of the nuclear receptors. OEA is a high affinity ligand of PPAR-α (Misto, Provensi, Vozella, Passani, & Piomelli, 2019; Rosen, 2003). PPAR-α is a nuclear receptor involves in maintaining the energy balance and lipid oxidation processes, as well as declining the inflammation (Guzmán et al., 2004; Payahoo et al., 2018a, b, c). Fig. 3 shows the potential interaction between OEA supplementation and

Table 2
The effect of OEA supplementation on the dietary intakes in people with obesity.

Variables	Intervention		Placebo		Mean Diff 95% CI pb
	Before	**After**	**Before**	**After**	
Energy (kcal)	2714.30 (423.06)	2379.07 (476.46)	2535.92 (461.77)	2640.74 (447.96)	272.226 (20.338–542.114)
p[a]	< 0.001		0.002		0.035
Carbohydrate (g)	422.25 (103.11)	368.44 (99.08)	394.05 (93.69)	423.15 (94.44)	55.990 (2.191–109.790)
p[a]	< 0.001		< 0.001		0.042
Fat (g)	80.77 (31.94)	74.77 (30.51)	70.47 (30.68)	71.11 (33.49)	3.613 (−13.933–21.159)
p[a]	< 0.001		0.685		< 0.681
Protein (g)	87.23 (20.94)	80.34 (18.59)	80.21 (17.00)	86.90 (16.64)	6.757 (−3.053–16.567)
p[a]	< 0.001		0.001		0.173
Fiber (g)	25.73 (6.62)	26.02 (6.48)	23.34 (3.90)	24.20 (3.66)	1.920 (−0.861–4.701)
p[a]	0.595		0.114		0.172

Data presented as mean (SD).
[a] Paired sample *t*-test.
b ANCOVA test adjusted for age, gender, baseline values, education and occupation.

Akkermansia muciniphila bacterium in the management of obesity.

There were some limitations in this study including lack of measuring the gene expression of GPR119, the serum concentration of SCFAs especially acetate and propionate and, the serum levels of OEA during the intervention. To the best of our knowledge, this study was the first clinical trial assessed the effects of OEA supplementation on the abundance of *A. muciniphila* and the dietray intakes in the obese people, although it can be considered as the strength of our study.

6. Conclusion

In this study, OEA supplementation for obese people (250 mg/day) for eight weeks caused a significant increase in the abundance of *A. muciniphila* in the gut microbiota of obese people, as well as a significant decrease in the energy and carbohydrate intakes. Considering the accumulating evidence identified OEA as a safe and efficacious pharmaceutical agent modifying the abundance of *A. muciniphila* bacterium, anthropometic measurements and biochemical biomarkers and decreasing the appetite, inflammation and energy intake, Therefore, it can be used as a supplement to help overweight and obese people manage their weight and beneficially modulate their microbiota composition. However, future studies are needed to confirm the positive results obtained in this study.

Conflicts of interest

The authors declare there is no conflict of interest in the content of this study.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.appet.2019.05.032.

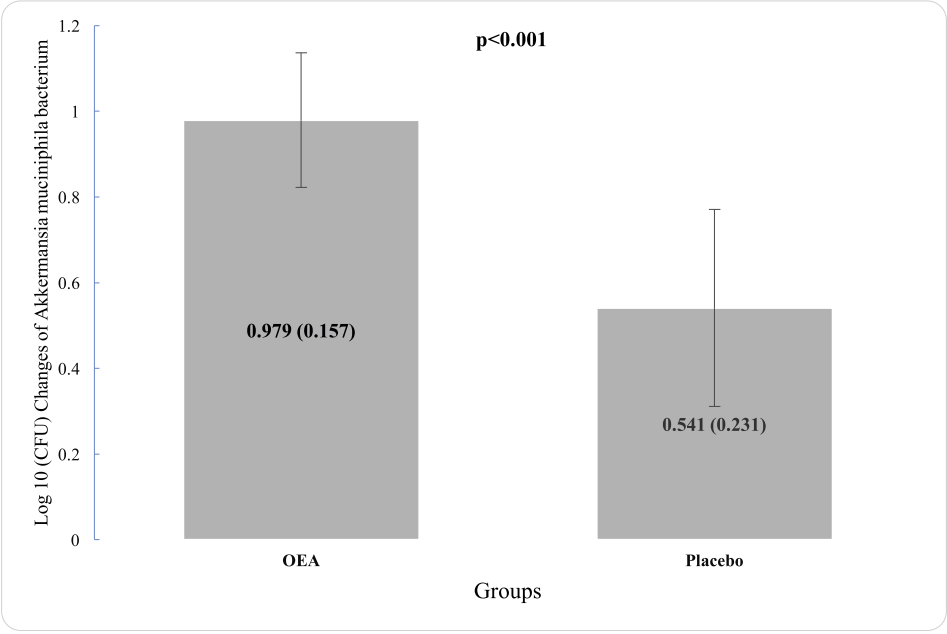

Fig. 2. The changes of *Akkermansia muciniphila* bacterium in people with obesity supplemented with OEA and placebo at the end of study.

Table 3
The compounds used to enhance the abundance of *A. muciniphila* bacteriaum

References	Results	Intervention	Target group
Lee and Ko (2014)	The abundances of *A. muciniphila* increased significantly after metformin treatment of mice on the HFD	A) Metformin group (300 mg/kg/day) during the HFD for 10 weeks B) Control group receiving HFD without metformin	Mice (n = 41)
Caesar, Tremaroli, Kovatcheva-Datchary, and Cani (2015)	The number of *A. muciniphila* increased significantly in the cecal of mice fed fish oil compared to lard oil	Lard or fish oil for 11 weeks	Mice (n = 15/group)
Zhang et al. (2012)	The number of *A. muciniphila* increased only in the metformin supplemented rats	(1) HFD group (n = 10) (2) HFD + 200 mg/kg berberine/daily (3) HFD + 100 mg/kg berberine/daily (4) HFD + 200 mg/kg bw metformin/day	Wistar rats (n = 40)
Shin et al. (2014)	The number of *A. muciniphila* increased in the metformin supplemented mice -Supplementation of mice without metformin resulted in attenuate adipose tissue inflammation and glucose tolerance improvement	1) A normal-chow diet (NCD) without metformin treatment (2) a NCD with metformin (300 mg/kg/day) (3) a High Fat Diet without metformin treatment (4) a HFD with metformin treatment for 6 weeks	Mice (n = 24)
Zhang et al. (2018)	more frequency of *A. muciniphila* and improvement of metabolic syndrome parameters compared to controls	Grape polyphenols and grape proanthocyanidins (360 mg/kg) for 12 weeks	C57BL mice (n = 24)
Anhê et al. (2015)	increased significantly the *A. muciniphila* frequency	Cranberry extract (200 mg/kg) for 8 weeks	C57BL/6J mice (n = 36)
De La Cuesta-Zuluaga et al. (2017)	The abundance of *A. muciniphila* bacteria in diabetic patients who consume metformin was higher than controls and diabetic patients who didn't consumed metformin	Metformin users	- Twenty-eight type 2 diabetic patients (14 with using metformin 14 not using metformin) - 84 subjects as controls
Roshanravan et al. (2017)	-The growth of *A. muciniphila* increased significantly both in the inulin and sodium butyrate groups	(A) 600 mg/day sodium butyrate 10 g (B) high performance inulin powder (C) combination of A and B (D) placebo for 45 days	- sixty type 2 diabetic patients

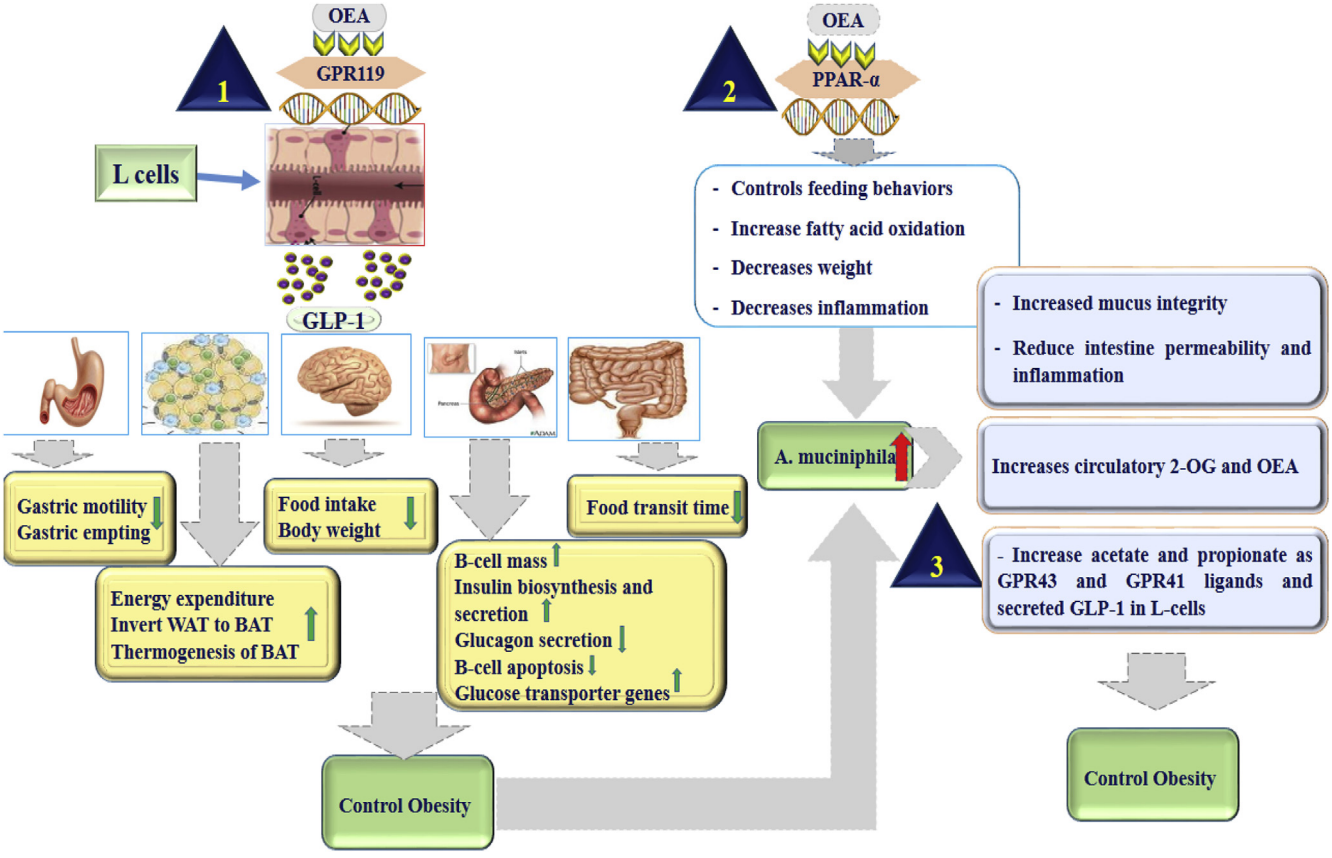

Fig. 3. The potential interaction between OEA supplementation and *Akkermansia muciniphila* bacterium in the management of obesity.

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