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Effects of daily functional acorn cake consumption on insulin resistance in individuals with obesity or overweight and the metabolic syndrome: a placebo-controlled randomised clinical trial

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Abstract

The metabolic syndrome is a multi-factorial condition and functional foods need more investigation as novel adjunct treatments for this group. This study aimed to determine the effects of daily consumption of a functional acorn cake in conjunction with energy restriction (119.50 kJ) on individuals with overweight or obesity and the metabolic syndrome. In this randomised double-blinded study, eighty-four participants were randomly allocated to either an energy-restricted diet plus two servings (2 × 30 g)/d of functional acorn cake (a cake made of acorn for the intervention group) (FC) (*n* 42) or an energy-restricted diet plus placebo cake (PC) (*n* 42). Body composition and biochemical parameters were measured before and after 10 weeks of intervention. Seventy-three participants completed this trial. No differences in loss of body weight, waist circumference, fat mass, fasting blood glucose and blood pressure were shown between two groups. Body weight decreased by 4·2 (SD 1·9) kg and 5·1 (SD 2·8) kg in PC and FC groups, respectively. Compared with PC, the consumption of FC resulted in a significant reduction in serum insulin ($P = 0{\cdot}02$), homoeostasis model assessment for insulin resistance ($P = 0{\cdot}02$), high-sensitivity C-reactive protein ($P = 0{\cdot}04$) and a significant increase in adiponectin concentration ($P = 0{\cdot}04$). Although lipid metabolism did not differ among groups, total cholesterol and HDL-cholesterol improved non-significantly in the FC group. Functional acorn cake as an adjunct to energy restriction could possibly improve insulin resistance in individuals with obesity. Further research is needed to elucidate whether functional acorn cake can be used as a preventive strategy for the metabolic syndrome in individuals with obesity.

Keywords: **Metabolic syndrome: Obesity: Acorn: Oak**

Obesity and insulin resistance are two important factors in the development and progression of the metabolic syndrome, which leads to many chronic related diseases, especially diabetes and CVD[1]. The global prevalence of obesity has doubled in the last 30 years, and in many developed countries, more than a third of adults are obese[2], which is why there is an urgent need to find solutions to treat the disease[3]. Using functional foods along with weight loss diets has increased to enhance more weight loss and reduce risk factors associated with obesity, especially insulin resistance. Based on these challenges in obesity management, a novel area of research consists of identifying functional foods that may facilitate the positive effects of energy-restricted diets[4].

Acorn, or oak fruit, is used as a food because of its high content of carbohydrates, proteins, amino acids, lipids and sterols. Besides, *Quercus* acorns are mainly used for making bread[5]. In a study by Molavi *et al.*, adding acorn powder instead of wheat powder along with increasing the nutritional value of the cake improved quality parameters[6]. Furthermore, in the study of Korus A *et al.*[7], replacing wheat or maize powder with acorn powder in preparing biscuits led to greater antioxidant activity and lower peroxide value of acorn biscuits. Numerous biological compounds are found in acorns, such as phenolic acids (gallic and ellagic acid), flavonoids (quercetin, catechin, naringin) and different galloyl and hexahydroxydiphenoyl derivatives[8], antioxidant vitamins (vitamin E and provitamin A)[9], saponins, especially tannins[10] and prebiotics[11], which are known for their role in the regulation of parameters of the metabolic syndrome. Studies have reported numerous biological features like antioxidant activity[12] and anti-inflammatory action[13] of the acorn. Although no human clinical trials have

Abbreviations: CRP, C-reactive protein; FC, functional acorn cake; HOMA-IR, homoeostasis model assessment for insulin resistance; PC, placebo cake.

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been performed on the effects of acorn on obesity and the metabolic syndrome, some animal studies have yielded promising results. In rats with obesity fed a high-fat diet, acorn powder had significant effects on lipid profile and increased antioxidant enzymes[14]. In addition, a significant decrease in insulin resistance was observed in rats fed acorn powder for 8 weeks[15]. The suppressive effects of acorn supplementation against obesity in differentiated 3T3-L1 cells because of its antioxidant properties were also observed[16]. Therefore, one of the main objectives of this study was to investigate the effects of a functional acorn cake compared with a control cake on improving metabolic parameters in adults with obesity or overweight and the metabolic syndrome on an energy-restricted diet. It is hypothesised that compared with the control cake, the consumption of the functional acorn cake would improve the metabolic syndrome parameters.

Materials and methods

Acorn gathering and treatment

Acorns were collected from Dosiran, a village in the south-west of Fars province, to the south of Iran, in December 2019. The voucher specimen was deposited in the herbarium of Pharmacy School at Shiraz University of Medical Sciences with Voucher No 3041. The plant sample was identified by a botanist as *Q. brantii* Lindl. The shell and internal layer (in Persian: Jaft) of dried acorn were removed, and, thereupon, acorns were soaked in water for about 48 h to reduce their astringent taste. Finally, the fruits were dried and milled. The dose used in this study was the maximum dose that did not adversely affect the taste of the cake and was tolerable for the participants in the study

Cake preparation

The cakes were made in 'Noono Namak' bakery workshop located on Hor Street, Shiraz. An amount of 10 g of treated acorn flour, whole egg, stevia, low-fat milk, rapeseed oil, baking powder, emulsifier and vanilla essence was used in preparing each intervention cake (functional acorn cake (FC)). All ingredients were similar and equal in amount for both case and control cakes except for the flour. Null flour (wheat flour without bran) was used in the control or placebo cake (PC) instead of acorn flour. Given that the energy of treated acorn flour was more than null flour, about 12 g null flour was applied to make the energy content of both cakes equal. Each cake weighed about 30–35 g. Also, because of the natural brown colour of acorn cakes, brown food colour was used to make the cakes similar considering their appearance. Despite of using the maximum dose of acorn, the cake had an acceptable palatability as it was assessed before starting the trial. In other words, the dose of acorn used in cake preparation did not affect the palatability.

Chemical analyses of flour and cakes

The following methods were applied to determine the basic chemical composition of the treated acorn flour and cakes: Kjeldahl method for protein content, Soxhlet method for fat content, AOAC-1995 method for ash and moisture and AOAC-2002 for fibre[17]. The total carbohydrates quantity was obtained by subtracting the sum of fat, protein, ash and moisture from 100 %. Tannins of acorn cakes were measured by titration method and the application of indigo solution as an identifier[18]. The energy values were calculated based on Atwater coefficients (carbohydrates and protein 4 kcal/g, fat 9 kcal/g)[19]. All of the analyses were performed two times.

Methods

Participants

Volunteers were recruited from Moslemin, Imam Reza and Motahari clinics that are all affiliated to Shiraz University of Medical Sciences, Iran. The inclusion criteria were as follows: men and women between 20 and 60 years of age and BMI of 25–35 kg/m^2 with a diagnosis of the metabolic syndrome according to the National Cholesterol Education Program Adult Treatment Panel III report[20]. Exclusion criteria were taking drugs or supplements that could affect appetite, body weight, blood glucose and lipid metabolism or having anti-inflammatory effect, pregnant and lactating women, diabetes, smoking, history of alcohol consumption, those with mental illnesses, cancer, thyroid, cardiovascular, pulmonary, renal, hepatic, eating disorders, weight loss > 10 % body weight within 6 months before the study initiation or a recent change in the intensity or frequency of physical activity (within 4 weeks).

The present research was performed based on the guidelines of the Declaration of Helsinki. The study protocol was approved by the ethics committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (IR.BMSU.BAQ.REC.1399.002) and was registered in the Iranian Registry of Clinical Trials (IRCT20170506033836N2). All participants read and signed an informed consent form before study enrolment.

Study design

In this randomised double-blinded controlled trial, 146 participants were screened, and eighty-eight eligible participants entered a 2-week run-in period to obtain detailed information about their dietary intakes and physical activities as well as medical history. During the run-in period, participants were asked to record their dietary intakes for three non-consecutive days. Four people declined to participate during the run-in period. Eighty-four participants were enrolled into this study (Fig. 1). At the end of the run-in period, participants were randomly assigned to the PC (n 42) or the FC group (n 42) using balanced block randomisation. In this method of randomisation, the size of the blocks was considered 4, and all possible scenarios were written for two groups A and B, and this process was repeated until the number reached eighty-four people. Then a number was given to each of the block components using a random number table. The numbers given to each of the block components represented the number assigned to each person. This identified the group assigned to each individual. Two daily servings (2 × 30 g) of FC or PC were given as snacks for 10 weeks to each participant (Table 1). The products were provided in

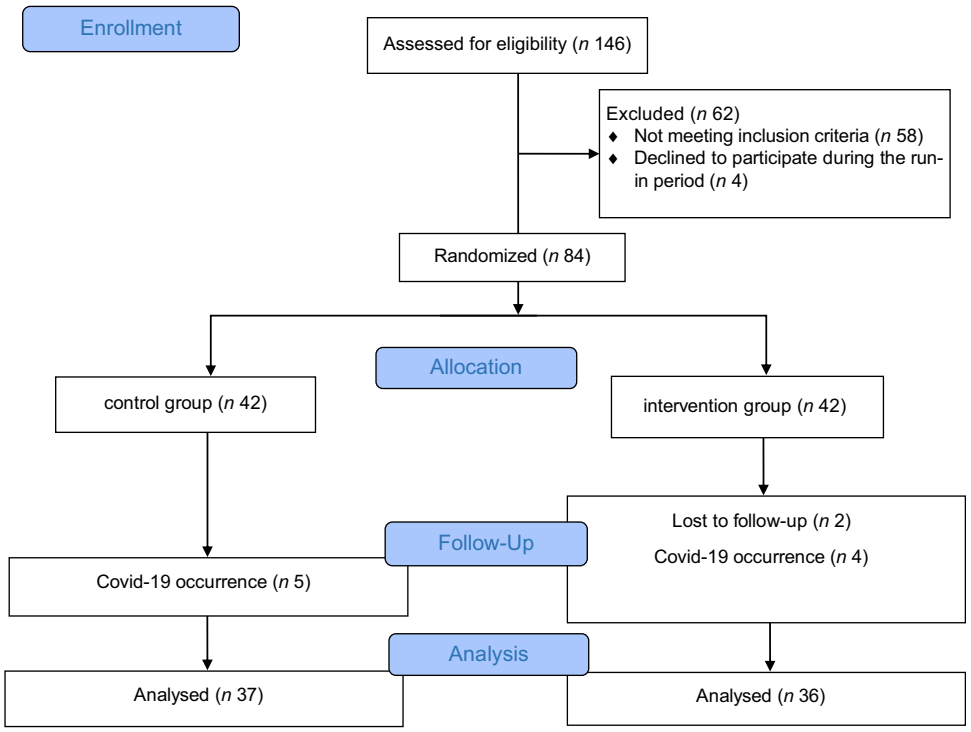

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

Table 1. Nutritional composition of cakes and treated acorn flour per 100 g

Sample	Protein (w.p)	Pro NRV (w.p)	Fat (w.p)	Fibre (%)	Ash (w.p)	Moisture (w.p)	Total CHO (%)	Tannins (g)	Energy (kcal)
Acorn cake	5·5*	11·1	16·8	0·5	1·6	44·2	31·2	0·05	303·4
Control cake	7·3	14·5	15·02	Not detected	1·3	44·9	31·5	Not detected	295·6
Treated acorn flour	4·9	9·8	6·2	1·8	1·2	6·7	79·2	Not examined	399·9

w.p., weight percent; Pro NRV, protein nutrient reference value; CHO, carbohydrate.
* Data are reported as mean.

identical packages to blind the participants and investigators to group allocation. The designed placebo cake was completely similar to the cake provided for the intervention group in terms of colour, size and model. For blinding, the packs of cakes were coded in A and B by a third party who was not involved in the research process. FC and PC were freshly produced every 2 weeks and given to the participants. In fact, participants were visited every 2 weeks to check their compliance as well as receive their cakes for consumption. Furthermore, 'cake consumption table' was given to the participants for recording their cake consumption. A daily short message was also sent via WhatsApp to remind the consumption of cakes. Participants consuming at least 90 % of the cake products were considered adherent, and if they missed consuming > 10 % of the cake products, they were considered non-adherent and were excluded. Any possible side effects during the bi-weekly visits were carefully examined, and if any serious side effects of cake consumption had been observed, they would have been excluded from the study.

A 3-d food record (two weekdays and one weekend) was used to assess food consumption and energy intake at three points during the study duration (baseline, week 5 and week 10). Nutritional data were analysed using Nutritionist IV software (First Databank) modified for Iranian foods. At baseline, the total energy expenditure was calculated using equations recommended for adults with overweight or obesity aged 19 years and older[21]. All participants in each group were given an energy-restricted diet for a 10-week study intervention (500 kcal less than total energy expenditure). The composition of diets was as follows: 55 % carbohydrate, 15 % protein and 30 % fat. An exchange list was given to each participant. The weight loss programme was similar for both groups.

Assessment of variables

Weight was measured by a digital balance scale in light clothing to the nearest 0·1 kg. Standing height was measured using a non-stretchable tape fixed to the wall to the nearest 0·1 cm, and fat mass and fat free mass were measured via bioelectric impedance analysis (InBody s10) in a supine position. BMI was calculated using the equation: weight (kg)/height^2 (m). Waist circumference was obtained to the nearest 0·1 cm at the midpoint of the lower rib and iliac crest at the end of normal expiration using a tape measure. Blood pressure was measured twice with a 5-minute interval using a mercury sphygmomanometer (BC08,

Beurer) after 15 min of rest in a sitting position. The same person took all the measurements to decrease the error rate. Physical activity levels were checked using the validated form of International Physical Activity Questionnaire before and after the study phase. Dietary intakes were assessed by a skilful dietitian at the beginning and at the end of the study using a validated 24-h recall questionnaire, and the average of food recalls was converted to grams and entered into Nutritionist IV software (based on food composition table of Agriculture Department of US that has been modified for Iranian foods).

Blood samples were collected after a 12-h fasting period. Fasting blood sugar, TAG, total cholesterol, LDL-cholesterol and HDL-cholesterol were measured by colorimetry kits (Pars Azmoon Co.) with an analyser system (Hitachi902, Roche). Serum insulin concentrations were measured by ELISA kits (Monobind). Plasma concentrations of adiponectin were determined using commercially available ELISA kits (Human adiponectin ELISA kit, AdipoGen Pharmaceuticals, Belmont). High-sensitivity C-reactive protein (hs-CRP) values were measured using an immunoturbidimetric assay (Pars Azmoon Co.). Homoeostasis model assessment for insulin resistance (HOMA-IR) index was used to determine the degree of insulin resistance using the following formula: HOMA-IR = (Fasting blood sugar (mg/dl) × Serum insulin level (mIU/l))/405.

Statistical analyses

The sample size was computed based on having an effect size of HOMA-IR as a key variable equal to 1·00. A minimum of thirty-five participants per group were calculated with a power of 80 % and a type I error of 5 %. This number increased to forty-two participants per group which accounted for an anticipated ~20 % dropout rate. Descriptive statistics are presented as mean and standard deviation. Kolmogorov–Smirnov test was used to verify normal distributions. Independent sample *t* tests and Mann–Whitney U tests were used to compare continuous variables of independent groups with normal and skewed distributions, respectively. Paired *t* test or Wilcoxon signed-rank test was used to compare the differences within groups for the normal or skewed data, respectively. To measure the effect of the 10-week intervention, a generalised linear model was applied with the baseline measures as covariates and the post-intervention values as a dependent variable (ANCOVA test). A χ^2 test was used to compare categorical variables between the PC and FC groups. Statistical analyses were performed using IBM SPSS Statistics, version 19 software, with the level of significance of $P \leq 0{\cdot}05$.

Results

Among eighty-four participants enrolled in the study, seventy-three subjects completed the study (PC group = 5 dropout, FC group = 6 dropouts) (Fig. 1).

The participants tolerated both cakes well, and no adverse reactions were reported. All FC and PC packages were returned empty biweekly, indicating full compliance with the cake consumption over the 10-week intervention. According to the participants' opinion, the taste of the cake was acceptable and palatable. Baseline characteristics of participants who completed the 10-week intervention are presented in Table 2. Comparing the anthropometric measures and biochemical parameters at baseline, no significant difference was observed between the two groups. Based on 3-d dietary recalls, no statistically significant difference was seen between the two groups regarding dietary intakes throughout the study (Table 3).

Table 2. Baseline participant characteristics
(Mean values and standard deviations)

	PC (*n* 42)		FC (*n* 42)		
	Mean	SD	Mean	SD	*P*
Age (years)*	42·9	7·4	44·2	7·9	0·49
Sex					0·175*
Male	11		16		
Female	31		26		
Height (cm)	164·3	8·8	166·7	9·8	0·27
Weight (kg)	82·9	10·9	83·2	12·3	0·93
BMI (kg/m^2)	30·7	2·2	29·8	2·6	0·26
WC (cm)	104·7	7·5	102·6	8·4	0·25
Fat mass (kg)	30·6	6·9	29·9	6·9	0·67
Body fat percent (%)	37·4	6·2	36·2	7·0	0·41
Fat free mass (kg)	50·9	8·4	53·2	11·1	0·32
Systolic BP (mmHg)	137	18·3	130·8	14·5	0·11
Diastolic BP (mmHg)	90·3	9·0	88·8	7·6	0·47
FBS (mg/dl)	97·8	19·3	101·1	17·4	0·45
Insulin (mU/l)	17·9	7·4	19·7	7·9	0·34
HOMA-IR	4·4	2·2	4·9	2·5	0·51
TAG (mg/dl)	174·2	77·1	178·6	81·4	0·81
Total cholesterol (mg/dl)	187·9	29·6	199·4	57·4	0·29
LDL (mg/dl)	113·05	28·3	113·6	35·2	0·94
HDL (mg/dl)	41·5	6·9	40·5	7·3	0·56
hs-CRP (mg/dl)	7·01	2·0	7·0	1·8	0·92
Adiponectin (μg/ml)	9·7	4·07	11·01	4·6	0·19
Energy intake, kcal	2189·5	512·3	2225·7	794·4	0·69
Physical activity (MET.min/week)	775	998	987	1488	0·43

CC, conventional cake group; FC, functional cake group; WC, waist circumference; BP, blood pressure; FBS, fasting blood sugar; HOMA-IR, homoeostasis model assessment for insulin resistance; hs-CRP, high-sensitive C-reactive protein; MET, metabolic equivalent.
All outcomes reported as mean ± standard deviation. *P*-value from independent samples *t* test.
* Chi-square test.

Table 3. Dietary intakes and physical activity of study participants throughout the study*
(Mean values and standard deviations)

	FC (*n* 36)		PC (*n* 37)		
	Mean	SD	Mean	SD	*P*
Energy intake, kcal	1564·2	487·3	1695·8	427·3	0·12
Carbohydrate intake, g	221·1	57·1	242·32	57·47	0·70
Protein, g	62·6	27·9	60·4	20·8	0·69
Fat, g	48·2	11·3	48·5	10·9	0·43
Cholesterol, g	235·4	150·4	241·1	140·7	0·84
SFA, g	11·06	6·1	11·1	4·3	0·95
MUFA, g	11·3	7·3	10·7	5·05	0·69
PUFA, g	14·08	6·1	12·7	5·5	0·37
Fibre intake, g	18·3	8·3	18·5	9·5	0·94
Physical activity (MET.min/week)	824·6	769·3	743·03	1043·9	0·68

PC, placebo cake group; FC, functional acorn cake group; MET, metabolic equivalent.
* All outcomes reported as mean ± standard deviation. Comparison of variables with normal distribution between two groups were analysed by *t* test and for inter groups paired *t* test. For variables without normal distribution between two groups Mann–Whitney was used and for inter-group analyses Wilcoxon was used.

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A decrease in body weight (kg), BMI (kg/m^2), waist circumference (cm), body fat mass (kg) and body fat percentage (%) was observed in both groups at the end of the study compared with the baseline (Table 4). No difference between groups in body weight, waist circumference, body fat mass and body fat percentage was observed ($P > 0{\cdot}05$) (Table 4). No differences were observed between groups regarding systolic and diastolic blood pressure.

The consumption of an FC, compared with the PC, resulted in a significant decrease in fasting serum insulin levels (changes from baseline: −5·8 (SD 5·3) *v.* −2·7 (SD 6·2) mU/l, $P = 0{\cdot}03$) and HOMA-IR (changes from baseline: −1·7 (SD 1·6) *v.* −0·8 (SD 1·7), $P = 0{\cdot}02$) (Table 4). No differences were observed between groups with regard to fasting blood sugar. Although we failed to find a significant effect of FC on fasting values of TAG and LDL-cholesterol ($P > 0{\cdot}05$), the effects on total cholesterol (−15·4 mg/dl; $P = 0{\cdot}05$) and HDL-cholesterol (+2·1 mg/dl; $P = 0{\cdot}06$) tended to be significant (Table 4). After the 10-week intervention, a significant reduction in serum hs-CRP levels (−2·3 (SD 1·2) *v.* −1·6 (SD 1·7) mg/dl, $P = 0{\cdot}04$) and adiponectin levels (+2·1 (SD 2·6) *v.* +1·1 (SD 1·5) μg/ml, $P = 0{\cdot}04$) was found following the consumption of FC compared with the PC group. After adjustment for baseline levels, no significant changes in our findings occurred. Inverse associations between serum adiponectin concentration changes and HOMA-IR changes ($r = -0{\cdot}2$, $P = 0{\cdot}03$) were observed.

Discussion

In this study, we examined the effect of a functional acorn cake on the metabolic status in participants with obesity or overweight and the metabolic syndrome. This randomised, double-blinded, placebo-controlled trial found that the consumption of functional acorn cake for 10 weeks led to significant improvements in insulin resistance indices, adiponectin and hs-CRP levels, while no significant change was observed in body composition, anthropometric measurements, lipid profile and fasting blood sugar compared with the control cake.

One of the important findings of the current study is related to the effects of functional cake on body weight as the participants of the study were individuals with obesity or overweight. Although participants in both groups lost significant weight (5–6 % of their body mass) because of an energy-restricted diet, weight loss was not different between groups. This finding could be possibly justified because of the effects of energy restriction that was implemented for both groups. The weight loss was more pronounced in the group receiving the functional cake. However, no beneficial additional effect on body weight because of the functional cake consumption was observed according to the statistical analyses. The weight loss observed in both groups can be clinically important as it can lead to improved health by reducing the complications associated with obesity[22]. No human clinical trial was done considering the effects of acorn or oak fruit on body weight or composition. However, it was reported previously that prebiotics available in the acorn could possibly affect gut–brain axis that is correlated with the microbiomes, and it could be helpful in treating obesity induced by the diet. It seems that prebiotics can have positive effects on obesity and metabolic disorders through various mechanisms, such as increasing the production of SCFA, reducing plasma endotoxin levels and also decreasing fat accumulation in adipocytes[23]. However, the components of the acorn may vary between different types or extracts, and this can affect the results[24]. Hence, we could hypothesise that the probiotic content of the acorn cake was not enough to affect the weight significantly or maybe the duration was not long enough to observe the intended effects.

As another finding, improvements in lipid profiles were also observed in both groups, probably because of the weight loss. However, total cholesterol and HDL-cholesterol improved non-significantly in the FC group. No human clinical trials have been done in this regard, but some animal studies have been conducted. In the studies done by Dogan[25] and Shaheen[26], which were performed on diabetic mice, lipid profile levels, including total cholesterol and HDL-cholesterol, improved after treatment with different doses of oak fruit extract. In these studies, higher doses of oak fruit extract showed better effects. The authors suggested that the positive effects of oak extract on lipid profile may be related to a decrease in cholesterol and fatty acid synthesis because of better glucose utilisation. The results of the current study also showed higher decreases in the LDL and total cholesterol of the FC group which were clinically significant but lacked statistical significance. This lack of significant effect could be attributed to the difference between the contents of the acorn used in the current study with that of other studies, which can affect the results significantly. Mostly, the studies showing the hypolipidaemic effects of the acorn used the pure extract rather than the acorn itself, and this could also affect the results as well.

Moreover, the current intervention showed significant positive effects of FC on insulin resistance. The positive and significant effect of functional acorn cake on reducing insulin resistance in this study is important and valuable because insulin resistance is the main feature and common root of the metabolic syndrome. This result was also confirmed by the study by Dogan *et al.* that showed the effect of acorn extract on reducing insulin level in diabetic mice[25]. Acorn, as a good source of saponins, could possibly reduce insulin resistance and improve insulin response by affecting insulin signalling[27]. Flavonoids, which are abundant in the acorn, could also play an important role in reducing insulin resistance by improving metabolic responses[25]. On the other hand, it seems that high concentrations of tocopherols in oak fruit can have positive effects on insulin sensitivity because studies showed that vitamin E can have a positive effect on insulin signalling pathways via its antioxidant capacity[28]. Under conditions of oxidative stress, insulin signalling pathway, that is, serine threonine kinase pathways, and insulin receptor substrate-1 are specifically phosphorylated and could diminish insulin signalling[29,30]. Acorn contents, especially antioxidants, could possibly improve insulin signalling pathways in those with the metabolic syndrome that have high levels of oxidative stress. Furthermore, the effect of acorn on intestinal microbiota could beneficially affect various conditions, including obesity, the metabolic syndrome and related disorders[31]. Acorn, as a food containing prebiotic, can

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Table 4. Body composition and metabolic measures at baseline and 10 weeks in adults with overweight/obesity and the metabolic syndrome (Mean values and standard deviations)

	PC (*n* 37)							FC (*n* 36)							Between groups		
	Baseline		End study		Change			Baseline		End study		Change					
Parameters	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	Mean	SD	*P**	*P*†	*P*‡	Mean difference
Weight (kg)	82·9	10·9	78·9	10·6	–4·2	1·9	<0·001	83·2	12·3	78·1	12·3	–5·1	2·8	<0·001	0·13	0·12	–0·1·02
BMI (kg/m^2)	30·7	2·2	29·2	2·4	–1·1	1·03	<0·001	29·8	2·6	27·9	2·8	–1·4	1·3	<0·001	0·26	0·27	–0·3
WC (cm)	104·7	7·5	101	7·8	–3·7	1·00	<0·001	102·6	8·1	98·2	8·4	–4·3	2·0	<0·001	0·13	0·13	–0·6
Fat mass (kg)	30·6	6·9	28·8	6·4	–1·7	2·4	<0·001	29·9	6·9	27·1	7·4	–2·8	3·5	<0·001	0·14	0·11	–1·03
Body Fat Percent	37·5	6·2	35·5	6·4	–1·9	3·2	0·003	36·2	7·0	33·4	6·8	–2·8	3·0	<0·001	0·23	0·16	–0·8
Fat free mass (kg)	50·9	8·4	48·8	7·0	–2·07	2·9	<0·001	53·2	11·1	51·7	8·7	–1·5	3·8	0·07	0·18	0·17	0·5
Systolic BP (mmHg)	137	18·3	120·9	9·8	–16·08	13·1	<0·001	130·8	14·5	118·4	7·9	–12·4	11·6	<0·001	0·2	0·87	3·7
Diastolic BP (mmHg)	90·3	9·04	87·1	10·8	–3·2	13·9	<0·001	88·8	7·6	84·6	8·2	–4·2	8·5	0·03	0·69	0·32	–5·01
FBS (mg/dl)	97·8	19·3	92·9	16·7	–4·9	10·8	0·006	101·1	17·4	93·4	13·7	–7·6	11·86	<0·001	0·31	0·34	–2·8
Insulin (mU/l)	17·9	7·4	15·2	6·5	–2·7	6·2	0·04	19·7	7·9	13·9	5·9	–5·8	5·3	<0·001	0·03	0·04	–3·08
HOMA-IR	4·4	2·2	3·6	1·8	–0·8	1·7	0·003	4·9	2·5	3·2	1·6	–1·7	1·63	<0·001	0·02	0·03	–0·9
TAG (mg/dl)	174·2	77·1	147·05	84·2	–27·2	61·8	0·008	178·6	81·4	133·3	54·5	–45·3	51·0	<0·001	0·18	0·16	–13·3
Total cholesterol (mg/dl)	187·9	29·6	181·3	30·6	–6·7	37·0	0·009	199·4	57·4	177·3	43·6	–22·1	22·9	<0·001	0·04	0·07	–15·4
LDL (mg/dl)	113·05	28·3	104·5	30·1	–8·5	31·7	0·005	113·6	35·2	98·7	29·2	–14·9	18·7	<0·001	0·29	0·25	–6·4
HDL (mg/dl)	41·5	6·90	43·3	7·5	+1·7	5·09	0·07	40·5	7·3	45·3	8·4	+3·9	4·7	<0·001	0·06	0·08	2·1
hs-CRP (mg/dl)	7·01	2·01	5·4	1·3	–1·6	1·7	0·002	7·06	1·8	4·7	0·9	–2·3	1·2	<0·001	0·04	0·004	–0·7
Adiponectin (μg/ml)	9·7	4·07	10·7	3·9	+1·1	1·5	0·02	11·01	4·6	13·1	4·1	+2·1	2·6	<0·001	0·04	0·009	1·03

PC, placebo cake group; FC, functional acorn cake group; WC, waist circumference; BP, blood pressure; FBS, fasting blood sugar; HOMA–IR, homoeostasis model assessment for insulin resistance index; hs-CRP, high-sensitive C-reactive protein.

All outcomes reported as mean ± standard deviation. Comparison of variables with normal distribution between two groups were conducted by *t* test and within groups paired *t* test and for variables without normal distribution between group Mann–Whitney was used and within-group analyses Wilcoxon was used.

* Difference from baseline (paired samples *t* test).

† Difference between groups (independent sample *t* test).

‡ ANCOVA adjusted for baseline value.

significantly affect the change of intestinal microbiota[24]. In a study by Ahmadi *et al.*, using acorn prebiotics was able to prevent high-fat-diet-induced insulin resistance[24]. Prebiotics can show various health-promoting effects through different mechanisms[23], such as improving insulin sensitivity[32], and the prebiotic content of the acorn could affect the insulin pathway as described.

Further, in the current study, a significant increase in adiponectin level was seen in the group receiving FC compared with the control group. An inverse relationship among changes in adiponectin concentration and HOMA-IR was observed in the present study, which was in agreement with previous reports[33,34]. Therefore, one of the possible reasons for the decrease in insulin resistance in this study could be attributed to a significant increase in adiponectin concentration. Adiponectin improves insulin sensitivity by increasing the oxidation of fatty acids and decreasing hepatic glucose production as well as decreasing the inflammatory status[35]. Besides, PPARγ, which has emerged as a potent insulin sensitisers[36], is strongly regulated and expressed by adiponectin[37] and adiponectin can strongly regulate insulin resistance and sensitivity[38]. Although an increase in adiponectin in both groups could be due to the significant weight loss observed in both groups[39]. Further, significant increase in the FC group can be attributed to the presence of several compounds in acorn, especially antioxidant compounds, such as gallic acid, epigallic polyphenols, tocopherols and the like that could enhance the secretion and expression of adiponectin in adipocytes[40]. Besides, the positive effect of vitamin E on adiponectin gene expression and enhancement of its secretion has been shown in several studies[41,42]. All the aforementioned mechanisms could possibly justify the effects of acorn on enhancing adiponectin level; however, no clinical trials have been conducted in this regard to compare the current results with them.

Moreover, considering the results of the current study about CRP level, a significant decrease in hs-CRP levels was observed in FC group compared with PC, which could possibly explain a part of the decrease in insulin resistance in the intervention group. However, no study was available for evaluating the effects of acorn on inflammatory markers in human interventions. Inflammation and oxidative stress caused by obesity are key factors in the pathogenesis of insulin resistance and the metabolic syndrome[43]. As a matter of fact, low-grade inflammation can affect insulin function. On the other hand, CRP as an inflammatory marker is directly related to insulin resistance and is increased people with obesity and insulin resistance[44]. Therefore, suppressing inflammation and oxidative stress could reduce the severity of insulin resistance. Oak fruit is rich in oleic acid which could show significant anti-inflammatory effects[12]. Besides, acorns are remarkable sources of tocopherols (α- and γ-tocopherol), which can reduce oxidative stress by accumulating in cell membranes[8,45]. Some researchers have indicated the role of tocopherols (α- and γ-tocopherol) in the modulation of inflammatory factors[46–48]. Therefore, the effects of FC on alleviating inflammatory markers can be described by its contents.

One of the limitations of the current study is the short duration of the intervention which has made it impossible for us to assess the long-term effects of FC on body composition. Second, since this study was, by design, under energy-restricted conditions, it is unknown whether FC can treat the metabolic syndrome or not. Considering that an energy-restricted diet was used in the design of this study, it still seems that the main reason for the improvement of some parameters of the metabolic syndrome is the reduction of energy content, and functional cake intake may benefit the participants more to reach the goal of treatment. Therefore, the answer to the question reading whether receiving this product can be useful to treat the metabolic syndrome or not requires further investigations, and the results should be interpreted with caution. In addition, body composition was measured via bioelectric impedance analysis which is not the gold standard for assessing body composition when compared with dual-energy X-ray absorptiometry. However, the present study had some strengths as well. One of the strengths of the present study was the high adherence rate of the consumption of cakes in both groups, as well as its randomised design. On the other hand, no clinical trials assessing the effects of acorn products on metabolic factors in human participants have been done till now, and this was the first intervention in this regard.

In conclusion, diet and physical activity are still key strategies to prevent and treat the metabolic syndrome. The current study indicates that FC consumption could possibly improve insulin sensitivity and improve CRP and adiponectin levels as an adjunct to an energy-restricted diet in those with the metabolic syndrome. Our promising findings indicated that further research with a longer duration and larger dose of oak is needed to elucidate whether FC can be used as a preventive strategy or adjunct treatment for the metabolic syndrome in individuals with obesity.

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There are no conflicts of interest.

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