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Effects of *Nigella sativa* supplementation on blood concentration and mRNA expression of TNF-α, PPAR-γ and adiponectin, as major adipogenesis-related markers, in obese and overweight women: a crossover, randomised-controlled trial

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Abstract

Adipocyte expansion through adipogenesis can offset the adverse metabolic effects of obesity. *Nigella sativa* (NS) (black seed) oil is shown to have therapeutic features in the management of obesity. NS oil might have beneficial changes in obese populations through mediating serum levels of adipogenesis-related parameters and relative transcriptional gene–diet interactions (nutrigenomics), though no previous studies assessed this mechanism in overweight/obese participants. This study assessed the effects of NS oil supplements on blood concentration and mRNA expression levels of TNF-α, PPAR-γ and serum adiponectin and expression of *AdipoR1*, as major adipogenesis and obesity-related parameters, in overweight/obese women using a cross-over design. Eligible women were randomised to receive either NS oil supplements (2000 mg/d) or placebo. Two periods of interventions (8 weeks in each) were cross-changed by a 4-week washout period. An individualised diet plan without calorie deficits was given to participants to match their energy/macronutrient intakes. The Pkcross procedure and intention-to-treat analysis were performed using Stata. *Cohen's d(d)* was estimated to measure the magnitude of the effects. Forty-six participants were included. NS oil capsules reduced transcription levels (($d = -2{\cdot}31$), $P < 0{\cdot}001$) and blood concentrations of TNF-α (($d = -0{\cdot}29$), $P < 0{\cdot}001$). AdipoR1 expression ($d = 2{\cdot}24$, $P < 0{\cdot}001$) and serum adiponectin ($d = 0{\cdot}88$, $P < 0{\cdot}001$) showed a significant augmentation with a medium-high effect size, as did gene expression ($d = 0{\cdot}69$, $P < 0{\cdot}001$) and serum levels of PPAR-γ ($d = 0{\cdot}97$, $P < 0{\cdot}001$). There was a moderate but significant decrease in body weight ($d = 0{\cdot}6$, $P < 0{\cdot}001$). The present beneficial findings would provide strong information for future nutrigenomics/clinical trial studies assessing the role of NS in the management of obesity and other comorbidities.

Key words: ***Nigella sativa*: Adipogenesis: Nutrigenomics: TNF-α: PPAR-γ: Crossover Studies**

WHO defines overweight and obesity as an abnormal or excessive fat accumulation that presents a health risk. According to the latest report by the WHO in 2016, 39 % of adults aged 18 years and over (39 % of men and 40 % of women) were overweight, and about 13 % of the world's adult population (11 % of men and 15 % of women) were obese[1]. Obesity is a multifactorial disorder determined by genetics and obesogenic environment, which refers to a high-calorie diet and a sedentary lifestyle.

Adipocyte expansion through the adipogenesis process can offset the adverse metabolic effects of obesity[2]. Among several transcription factors and adipogenic genes involved in the adipogenesis process, *PPAR-γ*[3], *TNF-α*[4], and adiponectin are on the top list and have been shown to play a substantial role in modulating adipogenesis and obesity[5,6]. Obesity predisposes to an inflammatory state via increased TNF-α, which inhibits the adipogenesis process[7]. Evidence suggests that *PPAR*-agonists reduce *TNF-α* expression and thus have a positive effect on

Abbreviations: NS, *Nigella sativa*; TQ, thymoquinone.

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adipogenesis modulation[4]. In addition, adiponectin is another key regulator in obesity and adipogenesis which is believed to be down-regulated in the presence of obesity and excess adipose tissue[8]. Adiponectin exerts its effects by binding to adiponectin receptors (AdipoRs), specifically AdipoR1, which is up-regulated by PPAR-γ activation[9]. According to the evidence, mRNA levels of AdipoR1 are significantly lower in overweight or obese individuals, and adiponectin has also been shown to have reduced biological effects in this population due to a decreased adiponectin-to-AdipoR1 ratio[6,10].

Meanwhile, with the rising direct and indirect expenses of obesity treatments[11], herbal medicine and naturally occurring phytochemicals have been widely used as low-cost alternatives and/or supplementary therapeutic options for the treatment of obesity. They exert their beneficial effects via acting as natural ligands and altering gene expression of various obesity-related factors[12]. Among these beneficial medicinal plants, *Nigella sativa* (NS) is an herb from the Ranunculaceae family that is mostly known as black cumin or black seed. The therapeutic and pharmacological properties of NS, including its anti-oxidant, anti-inflammatory, weight-management and cardio-protective effects, have been extensively reported in the literature[13,14]. However, the exact mechanisms of the effects of NS or its main active component, thymoquinone (TQ), on adipogenesis and obesity are unclear. A few animal and *in vitro* studies on adipose tissue samples have revealed significant improvements in *PPAR-γ* and *TNF-α* expression levels[15,16]. Concerning clinical trial investigations, a few randomised-controlled trial studies assessed the effects of NS oil on obesity-related parameters[17–20]. A previous parallel randomised-controlled trial looked at the effects of NS oil supplementation combined with a low-calorie diet on cardiometabolic markers in obese or overweight women, and despite a significant reduction in body weight, the authors found no significant changes in serum TNF-α after 8 weeks[17]. Another study showed that a daily intervention of 1000 mg of NS oil had no considerable effects on serum adiponectin levels[20]. In fact, similar clinical trial investigations either included populations with different types of diseases[18,20,21] or used NS oil supplements in combination with other dietary interventions among overweight and obese participants and did not evaluate the pure effect of NS oil[17,22,23]. Meanwhile, recent evidence strongly focuses on nutrigenomics science, which refers to evaluating the interaction between nutrients and gene expression at the molecular level as well as understanding the influence of dietary components on the genome, transcriptome, proteome and metabolome[24,25]. Hence, involving nutrigenomics science in human investigations would not only provide insights into the exact mechanisms of gene–diet interactions but also provide contributions to improved treatment and prevention strategies for future illness, especially nontransmissible chronic diseases such as overweight and obesity[25]. Notably, peripheral blood mononuclear cells have been shown to reflect the effects of dietary modifications at the level of gene expression and have been potentially used in nutrigenomics sciences[26]. Regarding that, no study has so far investigated whether blood mRNA levels of major factors involved in adipogenesis and obesity are impacted by NS oil supplementation in humans or whether the relative changes in serum concentration of these parameters are correlated with their transcriptional changes.

We conducted a double-blind, randomised, placebo-controlled clinical trial using a crossover design to determine the effects of NS oil supplementation on both serum concentration and mRNA gene expression levels of major obesity- and adipogenesis-related factors (in peripheral blood mononuclear cells), including *PPAR-γ*, *TNF-α* and adiponectin (and *AdipoR1* as the assessed gene), among women with overweight and obesity.

Methods

Ethical approval

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board and Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (code: IR.SSU.SPH.REC.1397·007, on April 17th, 2018[27]). Participant recruitment was performed in April 2019. The study protocol was explained to all the participants, and an informed consent form was obtained from all the volunteers at the beginning of the study. The study protocol was registered with the Iranian Registry of Clinical Trials under the code IRCT20180528039884N1, on July 2nd, 2018[28].

Study design and participants

All details of study design and participant recruitment have been extensively reported previously[14]. To give a brief description, two periods of an 8-week intervention were used in the present double-blind, placebo-controlled, randomised trial study, which was conducted with a crossover design. A 4-week washout period was administered to separate the intervention periods. This article was reported according to the Consolidated Standards of Reporting Trials statement, revised for randomised crossover trials[29]. A Consolidated Standards of Reporting Trials diagram detailing the timing of enrolment, interventions and assessments is provided in Fig. 1.

The study included 25–55-year-old adult Iranian females with overweight and obesity (BMI) between 25 and 35 kg/m^2 who were referred to the obesity clinic at Shahid Sadoughi University of Medical Sciences in Yazd, Iran. Inclusion criteria for blood pressure and fasting serum total cholesterol were less than 139/89 and 250 mg/dl, respectively. Participants were asked not to change their routine physical activity. Taking regular medications, such as lipid-lowering agents or blood pressure medications, was allowed as long as the dosages remained the same during the study.

Patients were not included if they had any of the following criteria: hyperlipidemia, diabetes, thyroid or renal problems, CVD, pancreatic or hepatic disorders, alcohol consumption, smoking, a history of any type of allergy or cancer, pregnancy and lactating, using anti-obesity drugs and using any kind of weight-loss procedures (including dietary plans or surgery) in the 6 months before the study initiation.

Eligible participants were randomised into two groups (1:1) of intervention and placebo. Stratified block randomisation,

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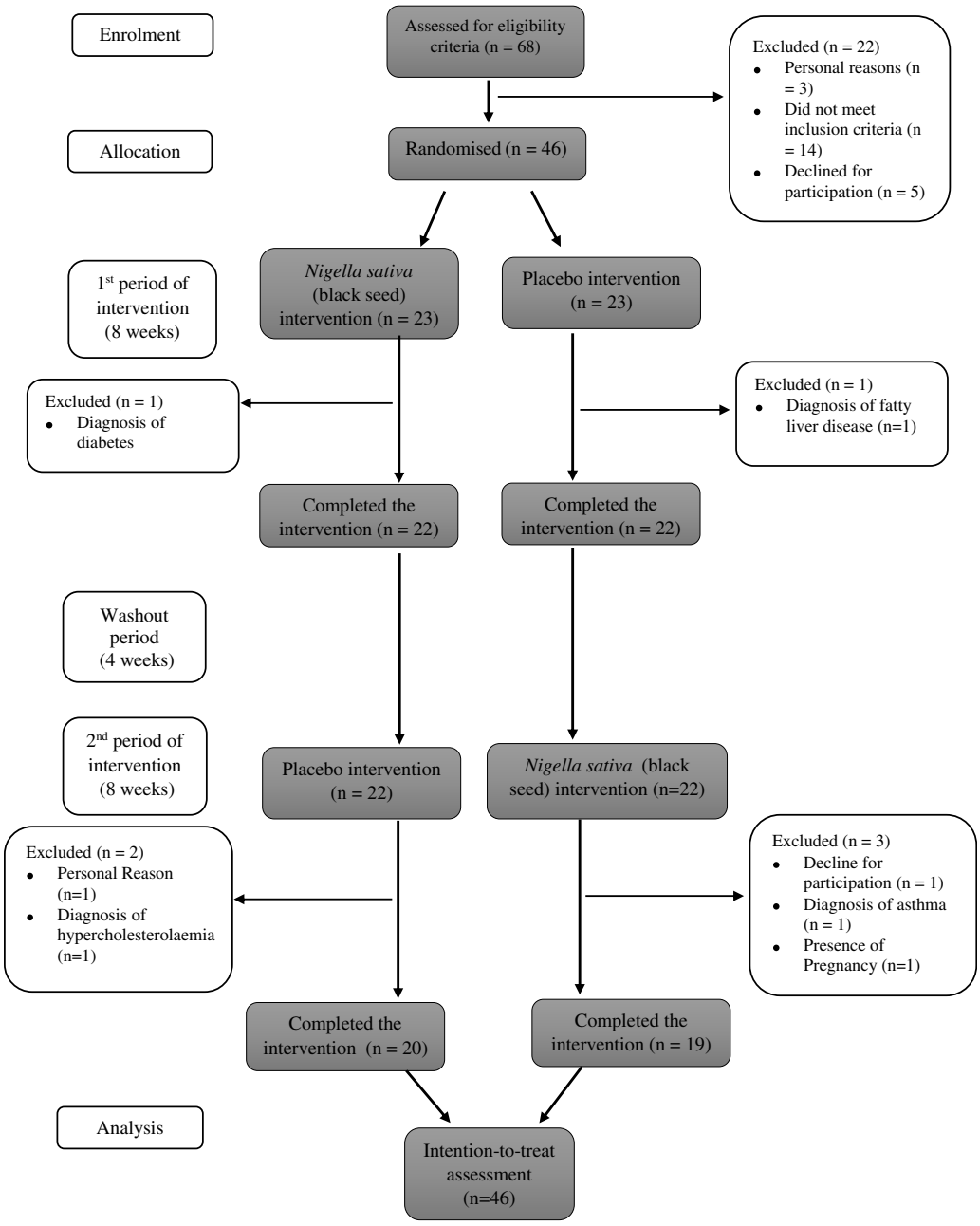

Fig. 1. Flow diagram.

based on age (25–40 and 40–55 years), was performed by an independent researcher using computer-generated random numbers and allocation concealment was performed using sealed envelopes. After the first 8 weeks of intervention, a washout period of 4 weeks was completed, and the second intervention period of 8 weeks was initiated. According to the evidence, a washout period should be long enough (at least five times the half-life of the treatment intervention with the maximum half-life in the study) to eliminate the carryover effect[30]. Given that, the elimination half-life of oral administration of TQ as the main constituent of NS oil was approximately estimated to be 275 min[31,32]. We extended the washout time to 4 weeks to ensure that the maximum elimination of carryover effects was achieved. Women were given either two separate capsules of 1000 mg of NS oil per day or two capsules of placebo (each containing 1000 mg of paraffin oil) because it has been reported that 2000 mg of NS oil per day is well tolerated and has no adverse reactions[33]. Participants were requested to take one capsule before lunch and another before dinner.

Both the NS oil and placebo capsules were produced by Barij Essence Pharmaceutical Co. The oil was extracted from the Iranian origin of *Nigella sativa* (Ranunculaceae family) and was prepared using the cold-press technique. Each capsule of 1000 mg of NS consisted of 1·1 % of TQ as the most active component. The intervention and placebo capsules had similar shapes and tastes, and the bottles were labeled with specific

codes. During the study, each participant received four bottles containing fifty-six capsules. The first two bottles were given at the beginning of the first treatment period, and the second two bottles were administered at the start of the second treatment period. The content of the bottles, study treatment and allocation was blinded to all the participants, administrators and researchers until the final analysis. Codebreaking was ultimately performed by someone unaware of the study after the statistical analysis was completed and the database was locked. Compliance with the intervention was monitored three times per week through phone and face-to-face interviews. All participants were requested to bring back the bottles with the remaining capsules at the end of each intervention period. All participants were requested to report any adverse reactions.

In addition to the capsules of NS oil, an individualised diet plan without any calorie deficit was given to every woman during the whole study to match all participants based on their dietary intakes. A registered dietitian designed the dietary program based on her body weight at the beginning of every intervention period. The required energy was initially calculated, and then the dietary plan of servings from each food group was developed to fulfill the macronutrient distribution, which was as follows: 55 % of total energy from carbohydrates, < 30 % from fat and 1·2–1·5 g of protein per kg of body weight. The compliance with dietary intakes of participants in both groups was monitored by a registered dietitian during each intervention period (at weeks 4 and 8).

More information regarding the details of the methodology, NS oil and placebo capsules, the individualised diet plan and the GC/MS of NS oil supplements has been published previously[14].

A body analyzer (Inbody, 770) machine was used for measuring weight (to the nearest 0·1 kg precision), height (to the nearest 0·1 cm precision) and body composition of participants in light clothing and in an overnight fasted state. BMI was calculated as weight (kg) divided by height (m^2). Every participant's dietary intake was assessed twice during the research using 3-day food records (each at the beginning of every intervention duration) and then household measures were used to convert the records to g/d[34]. Physical activity levels were evaluated by a questionnaire based on metabolic equivalent[35] at weeks 0 and 8 of each intervention period. The questionnaire includes nine categories of physical activity with different intensities. The total number of metabolic equivalent hours per day (h/d) was calculated using the sum of the average hours spent on 1 day for each activity based on the amount of metabolic equivalent.

Laboratory methods

Serum biochemical parameters. In a fasting condition, a venous blood sample (12 ml) was obtained from each participant at the start and end of each intervention period. After 10-min centrifugation of blood samples (3000 g, at room temperature; Eppendorf AG), aliquots of serum samples were separated and stored at −70°C. ELISA method was used to assess serum levels of TNF-α, PPAR-γ and adiponectin (Thermo Fisher Co.; inter and intra-assay CV were < 10 %).

RNA extraction and real-time PCR. Isolation of peripheral blood mononuclear cells was performed from whole blood using the standard Ficoll method[36]. Then, total RNA was extracted immediately using a Qiagen RNA purification kit (Qiagen Co., Cat No. 74 104). The quality and purity (260/280 nm ratio between 1·8 and 2·2) of the extracted RNA were checked by a spectrophotometer (NanoDrop, Thermo Scientific). Total mRNA was then reverse-transcribed to cDNA using a cDNA synthesis kit (Thermo Fisher Co., Cat No. RR037). Amplification of cDNA was performed using real-time PCR and the SYBR Green method (Takara Bio, Inc.) to determine the gene expression levels of *TNF-α*, *PPAR-γ* and *AdipoR1*. Primers of real-time polymerase chain reaction were designed using Primer Blast, Oligocalc and Gene-runner 5·0·99 (Table 1). The housekeeping gene Glyceraldehyde phosphate dehydrogenase (GAPDH) in all assessments. The efficacy of PCR was assessed using LinRegPCR software[37]. After obtaining cycle threshold (Ct) values, fold changes were calculated through ΔΔCt method[38]. The accession numbers of *TNF-α*, *PPAR-γ*, *AdipoR1* and *GAPDH* are provided from the Ensemble genome database, which are as follows, respectively: ENSG00000232810, ENSG00000132170, ENSG00000159346, ENSG00000111640.

Sample size and statistical analysis. Based on a formula specific for sample size calculation in crossover studies[39], after considering a minimum non-inferiority margin of 0·05, TNF-α gene expression as the key variable[40] with an acceptable mean difference of 0·5 between the intervention and the control groups, a significance level of 0·05, a power of 80 % and anticipating a 15 % of dropout rate, the overall sample size was calculated to be 40 (The exact methodology of sample size calculation in the present crossover-randomised controlled trial was previously provided in details[14]).

The continuous variables were reported as means ± SD. The intention-to-treat method was used in the final analysis. The intention-to-treat population is comprised of all randomised individuals for whom relative data were available for at least the first intervention period of the study. An independent samples *t* test was used to compare the baseline characteristics and outcomes of interest between groups. Skewness and Kurtosis test was performed to examine the normality of the data. If any baseline variables were found to depart from normality (*P* Skewness < 0·05), the BoxCox transform formula was used to normalise them. The Pkcross procedure specific for crossover analysis was used, considering four main indicators, including 'sequence', 'treatment', 'carryover', and 'period' effects. In addition, to assess the magnitude of the effect for final changes in each outcome, *Cohen's d* (*d*) was estimated, which was defined as the between-means differences after the intervention, divided by the pooled SD. The definition of the effect sizes was as follows: small ($d = 0·2$), medium ($d = 0·5$) and large ($d = 0·8$). Cohen's *d* of zero indicates that the mean between the two comparative groups had no differences, and 50 % of the observations in the control group were located below the mean of the experimental group. In contrast, the Cohen's *d* of 0·2, 0·5, and 0·8 locates at the percentile of 58th, 69th and 79th of the distribution of the control group, respectively[41]. In the presence of a significant carryover effect (residual P 0·05), data were analysed using

Table 1. Real-time PCR primer sequences

	Forward	Reverse
TNF-α	GCCCAGGCAGTCAGATCATC	GCTGGTTATCTCTCAGCTCC
PPAR-γ	TCAAGAGTACCAAAGTGCAATCA	AACTCCATAGTGAAATCCAGAAGC
AdipoR1	CCACCCAAAGCTGAAGAAG	TCATATGGGATGACCCTCCA
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

AdipoR1, adiponectin receptor 1, *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

ANCOVA only for the first intervention period. Participants' age and baseline BMI were considered covariates. *P* value of less than 0·05 was considered statistically significant. All the analyses were conducted using Stata software version 13·0 (Stata Corp LLC).

Results

Forty-six eligible participants were recruited for the present investigation. Figure 1 illustrates the complete flow of study. Seven participants were excluded mostly for personal reasons, pregnancy or the diagnosis of different diseases. Table 2 shows the participants' baseline characteristics. All the data was considered normally distributed. The mean ± SD of age, height and baseline BMI of participants were 36·4 ± 9·4 (years), 158·74 ± 6·41 (cm) and 31·26 ± 4·8 (kg/m^2), respectively. Based on the baseline data, 51·3 % of participants were overweight, and 48·7 % were obese. Nearly 17 % of the participants had mild fatty liver disease, 10 % had a family history of obesity and 7 % had high normal blood pressure. About 84·8 % of participants (*n* 39) were originally from Yazd province, Yazd, Iran and 15·2 % (*n* 7) were from other cities such as Tehran, Shiraz and Isfahan, Iran.

Participants taking NS oil or placebo capsules had no significant differences in food and medication intakes or physical activity levels. There were no significant changes between the two 3-day dietary records used in the study (data not shown). Furthermore, there was no significant change in the baseline measurements of outcomes before each treatment period. No serious adverse reactions were reported. Final assessments were conducted and compared with the pooled values of the baseline measures. Primary results showed that NS oil supplementation resulted in a moderate but significant decrease in BMI (*P* effect<0·001, Cohen's $d = 0·5$) and body weight (*P* effect < 0·001, *Cohen's* $d = 0·6$) in the intervention period compared with the placebo period[42]. Moreover, supplementation with NS oil resulted in an improvement in serum levels of HDL-cholesterol (Cohen's $d = 0·47$, $P = 0·009$), LDL-cholesterol (Cohen's $d = -0·33$, $P = 0·031$), aspartate aminotransferase (Cohen's $d = -0·5$, $P = 0·038$) and systolic blood pressure (Cohen's $d = -0·4$, $P < 0·001$). Furthermore, a significant reduction was found in the levels of TC/HDL-C ratio (Cohen's $d = -0·9$, $P < 0·001$). There was no significant effect on diastolic blood pressure measures ($P = 0·96$)[43].

Changes in mRNA gene expression of TNF-α, PPAR-γ and AdipoR1

The effect of the NS oil intervention on relative changes in mRNA expression levels of *TNF-α*, *PPAR-γ* and *AdipoR1* in peripheral blood mononuclear cells is shown in Fig. 2.

After controlling for treatment, period and carryover effects, the final crossover analysis revealed that NS oil supplementation resulted in a 0·47-fold decrease in TNF-gene expression compared with the placebo. The relative Cohen's *d* effect size was estimated to be very high, indicating that more than 85 % of mean fold changes in *TNF-α* gene expressions in the control group were below the mean measures in the intervention group (*P* effect < 0·001, *P* carryover effect = 0·93, $d = 2·31$). Furthermore, results from real-time PCR showed a 0·87-fold increase in *PPAR-γ* gene expression level with a medium-high effect size (*P* effect < 0·001, *P* carryover effect = 0·61, $d = 0·69$), showing that nearly 70 % of the mean measures in the control group were located below the mean measures in the intervention group. Moreover, a 0·77-fold increase with a relatively high effect size was observed in *AdipoR1* gene expression measures (*P* effect < 0·001, *P* carryover effect = 0·72, $d = 2·24$). More details are reported in Table 3.

Changes in serum concentrations of TNF-α, PPAR-γ and adiponectin

Consistent with results yielded from real-time PCR, NS oil supplementation resulted in a significant reduction in serum concentrations of TNF-*α* (*P* effect < 0·001). However, the carryover effect was shown to be significant (*P* carryover effect = 0·041). As a result, the final analyses were conducted using relative data from the first treatment period. The results remained significant, indicating that there was a significant reduction in serum TNF-levels at the end of the first intervention (29·8 ± 8·1 pg/ml in intervention group (*n* 22) *v.* 36·1 ± 9·2 pg/ml in the placebo group (*n* 22), *P* value = 0·035). In addition, Cohen's *d* estimation showed a low effect size in the final changes of blood concentrations of TNF-*α* ($d = -0·29$), determining that less than 58 % of serum TNF-*α* levels in the control group were below the mean measures estimated in the intervention group. Finally, the crossover analysis revealed a significant increase in PPAR-*γ* (*P* effect < 0·001, *P* carryover effect = 0·42, $d = 0·97$) and adiponectin (*P* effect < 0·001, *P* carryover effect = 0·71, $d = 0·88$) serum concentrations with very large effect sizes (Table 3).

Discussion

This crossover clinical trial study with an overall 5-month implementation period aimed to investigate the effects of NS oil supplementation on blood concentration and mRNA expression changes of major factors involved in adipogenesis in women with overweight and obesity. TNF-*α* mRNA expression was significantly reduced with a large effect size. Similarly, blood content of TNF-*α* was decreased significantly, albeit with a smaller

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Table 2. Baseline characteristics of participants*
(Mean values and standard deviations)

Variables	Total participants (*n* 46)		NS intervention (*n* 23)		Placebo intervention (*n* 23)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)†	36·4	9·4	37·7	10·91	34·6	9·6
Height (cm)†	158·74	6·41	156·5	4·51	159·60	7·2
Body weight (kg)	76·8	13·9	76·6	13·9	79·9·3	14·8
BMI (kg/m^2)†	31·26	4·8	31·15	4·13	31·40	5·5
Physical activity (MET-h/day)†	25·72	3·25	25·91	3·1	25·54	3·9
Menopausal status, (perimenopause) *n* (%)	8	17·17	3	13·1	5	20·9
High normal blood pressure, *n* (%)	7	14·9	2	8·7	5	20·85
Mild fatty liver, *n* (%)	17	36·17	8	34·78	9	37·5
Family history of obesity, *n* (%)	10	25·64	5	26·31	5	25·10
Dietary intake parameters†						
Daily energy intake (Cal)	1780·34	190·54	1765·86	212·82	1794·25	169·56
Carbohydrate (g)	222·14	34·153	217·25	31·89	226·82	37·1
Protein (g)	50·9	7·8	52·68	8·21	49·21	7·3
Fat (g)	79·3	12·57	79·7	12·3	78·9	13·1
Fibre (g)	13·11	2·80	12·5	2·95	13·85	2·51
Cholesterol (mg)	144·98	862·7	162·43	93·54	128·26	78·8
PUFA (g)	25·43	5·1	25·76	4·74	25·11	5·4
MUFA (g)	32·2	6·5	31·33	6·21	33·1	6·75
SFA (g)	14·9	2·8	15·26	2·25	14·64	3·31
Vitamin E (α-tocopherol) (IU)	26·01	5·6	25·93	5·3	26·11	5·96
Vitamin C (mg)	59·95	24·38	56·71	20·61	63·14	27·59
Vitamin D (μg)	3·8	2·7	4·1	2·8	3·5	2·5
Medication use						
Statins, *n* (%)	10	21·27	5	21·73	5	20·84
Blood pressure lowering drugs, *n* (%)	3	6·38	0	0·0	3	12·5
Both drugs, *n* (%)	6	12·78	3	13·10	3	15·50
No drugs, *n* (%)	20	421·55	11	47·82	9	37·5
Serum biomarkers†						
IL-1β (pg/ml)	2·95	1·42	2·75	1·45	3·0	1·4
IL-6 (pg/ml)	2·85	1·52	2·7	1·50	1·85	0·5
Leptin (ng/ml)	41·3	6·8	41·9	6·22	40·8	7·31

* Between-group changes were assessed using independent *t* test.
† Data are reported as mean (standard deviations).
MET, metabolic equivalent.

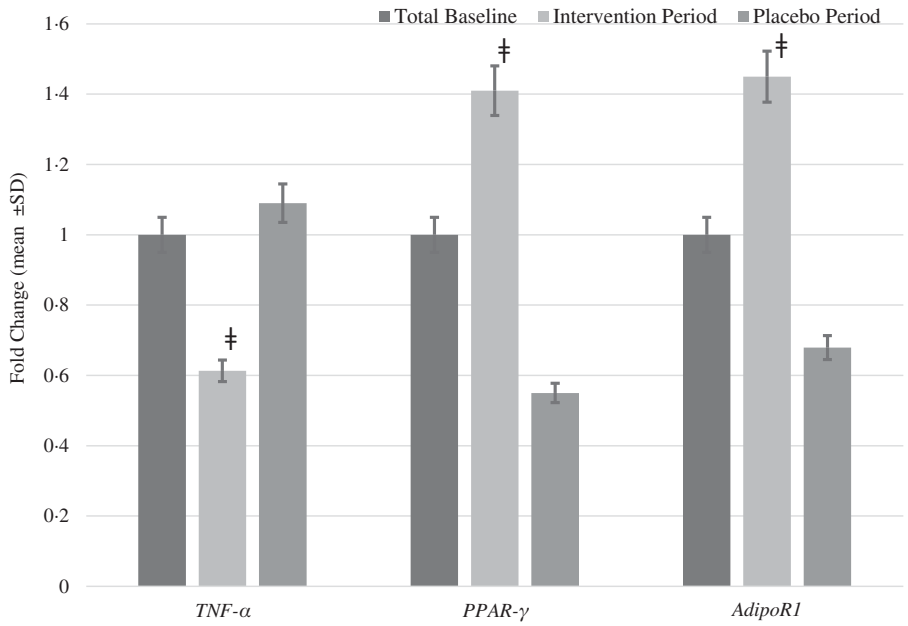

Fig. 2. Changes in mRNA expression levels in PBMCs of *TNF-α*, *PPAR-γ*, *AdipoR1* during NS intervention period *v.* placebo period among women with overweight and obesity (*n* 46). NS, *Nigella sativa*; AdipoR1, adiponectin receptor 1. ‡ Statistically significant changes were observed in the NS oil intervention period compared with the placebo period (*P* value < 0·001)

Table 3. Changes in mRNA expression levels of *TNF-α*, *PPAR-γ* and *AdipoR1* in PBMC and serum concentrations of TNF-α, PPAR-γ and adiponectin during NS intervention period *v.* Placebo period among women with overweight and obesity
(Mean values and standard deviations, *n* 46)

		Treatment periods										
		Placebo period		NS period		Within-participant difference					*P* value in Period effect	*P* value in Carryover effect
Outcome*		Mean	SD	Mean	SD	Mean	SD	*P* value†	*Cohen's d*‡	F		
mRNA gene expression changes in PBMC	*TNF-α* (fold change)	1·1	0·08	0·62	0·02	–0·48	0·21	< 0·001	–2·31	175·8	0·26	0·95
	PPAR-γ (fold change)	0·55	0·17	1·41	0·8	0·86	1·25	< 0·001	0·69	31·4	0·98	0·61
	AdipoR1 (fold change)	0·68	0·13	1·46	0·18	0·78	0·35	< 0·001	2·24	106·15	0·73	0·78
Serum concentrations	TNF-α (pg/ml)	31·7	6·4	28·1	7·7	–3·6	12·4	< 0·001	–0·29	177·5	0·71	0·04
	PPAR-γ (pg/ml)	2·7	0·67	3·4	0·77	0·72	0·74	< 0·001	0·97	208·5	0·32	0·42
	Adiponectin (μg/ml)	16·8	3·3	21·7	0·98	4·9	5·6	< 0·001	0·88	104·5	0·22	0·71

PBMC, peripheral blood mononuclear cell; NS, *Nigella sativa; AdipoR1*, adiponectin receptor-1.
* Data are reported as mean (standard deviations).
† *P* value of the treatment effect resulted from the crossover analysis. Pkcross procedure was used for crossover analysis.
‡ The effect size was estimated by calculating the Cohen's *d* values, which were defined as the difference between the means after the intervention, divided by the pooled SD. Cohen's *d* of 0·2, 0·5 and 0·8 locate at 58th, 69th and 79th percentile of the distribution of the control group, respectively.

effect size. NS oil supplementation also significantly increased both transcription and serum concentration of PPAR-γ, with high and medium-high effect sizes, respectively. Furthermore, an increase in the mRNA level of *AdipoR1* and the serum concentration of adiponectin were observed, both with high effect sizes.

Although no similar human study has been published so far, some cell biology and animal experiments have evaluated the effect of NS oil or similar compounds (e.g., black seed, black cumin, caraway, kalonji, TQ) exposure on mRNA expression levels of *TNF-α*, *PPAR-γ* and *AdipoR1*. In line with our findings, an animal study in rats reported a considerable reduction in blood *TNF-α* transcription after 12 weeks of NS oil intervention (1000 mg/d)[44]. Consistent with the current study, an intervention of 100 mg/kg TQ for 42 days prevented a decrease in blood mRNA expression of PPAR-γ in rats with metabolic syndrome, demonstrating TQ interaction with the ligand-binding pocket of PPAR-γ, which has been reported to be critical for its activity[45]. Furthermore, a recent study found that a 54-day intervention of NS extracts resulted in a significant decrease and increase in serum TNF-α and adiponectin levels in rats with metabolic syndrome[46]. A few clinical trial studies have looked into the effect of NS oil on obesity-related markers[17,18,21,47]. Mahdavi *et al.* demonstrated that a combination of 3 g/d of NS oil supplement and a low-calorie diet for 8 weeks resulted in a significant reduction in serum TNF-α in women with obesity. However, they declared that using a low-calorie diet throughout the study, and not NS oil supplementation, might be the main reason for the decreased level of serum TNF-α, because there was a significant decrease in dietary energy intake levels[17]. Other studies also revealed that serum concentrations and mRNA expression of *TNF-α* decreased significantly in participants with overweight and obesity after a low-calorie diet intervention[48]. The current study discovered a significant decrease in serum levels and mRNA expression of TNF-α after NS oil supplementation without any low-calorie diet program, but the decrease in blood TNF-α concentration was of medium effect size, indicating that more evidence is needed to confirm the potential role of NS oil supplementation alone in reducing TNF-α blood concentration. Contrary to the above-mentioned studies, we assessed the single effects of NS oil supplementation on modulating factors related to obesity and adipogenesis at a relatively safe dose and for a longer duration while giving every participant an individualised diet plan without any calorie deficit, in order to reduce the heterogeneity between the dietary intakes of participants. As a result, our findings regarding the single effects of NS oil intervention on serum TNF-α levels in obese participants would be more reliable.

With regard to the effects of NS oil supplementation on serum adiponectin, Hosseinpour-Niazi showed that using NS oil capsules at a daily dose of 1000 mg for 8 weeks had no significant effects on serum adiponectin in patients with type 2 diabetes mellitus[20]. One might say that this conflicting finding might be due to the different population or the lower intervention dose of NS oil capsules compared with the present study. Although none of the previous investigations evaluated the effects of NS oil supplements on serum levels of PPAR-γ or mRNA expression of *AdipoR1* and *PPAR-γ*, we observed a significant increase with a relatively high effect size in both mRNA expression and serum levels of these parameters, which further proves the beneficial effects of NS oil on obesity, specifically through nutrigenomics pathways. According to evidence, increased *Adipor1* and *PPAR-γ* expression and serum adiponectin and PPAR-γ concentrations have the greatest potential for reducing the burden associated with obesity and related chronic diseases, such as diabetes and CVD[49–51].

In terms of molecular mechanisms of action, adipocyte differentiation via the adipogenesis process is controlled by a tightly regulated transcriptional cascade in which PPAR-γ plays a key role[52–54] Increased *PPAR-γ* transcription increases adiponectin concentrations via increased expression of *AdipoRs*, specifically *AdipoR1*[55]. Obesity is also associated with higher TNF-α levels in the blood, which lowers serum PPAR-γ levels by activating the JNK pathway[56]. Besides, *TNF-α* suppresses *PPAR-γ* gene expression through activating NF-κB[57,58]. Consistent with these observations, we found that NS oil supplementation decreases both blood mRNA expression levels and serum concentrations of TNF-α, and PPAR-γ, and increases serum levels of adiponectin and mRNA expression of *AdipoR1*. These findings suggest that

alterations in both transcriptional and serum levels of major factors involved in adipogenesis and obesity in response to NS oil supplementation may partly explain the significant beneficial changes in body weight and body composition[42]. In fact, weight gain and adipogenesis status are interrelated. Any conditions that have a positive impact on adipogenesis status would undoubtedly result in obesity prevention and weight loss. A previous study showed that a combination of NS oil supplementation with a low-calorie diet resulted in a significant decrease in body weight among overweight and obese women[23]. This might strongly be due to the low-deficit diet plan and not NS oil supplements alone, while the promising results observed in the present investigation were yielded following NS oil supplementation alone. The metabolic health benefits of NS oil supplements may also be due to the positive role of NS in modulating the gut and intestinal microbiota composition[59,60].

The present investigation has several strengths. This study is the first study of its kind that assessed the effect of NS oil supplementation *v.* placebo on both serum and blood mRNA levels of adipogenesis and obesity-related parameters. A crossover design was used, which would be more reliable due to the longer duration and the double assessments performed for every participant[61]. Moreover, we analysed our data by estimating not only the treatment, period and carryover effects but also the effect sizes (Cohens' *d*) for each outcome, which resulted in estimating a more precise magnitude of the effects. An individualised diet plan was also given to every participant to minimise any possible confounding effects of dietary intake. Moreover, compared with a few previous studies which used other dietary interventions along with the NS oil supplements, such as honey[62] or vitamins and herbs[17,22], we assessed the single effect of NS oil supplement on obesity and adipogenesis parameters in participants with overweight or obesity. According to previous evidence, a therapeutically effective daily dosage of capsules of NS oil was considered in the present study[13,33]. Furthermore, we assessed both serum and blood mRNA expression changes of the above-mentioned parameters, which would give valid information regarding molecular mechanisms and nutrigenomics aspects of the effects of NS oil supplements on obesity and adipogenesis, specifically for future investigations. This study has some limitations. We did not measure the metabolite content of NS oil or its active component, TQ in blood or urine. We also did not assess the exact measures of dietary antioxidants. It should be noted that NS oil capsules may have different effects on other ethnic groups or male populations. We have enrolled women with overweight and obesity, but we are aware that enrolling only women with obesity may have added more homogeneity to our experimental design. Although we used a long washout period needed for the elimination of the carryover effects after the first period of NS oil intervention, we observed a significant carryover effect for the changes in serum TNF-α, and hence, more assessments should be performed regarding the half-life of NS oil supplements. Finally, we acknowledge that we used liberal cut-offs for significance (two-sided $P < 0{\cdot}05$). Therefore, our findings need further validation in independent studies.

Conclusions

In conclusion, daily supplementation of 2000 mg of NS oil capsules resulted in beneficial changes in blood mRNA expression levels of obesity and adipogenesis-related parameters such as *TNF-α*, *PPAR-γ* and *AdipoR1*. Consistent improvements were observed in the serum concentrations of TNF-α, PPAR-γ and adiponectin, though the estimated effect sizes were lower than the transcription changes. Overall, the present study showed beneficial findings on the interaction between NS oil supplements and gene expression of adipogenesis-related parameters at the molecular level and nutrigenomics, as well as positive changes in their serum concentration, which all provide detailed information for the treatment and prevention of obesity and its comorbidities. However, more investigations into both genders and different ethnic groups, with longer follow-ups and possible higher doses of NS oil supplements, are needed. Exploring adipose tissue remodeling, as well as measuring gene expression and blood levels of other obesity and adipogenesis-related factors, may help identify novel regulatory mechanisms.

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There are no conflicts of interest.

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