ORIGINAL ARTICLE

Dietary approaches to stop hypertension influence on insulin receptor substrate-1gene expression: A randomized controlled clinical trial

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Background: Insulin receptor substrate (IRS) Type 1 is a main substrate for the insulin receptor, controls insulin signaling in skeletal muscle, adipose tissue, and the vascular, so it is an important candidate gene for insulin resistance (IR). We aimed to compare the effects of the Dietary Approaches to Stop Hypertension (DASH) and Usual Dietary Advices (UDA) on IRS1 gene expression in women at risk for cardiovascular disease. **Materials and Methods:** A randomized controlled clinical trial was performed in 44 women at risk for cardiovascular disease. Participants were randomly assigned to a UDA diet or the DASH diet. The DASH diet was rich in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fat, total fat, cholesterol, refined grains, and sweets, with a total of 2400 mg/day sodium. The UDA diet was a regular diet with healthy dietary advice. Gene expression was assessed by the real-time polymerase chain reaction at the first of study and after 12 weeks. Independent sample *t*-test and paired-samples *t*-test were used to compare means of all variables within and between two groups respectively. **Results:** IRS1 gene expression was increased in DASH group compared with UDA diet ($P = 0.00$). Weight and waist circumference decreased in DASH group significantly compared to the UDA group ($P < 0.05$) but the results between the two groups showed no significant difference. **Conclusion:** DASH diet increased IRS1 gene expression and probably has beneficial effects on IR risks.

Key words: Diet, Dietary Approaches to Stop Hypertension diet, insulin receptor substrate 1gene expression, insulin resistance

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INTRODUCTION

Insulin resistance (IR) is a complicated metabolic disorder with a wide range of clinical traits. IR means that circulating insulin does not do its function in insulin sensitive tissues such as skeletal muscle, adipose tissue, liver, and endothelium.[1-3] IR is related with central obesity, low high-density lipoprotein cholesterol, high triglycerides, raised blood pressure, and hemostatic defects.[4] IR is regulated by genetic and environmental factors and their interaction. Diet, body composition, and usual physical activity (PA) levels are known factors. Diet management is a key component in the long-term health and quality of life of people with IR, and many features of the diet composition have been considered to be critical in the alteration of IR.[5-8] One of these diets is the Dietary Approaches to Stop Hypertension (DASH), which encourages the eating of vegetables, fruits, and low-fat dairy products with sodium restriction. It is a balanced diet that is currently recommended for all adults. This diet is high in unsaturated fatty acids, fiber, antioxidant constituents, and low-fat dairy, which may be beneficial for decreasing IR. The metabolic efficiency of the DASH diet, including its impact on insulin sensitivity is less known.[9] Genetic variations with affecting on insulin signaling, as well as environmental factors such as diet and PA, plays an important role in insulin sensitivity.[10] Candidate genes

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for insulin sensitivity either directly or indirectly encode the proteins, which are participated in glucose metabolism. Insulin receptor substrate (IRS) Type 1 which is a main substrate for the insulin receptor, controls insulin signaling in skeletal muscle, adipose tissue, and the vascular, so it is an important candidate gene for IR.[11,12] It is determined that decreased tyrosine phosphorylation of the IRS1 proteins and dysregulation of the insulin receptor participates in peripheral IR and B-cell malfunction.[13] The gene-environmental interaction may effect on insulin sensitivity, clarify the fundamental mechanisms of Type 2 diabetes, and provide effective prevention strategies. Dietary factors and nutrient intake are the main environmental factors in the incident and expansion of the typical polygenic, diet-related diseases such as IR.[14,15] There are few data regarding the effect of diet on gene expression.[12-14] To date, no intervention studies in humans have thoroughly explored the effects of DASH diet on gene expression. The purpose of this study, therefore, was to evaluate whether DASH diet effects on IRS1 gene expression and IR.

MATERIALS AND METHODS

Fifty-one healthy, overweight, or obese (body mass index >25 kg/m^2) female volunteers aged 20-50 years that were referred by the general health center were recruited. Subjects were not included by nutritionist if they were pregnant or lactating women or they had history of incidence of cardiovascular, gastrointestinal, hepatic, renal, thyroid, diabetes, rheumatoid arthritis, lupus, severe infection, trauma, allergy, and if they consumed of multivitamin and mineral or omega-3 fatty acids supplements, antacid drugs that containing magnesium and calcium, aspirin and nonsteroidal anti-inflammatory drugs, anti-inflammatory and anti-depressant, and hormone drugs.[16] They were excluded if they had changed their normal PA or they had not complete compliance. The nature of the trial was explained to the individuals, and all subjects provided written informed consent. The ethical reviewer committee of the Isfahan University of Medical Sciences approved the study. This trial was registered at Iranian clinical trial registry as IRCT2014090719072N1.

Study design

This study was a randomized, parallel clinical trial. After a 2-week run-in period with the Usual Dietary Advices (UDA) diet participants were randomly assigned to 12 weeks to a UDA diet or the DASH diet. We conducted a run-in to homogenize in the consumption of macronutrients and base of diets. In this period, patients consumed a UDA diet. Group assignments were made by statistic using random sequencing created in SPSS (SPSS Inc., Chicago, IL, USA). The nutritionist that prescribed the diets had to be aware of the group assignment, but laboratory staff members were not aware of that. The socioeconomic status was evaluated using a questionnaire, which validated for Iranian.[13] Participants were controlled every 2 weeks, and measurements were taken. During the study period, patients were prescribed the diet and prepared their own meals while living independently. They were demanded to record their PA for 3 days every month and not to change and it was assessed by using compendium of PA.[17]

Diets

Participants were randomly assigned to one of two diets: A UDA diet and the DASH diet. The UDA group was not given a diet and simply recommended to eat as regular and the intervention group was prescribed DASH diet that was rich in fruits, vegetables, whole grains, and low-fat dairy products, and low in saturated fat, total fat, cholesterol, refined grains, sweets, and red meat. Furthermore, it contained 2400 mg sodium per day [Table 1].[15] To calculate the energy requirement for each participant, we used Mifflin-St equation.[16] The participants were assessed every 2 weeks. The diets were individually prescribed using a calorie count system, and an exchange list was given to each patient for changing food items and counting the calories. A nutritionist explained subjects how to apply the exchange list and writing food diaries. Every participant had to bring her 3-day dietary records every month, and trained nutritionist reviewed the diaries. In these sessions, they were questioned about their diets and the food items that they should be eaten and their compliance was assessed by analyzing the three food record diaries by the NUTRITIONIST IV software (version 7.0; N-Squared Computing, Salem, OR, USA) that was modified for Iranian food items.

The anthropometric measurements

Participants were weighed wearing minimal clothing and without shoes, using digital scales (Seca, Germany)

Table 1: Dietary goals of the DASH intervention versus usual dietary advice

DASH diet	Usual dietary advice
At least eight servings/day of fruits and vegetables	Try to have a variety of foods in your daily diet
Two to three servings/day of low-fat dairy foods	Do not skip any meals
1/2 to 1 serving of nuts, seeds, and legumes daily	Minimize the eating of sugar, sweets, and sweetened drinks
<2400 mg/day of sodium	Before cooking, remove fats and skin of the chicken and meat
	Try to use whole-wheat and barley bread instead of rice

DASH = Dietary Approaches to Stop Hypertension

and recorded to the nearest 0.1 kg. Height was measured without shoes in a standing position while the shoulders were in a normal state. Waist circumference was measured where the waist was narrowest and the hip at the maximum level over light clothing, using an outstretched tape, without any pressure to the body surface and measurements were recorded to the nearest 0.1 cm.

Peripheral blood mononuclear cells isolation, RNA isolation, and real-time polymerase chain reaction

Blood samples (5cc) were collected at baseline and at 12 weeks, and human peripheral blood mononuclear cells (PBMC) was isolated by centrifugation on a Ficoll-Paque Plus (Amersham Biosciences) density gradient. Total RNA was isolated from the PBMC using Trizol® reagent (Invitrogen) according to the manufacturer's instructions. Briefly, 1.0 ml TRIZOL reagent and 200 µl chloroform were added to the sample, and the admixture was vortexed for 15 s and stood at 25°C for 3 min. The supernatant was transferred to a fresh tube after centrifugation at 12,000 ×*g* for 15 min at 4°C, and then 500 µl isopropanol was added. The mixture was centrifuged at 12,000 ×*g* for 10 min at 4°C after incubation at −20°C for 20 min, to remove the supernatant and the RNA pellet was washed with 75% ethanol. Ethanol was removed by centrifugation at 7500 ×*g* for 5 min at 4°C, and RNA was air-dried for 5 min and then dissolved in 25 µl RNase-free water. The purity of isolated RNA was ascertained by OD260/280 using a Nanodrop ND-1000 (Thermo Scientific, Worcester, MA). Isolated RNA was dissolved in RNase-free water, and the amount of RNA was determined by measuring absorbance at 260 nm with a spectrophotometer. The RNA samples were treated with DNase I (Thermo Scientific) in order to avoid potential contamination with genomic DNA. Two micrograms of total RNA were used to synthesize double-stranded cDNA by Revert Aid First Strand cDNA synthesis kit (Thermo Scientific) and oligo dT primers. The primers for all assayed genes were designed using the Allele ID 7 software (Premier Biosoft International, Palo Alto, USA) [Table 2]. The real-time polymerase chain reaction (PCR) was performed using SYBR Green PCR Master mix (Thermo Scientific) and the Step One Plus™ Real time PCR detection System (Applied Bio systems). Glyceraldehydes-3-phosphate dehydrogenase was used as an endogenous control. The expression level of each target gene was calculated as $2^{-\Delta\Delta Ct}$, as previously described.[18]

Table 2: Primers used in real-time PCR

Gene	Primer sequences	Size (base pair)
IRS1-F	CCACTCGGAAAACTTCTTCTTCAT	24
IRS1-R	AGAGTCATCCACCTGCATCCA	21
GAPDH-F	AAGCTCATTTCCTGGTATG	19
GAPDH-R	CTTCCTCTTGTGCTCTTG	18

IRS1 = Insulin receptor substrate 1; GAPDH = Glyceraldehydes-3-phosphate dehydrogenase; PCR = Polymerase chain reaction

Statistical analysis

The normality of continuous variables was assessed by normal probability plots and by one-sample Kolmogorov-Smirnov test. Paired-samples *t*-test and χ^2 tests were used to determine the significance of any baseline differences between diet groups. Energy-adjusted dietary intake of nutrients was computed using the residual method and compared using analysis of covariance. We used independent sample *t*-test and paired-samples *t*-test to compare means of all variables within and between two groups, respectively. Statistical analyses were performed by using the SPSS-software package 16.0 (SPSS Inc., Chicago, IL), statistical power was 90%, and a priori defined value of $P < 0.05$ was considered statistically significant.

RESULT

Of the 51 participants, 44 persons completed the study. During the study, one patient was diagnosed with polycystic syndrome and another with high weight change, so these two patients had to be excluded from the analyses. Five patients deviated from the study protocol and therefore, their data were not available. Consort diagram is shown in Figure 1. Differences in distribution of several characteristics among 22 individuals in DASH group and 22 subjects within the UDA group are shown in Table 3. The mean patient age was 38 ± 8 years in UDA group and 37 ± 9 years in the intervention group. There was no difference between groups regarding age, socioeconomic status, weight, PA, and gene expression at the baseline.

Analysis of diet showed that calorie and protein intake of two groups was not significantly different between groups, but these two diets were different in total fat and fat composition intake, as well as the percentage of the carbohydrate intake. These two diets were different in sodium content although these differences were not statistically significant but nutritionally are important. The DASH diet had a higher amount of calcium, potassium, and fiber [Table 4].

The result of reverse transcription-PCR showed that DASH diet significantly increased the expression of IRS1 compared to UDA group ($P = 0.000$) and after adjusting for weight change, the results did not appreciably alter. This suggests that DASH diet may increase the expression of IRS1 [Table 3].

DISCUSSION

The finding of this study indicated that the DASH diet could induce the expression of IRS1, which is an early substrate for the insulin receptor and plays a key role in mediating some of the insulin's actions. However, to the best of our knowledge, effect of the DASH diet on gene expression has not been reported previously, but a number of studies

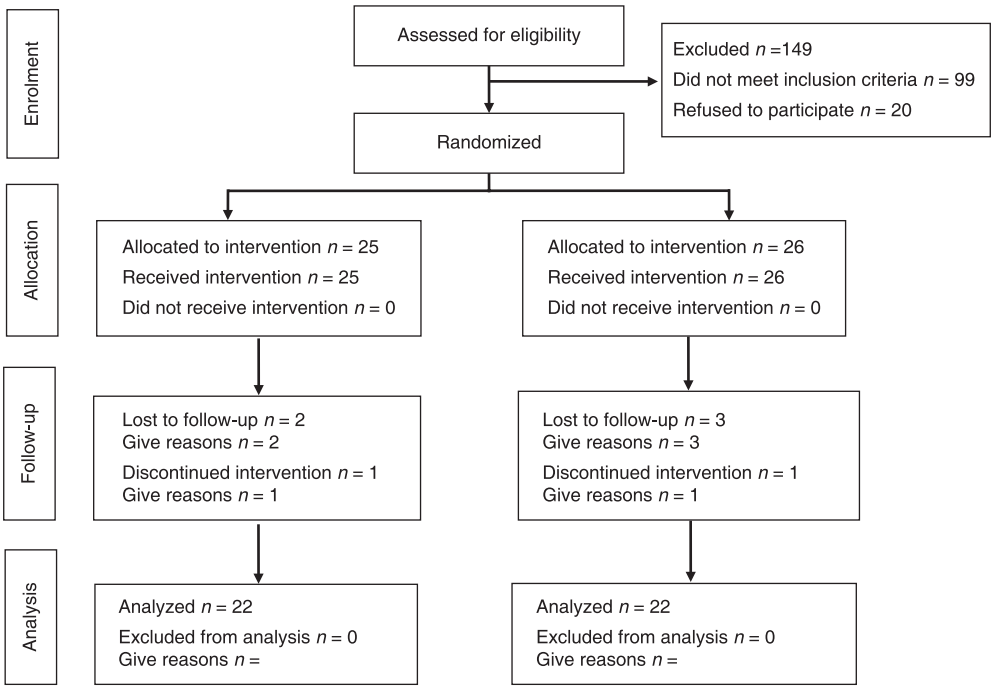

Figure 1: Consort diagram template

Table 3: Effects of recommendations to follow the DASH diet and UDA diet on anthropometric, gene expression (mean values with their SD)

Variables	DASH diet*				UDA†				
	Mean ± SD			$P^{\S}$	Mean ± SD			$P^{\S}$	$P^{\|}$
	Baseline	12th week	Change‡		Baseline	12th week	Change‡		
Weight (kg)	84.5±9.3	83.8±9.3	−0.73±1.8	0.06	82±8.4	82.3±7.4	0.32±3	0.6	0.4
BMI (kg/m^2)	33.5±3.6	33.1±3.8	−0.4±0.7	0.01	32.9±2.8	32.6±2.6	−0.3±1.5	0.4	0.7
Waist circumference (cm)	102.3±10.9	100±8.7	−2.3±5.3	0.04	99.8±6.7	100±6.7	−0.2±3.5	0.9	0.9
PA (MET)	40±4.2	38.9±3.5	−1.04±3.7	0.2	42±5.9	41.8±6.3	−0.17±2	0.7	0.07
Gene expression	1.11±2.6	3.6±2.9	2.5±2.2	0.00	1.75±1.8	2.2±1.9	0.55±2.8	0.44	0.00

*The DASH diet was high in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fats, total fats, cholesterol, refined grains, and sweets. The amount of sodium intake was 2400 mg/day; †The UDA group had the usual diet; ‡Calculated by subtracting the values at baseline from the values at the 12th week; §For comparison of within-group differences by a paired *t*-test. For comparison of between-group differences by an independent *t*-test. DASH = Dietary Approaches to Stop Hypertension; UDA = Usual Dietary Advices; PA = Physical activity; BMI = Body mass index; SD = Standard deviation; MET = Metabolic equivalent

show the DASH diet is high in fiber, unsaturated fatty acids, antioxidant components, and low-fat dairies improve IR.[19]

The mechanism behind this effect is not clear, but it may be due to the increased expression of some genes such as IRS1 whose activation leads to translocation of GLUT4-containing vesicles and subsequent increase in glucose uptake.[20] Because IRS1 is a membrane-bound protein and its function is membrane-dependent, it is logical to hypothesize that alteration in membrane lipid induced by dietary fat may impression on the function of the plasma membrane insulin receptor. As it can be seen from the Table 4, fat intake is significantly less in DASH group, and its composition is different between two groups such as polyunsaturated fat (PUFA), saturated fatty acid (SFA) and monounsaturated fatty acids ($P < 0.05$). These findings further support the idea of previous studies have shown that long-term high-fat diets and increased plasma free fatty acid levels impair insulin signaling by alteration in IRS1 expression and tyrosine/serine phosphorylation of IRS1, leading to decreased IRS1-associated PI3K activity.[21] In fact, the insulin-stimulated uptake of glucose in visceral fat deposits and muscle were damaged by the high fat diet (HFD) as corn oil-based HFD intervention causes the expansion of IR concurrently in the liver, adipose tissue, and skeletal muscle in young adult C57BL/6 mice.[21] Furthermore, the evidence shows that the diet composition, such as quality and quantity of fat, plays a notable role in glucose homeostasis and insulin sensitivity. It is commonly approved that saturated fats have a negative effect on insulin sensitivity, whereas unsaturated fats have a positive effect. Accordingly, animal and human studies have confirmed that SFAs decrease insulin sensitivity.[22] It has been established that a high-PUFA diet can increase receptor tyrosine kinase activity and a high PUFA:SFA diet also improved insulin receptor function, glucose oxidation, and glucose transport in rats.[23]

Table 4: Daily energy and nutrient intakes in DASH group and UDA group at baseline an at end of the study

Intake	UDA group (*n* = 22)	DASH group (*n* = 22)	*P**
Energy (kcal)	1688.33±799.76	1633.36±391.83	0.77
Protein (g/day)	63±34.5	66.9±24	0.27
Total fat (g/day)	69±35	48±21	0.00
Carbohydrate (g/day)	211±108	239±50	0.00
Saturated fat (g/day)			
Crude‡	15.2±3.3	13.42±3.7	0.2
Model 1†	15.2±4.9	13.3±5	0.05
PUFA[a] (g/day)			
Crude	26.5±12	16.3±9	0.00
Model 1	26±6.8	16.7±6.8	0.00
MUFA[b] (g/day)			
Crude	13±6	15.8±6	0.14
Model 1	13.3±4.4	15.6±2.9	0.04
PUFA/SFA ratio			
Crude	1.75±0.63	1.23±0.67	0.00
Model 1	2.4±2.1	0.92±3.5	0.09
Fiber (g)			
Crude	14.64±6.69	14.79±5.23	0.94
Model 1	11.2±4.5	14.3±5.8	0.05
Potassium (mg)			
Crude	2362.17±1039.69	2796.51±1086.65	0.19
Model 1	2325±542	2831±769	0.01
Calcium (mg)			
Crude	674.13±318.93	875.07±378.91	0.06
Model 1	664.5±260	884±287	0.01
Magnesium (mg)			
Crude	249.32±207.01	255.29±115.15	0.91
Model 1	246±185	259±93	0.8
Sodium (mg)			
Crude	1544.34±1151.20	1613.71±1625.39	0.87
Model 1	1682±1242	1645±849	0.7
Vitamin C (mg)			
Crude	104.57±73.88	138.25±94.73	0.2
Model 1	102.9±64	140±87	0.12

†Model 1 adjusted for energy intake (data are means ± SD); ‡Data are mean ± SD; Obtained from independent *t*-test; [a]PUFA = Polyunsaturated fat acid; [b]MUFA = Monounsaturated fatty acids; SFA = Saturated fatty acid; DASH = Dietary Approaches to Stop Hypertension; SD = Standard deviation; UDA = Usual Dietary Advices

Some studies established that dietary supplementation with calcium and magnesium reduce IR and low amount of potassium, magnesium, calcium, and fiber in the diet are correlated with diminished insulin sensitivity,[24] so fruits and vegetables; rich sources of magnesium, potassium, and fibers may improve IR.[25] Thus, as regard to a transactional study that shown the fruit and vegetable consumption was inversely associated with mRNA expression of some pro inflammatory indicators in healthy young adults[23] a possible explanation for our results may be higher fruit and vegetable consumption in DASH diet that may similarly effects on IR related genes. In the same way, epidemiologic research data show a useful effect of raised whole grain, nuts, and dairy on diabetes risk and insulin sensitivity.[26-28] Thus, other possible explanations for IRS1 up-regulation are consumption more dairy product, and so more Ca intake that may be supported by study which show high-calcium whey, causes smaller adipocyte size, can also partly explain the clustering of up-regulated genes in the insulin signaling pathway.[29]

Another important finding was that weight for the DASH diet group significantly has reduced in spite of fixing calorie intake and no change in PA during the study. As regards to the dairy product were higher in the DASH diet than the UDA diet, this finding are consistent throughout the study which has indicated the macronutrient variability of the DASH diet might be related to weight reduction.[18]

Although the study has successfully demonstrated that DASH diet induces IRS1 expression, it has certain limitations in terms of dietary intake in this study was self-reported, and patients were advised to follow a special diet rather than getting prepared foods, thus possibly lead to incomplete adherence to the diets.

CONCLUSION

DASH diet increased IRS1 gene expression and it may be one of the mechanisms, which diets influence on IR.

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Conflicts of interest

There are no conflicts of interest.

AUTHOR'S CONTRIBUTION

MK involved in conception, design, data collection,statistical analysis, and data interpretation. ME supervised the study. MJ participated in the statistical analysis and data interpretation. RS and MK supervised genetic experiments. All authors contributed in manuscript drafting and approval of final manuscript for submission.

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