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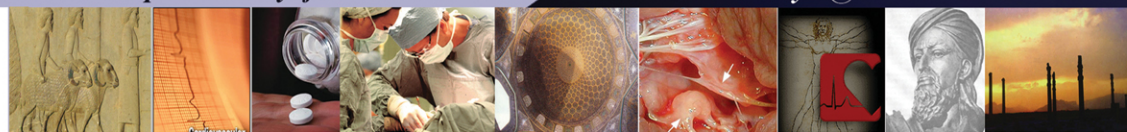

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Effect of conjugated linoleic acid and omega-3 fatty acid supplementation on inflammatory and oxidative stress markers in atherosclerotic patients

Mohammad Hassan Eftekhari[(1)], Fereshte Aliasghari[(2)], Mohammad Ali Babaei-Beigi[(3)], Jafar Hasanzadeh[(4)]

Original Article

Abstract

BACKGROUND: Cardiovascular disease is the major cause of morbidity, mortality, and disability in Iranian people. Inflammation and oxidative processes are key components of cardiovascular disease. The aim of this study was to evaluate the effect of conjugated linoleic acids (CLA) and omega-3 fatty acid (ω-3 fatty acids) supplementation on inflammation markers and oxidative stress in atherosclerotic patients.

METHODS: This study was a two-month clinical, randomized trial. 90 volunteers who referred to Emam Reza Heart Clinic of Shiraz University of Medical Sciences (Shiraz, Iran) from February to March 2011 and had the inclusion criteria of this study were selected. Participants were classified into 3 groups receiving 3 g/d CLA, 1920 mg/d ω-3, or placebo for 2 months. C-reactive protein (CRP), interleukin-6 (IL-6), malondialdehyde (MDA), and glutathione peroxidase (GPx) were measured before and after supplementation.

RESULTS: The hs-CRP level decreased significantly in both the omega-3 and CLA group ($P < 0.05$). IL-6 reduced significantly in the ω-3 group, but the reduction of IL-6 levels in the CLA group was not significant. GPx increased in the CLA and omega-3 groups ($P < 0.05$). MDA level decreased significantly in both omega-3 and CLA groups ($P < 0.05$). Comparison between the groups indicates a significant change in CRP levels in the ω-3 group relative to the control group. However, other indices did not cause any significant change in the ω-3 and CLA groups in comparison to the control group.

CONCLUSION: Diet supplementation with CLA and ω-3 can have a beneficial effect on some indices of inflammatory and oxidative stress.

Keywords: Atherosclerosis, Inflammation, Oxidative Stress, Conjugated Linoleic Acids

Date of submission: 04 Feb 2013, ***Date of acceptance:*** 15 Oct 2013

Introduction

Cardiovascular disease is the serious cause of mortality in developed and developing countries.[1] This disease is also the major cause of morbidity, mortality, and disability in Iranian people and accounts for nearly 50% of mortality each year.[2] Recent research has found atherosclerosis to be a chronic inflammation that leads to an acute clinical event by plaque rupture.[3] Inflammation appears by different stimuli, such as oxidative stress. Oxidative metabolites can activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and increase induction of proinflammatory cytokines.[4-6] NF-κB is a group of transcription factors that regulate the inflammatory and immune response.[7] inflammation and oxidative stress are crucial in the atherosclerosis process.[8]

The anti-inflammatory and antioxidant effect of nutrients may improve cardiovascular disease.[7,8] Today, there is a widespread interest in the health beneficial properties of conjugated linoleic acids (CLA) and ω-3 fatty acids.[9] CLA was found naturally in food from ruminant animals such as dairy and meat products.[10] For nearly a decade, the health benefits of CLA have been investigated in animal models. It has been observed that animals fed an atherogenic diet and supplemented with CLA had significantly less aortic lesions.[11,12] Nagao and

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Yanagita have also found 30% regression of atherosclerotic lesions in CLA supplemented rabbits.[11] ω-3 fatty acids are essential fatty acids that the human body needs for metabolic function.[13] There are considerable evidence from randomized controlled trials (RCTs) indicating that ω-3 fatty acids from fish and fish oil are protective against atherosclerosis.[14] Some studies attributed these prospective properties of omega-3 fatty acids on atherosclerosis to its anti-inflammatory effect.[15,16] To the best of our knowledge, this paper was the first human study which assessed the effect of CLA supplementation on atherosclerosis patients. Due to high prevalence of atherosclerosis in the Iranian population, this study was carried out to evaluate the effect of omega-3 fatty acids and CLA on inflammatory and oxidative stress markers in atherosclerotic patients.

Materials and Methods

This was a 2-month clinical randomized trial. To determine the sample based on power = 80% and $\alpha = 0.05$, the results of the study of Omrani et al. was used.[17] The sample size in each group was calculated as 30. Therefore, 90 atherosclerotic patients (40 males and 50 females) aged 30 to 60 years with angiographically diagnosed coronary atherosclerosis who were referred to Emam Reza Heart Clinic from February to March 2011 were recruited for this study. Volunteers had the following criteria: history of angina, myocardial infarction or bypass surgery, body mass index (BMI) of 18.5-24.9 kg/m^2, no pregnancy, and no dietary supplements. Volunteers with acute heart failure, acute arrhythmia, or chronic inflammatory disease were excluded from the study. Most of the patients consumed lipid lowering drugs; thus, the dosage and type of these drugs were kept consistent. Participants followed their regular diet and physical activity during the study. To determine the food intake and macro- and micronutrient consumptions of participants, the food frequency questionnaire (FFQ) was completed for each patient at the beginning of the study.

The study was approved by the Research Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran. All participants gave a written informed consent. The volunteers were randomly divided into 3 groups using balanced block randomization (BBR) protocol. They were allocated to receive 3 g/d CLA (3 × 1 g soft gel, a 50:50 isomer blend of cis-9 trans-11 and trans-10 cis-12), 1920 mg/d omega-3 fatty acids (3 × 640 mg soft gel blend of 210 mg DHA and 310 mg EPA), and the placebo.

CLA soft gel was obtained from Puritan's Pride (USA) and omega-3 fatty acids soft gel was produced by Seven Seas Ltd (UK). Placebo (olive oil) was produced by Zahravi Pharmaceutical Company (Tehran, Iran). Each group was invited separately to take their supplements every two weeks and the researcher supervised ingestion of supplements every week.

Procedure

Blood sampling: Fasting blood samples (5 cc) were collected at the beginning and the end of the study and immediately centrifuged (3000 × g, 10 min, 4ºC); then, the plasma was placed into a tube and stored at -70ºC until analysis for high sensitivity C-reactive protein (hs-CRP), IL-6, malondialdehyde (MDA), and glutathione peroxidase (GPx).

Anthropometric assessment: Body weight was measured by Seca 713 scale while the subjects were minimally clothed and their height was determined using measuring tape without shoes. Then, BMI [weight (kg) / hight^2 (m)] was calculated.

Biochemical analysis: Hs-CRP measurement was done by a highly sensitive enzyme-linked immunosorbent assay kit (IBL, Minnesota, USA), and IL-6 assay was performed by radioimmunoassay kit (IRMA source, Belgium, Louvain-la-Neuve). GPx enzyme activity was measured by the coupled enzyme assay commercial kit (Cayman, Michigan, USA). GPx catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide, in the presence of glutathione reductase (GR) and Nicotinamide adenine dinucleotide phosphate (NADPH); oxidized glutathione (GSSG) is immediately converted into the reduced form with concomitant oxidation of NADPH to $NADP^+$. Decrease in absorbance at 340 nm is measured.[18] MDA was determined using the thiobarbituric acid (TBA) method.

Statistical analysis

Data were analyzed using SPSS for Windows (version 19; SPSS Inc., Chicago, IL, USA). Normality of the data was evaluated by the Kolmogorov-Smirnov test. Normality distributed data were expressed as mean ± standard deviation. Paired t-test was used for within-group effects from baseline. Differences between groups from baseline to 8 weeks were assessed using ANOVA followed by a post-hoc Dunnett analysis. FFQ was analyzed using Food Processor Nut4 software. P values < 0.05 were considered statistically significant.

Results

As shown in figure 1 three patients were excluded during the study and finally data from 87 patients (39 men and 48 women) were collected and analyzed and with on average over 95% of supplements being apparently consumed by trial participants. Moreover, there were no significant differences in terms of dosage and type of lipid lowering drugs between the groups. As shown in tables 1 and 2, age, weight, height, body mass index, disease duration, and biochemical markers did not differ significantly between the groups. Concerning differences in food intake between patients, analysis of food frequency questionnaire showed no differences in food intake between the patients (results will be presented in a separate article). At the end of the study, CRP differed significantly in CLA group as compared to baseline. However, this was not the case with IL-6, although a decreasing trend was seen in IL-6 status (16.1 ± 10.2 vs 12.9 ± 8.1). ω-3 supplementation reduced both CRP and IL-6 significantly during the study compared to baseline (Table 3).

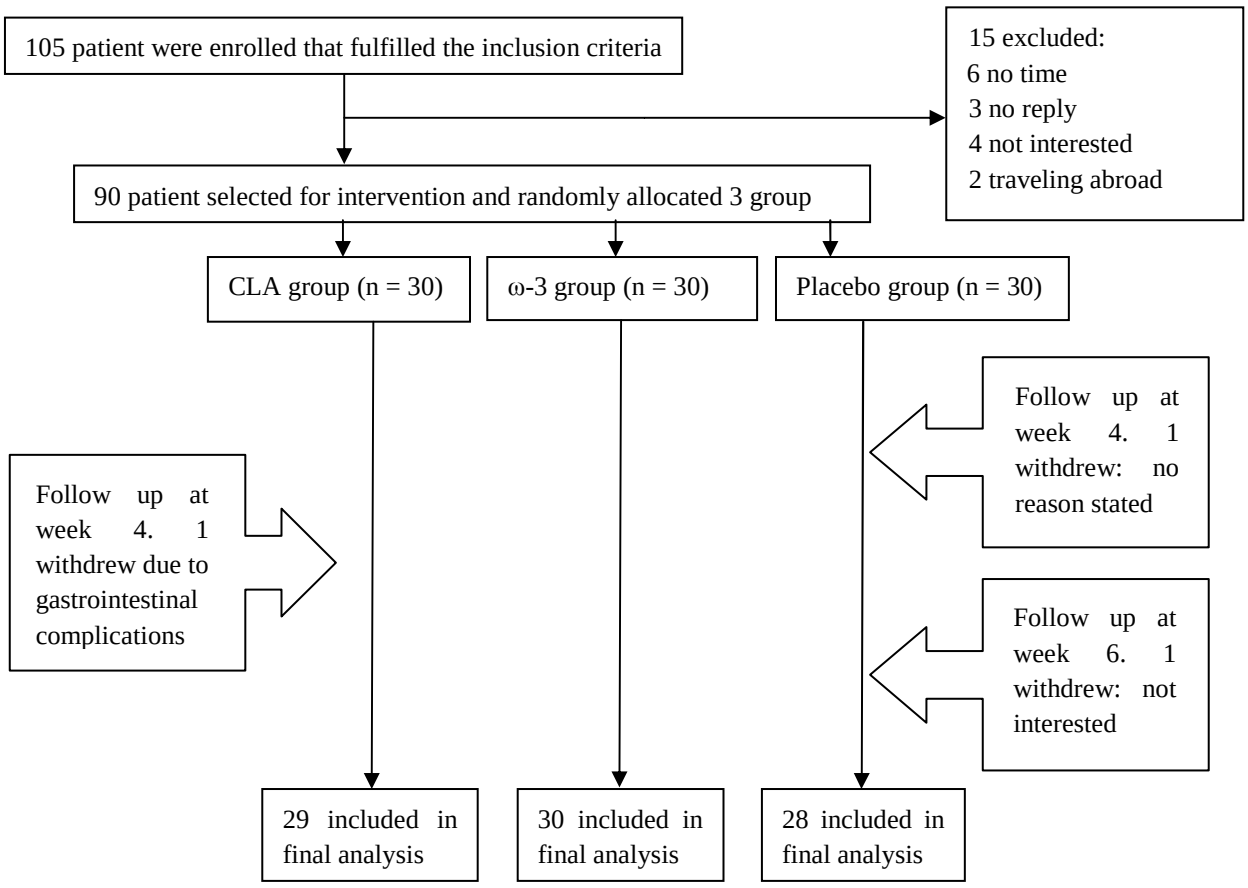

Figure 1. Flow chart of a randomized control trial
CLA: Conjugated linoleic acids

Table 1. Baseline characteristics of the study population

	Control group (n = 28)*	CLA group (n = 29)	Omega-3 group (n = 30)	P***
Age (year)	55.85 ± 14.13**	52.79 ± 14.11	54.53 ± 15.21	0.29
Weight (kg)	68.21 ± 7.82	67.06 ± 8.01	67.66 ± 7.96	0.89
Height (cm)	166.21 ± 5.75	167.51 ± 9.57	166.80 ± 6.33	0.48
BMI (kg/m^2)	24.66 ± 2.34	24.02 ± 2.76	24.30 ± 2.34	0.68
Cardiovascular disease duration (year)	3.89 ± 2.00	3.50 ± 2.05	4.10 ± 1.96	0.56

CLA: Conjugated linoleic acids; BMI: Body mass index; * N refers to the number of participants in each group
** All values are mean ± SD; *** Significance was determined using one-way ANOVA

Table 2. Baseline biochemical markers of the study population

	Control group (n = 28)*	CLA group (n = 29)	Omega-3 group (n = 30)	P***
hs-CRP (mg/l)	5.08 ± 5.02**	7.48 ± 5.64	4.43 ± 4.13	0.05
IL-6 (pg/ml)	12.88 ± 9.13	16.13 ± 10.21	18.59 ± 11.12	0.11
MDA (mol/l)	4.46 ± 2.52	3.7 ± 1.77	3.98 ± 1.50	0.37
GPx (nmol/ml/min)	172.06 ± 55.84	125 ± 46.06	144.57 ± 56.89	0.07

CLA: Conjugated linoleic acids; Hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; MDA: Malondialdehyde
GPx: Glutathione peroxidase; *N refers to the number of participants in each group; **All values are mean ± SD
*** Significance was determined using one-way ANOVA

In both CLA and omega-3 groups, MDA and GPx reduced significantly as compared to the baseline (Table 2). As shown in table 4, there were no significant changes in mean differences of MDA and GPx between the groups. Although hs-CRP and IL-6 differed significantly in the ω-3 group relative to the placebo group, there was no significant change in mean differences of hs-CRP and IL-6 in CLA groups in comparison to the placebo (Table 4).

Discussion

The present study determined the effect of CLA and omega-3 fatty acid supplementation on some key atherosclerosis risk factors in a group of atherosclerosis patients. Considering the association between inflammation and atherosclerosis, we evaluated several plasma markers of inflammation, such as hs-CRP, as potential tools for prediction of the risk of coronary disease.[19] Evidence indicated that oxidative stress may cause pro-inflammatory effects.[20,21] Some reports demonstrated that oxidative stress is necessary for NF-κB pathway.[5] Peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors whose activation suppresses the production of pro-inflammatory cytokines by inhibiting NF-κB pathway.[5] CLA and ω-3 fatty acids increase the peroxisome proliferator-activated receptor (PPAR).[20,21]

A few studies have investigated the effect of CLA isomers on inflammation in the human population. As a report by Steck et al. showed, CLA isomers increase the CRP level.[22] However, t10,c12 CLA (albeit at a high dose) had more significant effects on increased CRP in recent studies.[23] Raff et al. reported the non-significant effect of CLA supplementation on CRP concentration.[24] In the present study, supplementation with CLA for 2 months reduced the hs-CRP level in this group during the study. The dose of t10,c12 CLA used in this study (1.27 g/d for 8 weeks) was lower than that used in the studies by Steck et al.[22] (3.2 g/d for 12 weeks) and Raff et al.[24] (2.1 g/d for 5 weeks), which may account for the effect of CLA on CRP in our study. As reported by LaRosa et al. t10,c12 CLA supplementation increases IL-6 level in rats.[25] Although, in the current study, CLA had no effect on IL-6, which is consistent with the study of Raff et al.[24]

Our data suggest that supplementation with ω-3 decreases hs-CRP and IL-6 measurement. A similar result was gained by Rallidis et al. who reported that 3 months of α-linolenic acid supplementation in dyslipidaemic patients decreases CRP and IL-6 levels.[26] In a study by Chan et al. 6 weeks of ω-3 supplementation did not cause any significant change in CRP levels.[27] Several studies indicated that ω-3 fatty acid anti-inflammatory effect may result from activation of PPAR-γ. This fatty acid also directly decreases the inflammatory cytokine production. However, the mechanism is unclear.[28]

Extensive evidence from studies in animal models and data from human studies have indicated the role of oxidative stress in cardiovascular disease. In this study, we showed the significant effect of CLA and ω-3 on oxidative stress. CLA and ω-3 increase the levels of GSH with over-expression of gamma–glutamylcysteine ligase which was accepted as an antioxidant response.[29] In the study of Choi et al. CLA supplementation increased GPx activity.[30] Glutathione peroxidase (GPx) is an antioxidant enzyme that reduces hydrogen peroxide by reduced glutathione.[31]

On the other hand, the study by Taylor et al. demonstrated that CLA increase oxidative stress.[23] There is conflicting evidence about the effects of CLA supplementation on oxidative stress. According to a previous study, CLA increases the oxidative stability of the liver which suggests CLA supplementation enhances the protection to oxidative stress.[32] Park et al. indicated that CLA supplementation reduces oxidative stress in mice.[32]

Table 3. Effect of supplementation on biochemical indices at the end of the study

Indices	Control group (n = 28)*			CLA group (n = 29)			Omega-3 group (n = 30)		
	Week 0	Week 8	P***	Week 0	Week 8	P***	Week 0	Week 8	P***
Hs-CRP (mg/l)	5.08 ± 5.02**	5.03 ± 4.46	0.90	7.48 ± 5.64	5.95 ± 5.87	0.010	4.43±4.13	1.60±1.41	0.010
IL-6 (pg/ml)	12.88 ± 9.13	13.51 ± 8.86	0.70	16.13 ± 10.21	12.95 ± 8.10	0.060	18.59±11.12	13.37±9.44	0.040
MDA (mol/l)	4.46 ± 2.52	3.64 ± 1.32	0.09	3.7 ± 1.77	2.4 ± 0.80	< 0.001	3.98±1.50	2.87±1.55	0.001
GPx (nmol/ml/min)	172.06 ± 55.84	194.13 ± 105.42	0.14	125 ± 46.06	171.4 ± 68.90	< 0.001	144.57±56.89	174.61±62.80	0.001
BMI (kg/m^2)	24.66 ± 2.34	24.70 ± 2.26	0.31	24.02 ± 2.76	23.98 ± 2.78	0.370	24.30±2.34	24.40±2.34	0.450

CLA: Conjugated linoleic acids; Hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; MDA: Malondialdehyde; GPx: Glutathione peroxidase; BMI: Body mass index
* N refers to number of participant in each group; ** Values are mean ± SD; *** Significance was determined using paired t-test

Table 4. Mean differences† and changes in biochemical indices

	Control group (n = 28)*		CLA group (n = 29)		Omega-3 group (n = 30)		P
	Mean difference	Changes** (%)	Mean difference	Changes** (%)	Mean difference	Changes** (%)	
hs-CRP (mg/l)	-0.05 ± 0.78***	-0.98	-1.52 ± 0.77	-20.00	-2.80‡ ± 0.76	-63.88	0.04
IL-6 (pg/ml)	0.62 ± 1.66	4.89	-3.17 ± 1.63	-19.71	-5.18 ± 1.60	-28.07	0.41
MDA (mol/l	-0.81 ± 0.36	-18.16	-1.30 ± 0.36	-35.13	-1.10 ± 0.35	-27.88	0.62
GPx (nmol/ml/min)	22.07 ± 12.76	12.82	45.45 ± 12.54	36.80	30.04 ± 12.33	20.77	0.41

CLA: Conjugated linoleic acids; Hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; MDA: Malondialdehyde; GPx: Glutathione peroxidase
†Difference between values after and before the study; *N refers to number of participant in each group; **The percent of changes in biochemical before and after study
***Values are mean ± SD; ‡Significance was determined using ANOVA

Other studies have shown that supplementation with ω-3 fatty acids slows the progression of oxidative stress.[33] As reported by Tayyebi-Khosroshahi et al. ω-3 fatty acids increase the level of glutathione peroxidase and decrease the level of MDA in hemodialysis patients.[33] In the study by Bhattacharya et al. ω-3 supplementation increased GPx activity.[34] In another study by Iraz et al. ω-3 supplementation decreased MDA levels.[35] However, the study by Oarada et al. has revealed increased lipid peroxidation due to ω-3 supplementation in mice.[36] Shidfar et al. in their study, suggest that these contradictory results of plasma MDA and lipid peroxidation with ω-3 supplementation may be due to the level of antioxidants in the plasma or supplement content to suppress free radical production, differences in the population of studies, and the duration of the study.[37]

Conclusion

In conclusion, this study showed the beneficial properties of CLA and the many more of ω-3 fatty acids on inflammatory and oxidative stress markers in atherosclerosis. However, more research, particularly on CLA supplementation, is necessary in order to give definite comments in this regard.

To determine the food intake of participants the FFQ was completed for each patient at the beginning of the study. However, the limitation of this study was that we did not assess dietary intake and physical activity during the study, although the randomized design should have clearly lowered the risk of such bias, and all subjects were instructed to maintain their usual lifestyle habits. On the other hand, patients had normal BMI, so this might have affected our results.

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Conflict of Interests

Authors have no conflict of interests.

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Relationship between dietary approaches to stop hypertension score and presence or absence of coronary heart diseases in patients referring to Imam Hossein Hospital, Tehran, Iran

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Original Article

Abstract

BACKGROUND: The dietary approaches to stop hypertension (DASH) dietary pattern reduces blood pressure. However, there is little information about the relationship between DASH and coronary heart diseases. This study aimed to assess the relationship between a DASH-style diet adherence score and coronary heart diseases (CHD) in patients referring for coronary angiography.

METHODS: In this study, 201 adults (102 males, 99 females) within the age range of 40-80 years who referred for coronary angiography were selected. Diet was evaluated using a validated food frequency questionnaire. DASH score was calculated based on 8 food components (fruits, vegetables, whole grains, nuts and legumes, low fat dairy, red/processed meats, soft drinks/sweets, and sodium). The relationship between DASH score and CHD was assessed using logistic regression analysis.

RESULTS: Mean of DASH score was 23.99 ± 4.41. Individuals in the highest quartile of DASH score were less likely to have CHD [odds ratio (OR) = 0.38, 95% confidence interval (CI): 0.16-0.86]. However, after adjustment for gender or smoking, there was little evidence that coronary heart disease was associated with DASH diet score. There was a significant negative correlation between DASH score and diastolic blood pressure ($P \leq 0.05$).

CONCLUSION: In conclusion, having a diet similar to DASH plan was not independently related to CHD in this study. This might indicate that having a healthy dietary pattern, such as DASH pattern, is highly related to gender (dietary pattern is healthier in women than men) or smoking habit (non-smokers have healthier dietary pattern compared to smokers).

Keywords: Coronary Heart Disease, Dietary Approach to Stop Hypertension, Blood Pressure

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Introduction

Coronary heart diseases (CHD) are the main cause of disability and mortality in Iran, since half of annual mortality is due to CHD.[1] The etiology of coronary diseases is largely known and hypertension is its main and independent risk factor.[2] Lifestyle modification is the primary approach to treatment of hypertension and includes dietary changes, weight loss, and physical activity.[2]

Diet plays an important role in hypertension.[3] Previous studies have shown the importance of nutrients such as potassium, calcium, and magnesium in blood pressure regulation.[4] However, compared to micronutrient supplementation, changing dietary pattern appears to be more effective in controlling hypertension.[5] Dietary approach to stop hypertension (DASH) is one of the strategies that have been effective in controlling hypertension.[6] In DASH diet,

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increased consumption of vegetables, fruits, whole grains, nuts, and low-fat dairy and decreased intake of saturated fatty acids and sugar are encouraged.[7] Several studies have suggested that DASH diet decreases blood pressure, low-density lipoprotein cholesterol (LDL-C), and fasting blood glucose.[6,8,9]

Since studying the effect of DASH on cardiovascular events is costly and time consuming, instead of clinical outcomes such as CHD events, most DASH studies have focused on risk factors.[10] However, the relationship between DASH dietary pattern and outcomes of heart diseases could also be investigated through observational studies. Constructing a score which indicates how well an individual follows a diet similar to the DASH plan could be helpful in assessing the relationship between a DASH-style diet and CHD.[11] A study showed that risk of CHD decreased in women, the dietary pattern of whom was similar to DASH pattern.[12] In addition, another study reported that greater concordance with DASH diet guidelines was associated with a somewhat lower incidence of hypertension and mortality due to coronary heart diseases, stroke, or total cardiovascular diseases. However, these relationships were eliminated after adjustment for other risk factors.[6]

The DASH dietary pattern is recommended by the American Heart Association[13] and is included as an example of healthy eating in the 2005 Dietary Guidelines for people.[14] Moreover, no studies have previously investigated the relationship between DASH dietary pattern and coronary artery stenosis in Iran. Therefore, the present work aimed to assess the association between a DASH-style diet adherence score and coronary artery stenosis in patients referring for coronary angiography.

Materials and Methods

Participants

This cross-sectional study was conducted on individuals who referred for coronary angiography to Imam Hossein Hospital in Tehran, Iran, from May 2011 to March 2012. The initial criteria for eligible subjects were age of 40–80 years, no change in diet in the past year, and no history of cancer, kidney, or liver disease. Out of the 215 potential participants, 201 (93.4%) agreed to participate in this study. Moreover, the subjects who reported extremely low or high energy intakes (< 500 or > 5000 kcal/d) were excluded.[15] The final sample consisted of 201 subjects, for whom complete data were available. The ethical committee of Shahid Beheshti University of Medical Sciences approved this study (no: 043426) and an informed written consent was obtained from all the participants.

Assessment of clinical measures

Angiography results were determined and recorded by a cardiologist. The criterion for CHD was 50% stenosis in coronary arteries.[16] History of diabetes mellitus, hypertension, and hyperlipidemia was obtained from the patients' medical records. Seated blood pressure after 5 min of resting was measured using a random zero sphygmomanometer.

Assessment of anthropometric measures

Weight was measured and rounded to the nearest 100 g by digital scales while the subjects were minimally clothed and not wearing shoes. Height was measured while the subjects were standing, not wearing shoes, and the shoulders were in a normal position using a tape measure. Body mass index (BMI) was calculated and expressed in kg/m^2.

Assessment of dietary intake

The patients' dietary intake was assessed through a valid and reliable semi-quantitative food frequency questionnaire (FFQ), which included 168 items of food with standard serving sizes, as commonly consumed by Iranians.[17] Consumption frequency of each food item was questioned on a daily, weekly, or monthly basis and converted to daily intakes; portion sizes were then converted to gram using household measures.[18]

The collected data were analyzed using Nutritionist V (First Databank, Hearst Corp., San Bruno, CA, USA). The patients who had incomplete dietary questionnaires were excluded from the study. A DASH score was calculated for each FFQ. DASH score was constructed based on the food and nutrients emphasized or minimized in DASH diet, focusing on 8 components: 1. High intake of fruits (all fruits and fruit juice), 2. Vegetables (all vegetables except potatoes), 3. Low fat dairy products (skimmed milk, yogurt, doogh/yogurt drink, cheese, and kashk (a traditional high protein dairy product)), 4. Whole grains (all whole and dark breads, popcorn, cooked barley, bulgur, and corn), 5. Nuts/legumes (nuts, dried beans, and pear), 6. Low intake of soft drinks and sweets (all soft and sweet drinks, non-alcoholic beer, syrup sugar, cube sugar, noghl (a traditional small white candy), candy, honey, and jams), 7. Red/processed meats (tuna, egg, hamburger, sausage, organ meat, poultry, fish, red meats (beef, and lamb) and 8. Sodium (sum of sodium content of all foods in FFQ).

For calculation, the intake of fruit, vegetable, dairy, bean and nuts, whole grains, red and processed

meat, and soft drinks and sweets was transformed to the servings. Due to the lack of data on the salt intake, only the data on the sodium in foods were used to estimate sodium intake (without considering the received salt). For each 8 components, intake (in serving) was ranked to quintiles.[12]

For each food group, a maximum score of 5 points could be achieved when the intake met the recommendation, whereas lower intakes were scored proportionately. For the 5 groups of fruit, vegetable, dairy, and beans/nuts minimum intake of quintile received a score of 1 point and maximum intake received a score of 5 points. For the remaining components (red/processed meat, soft drinks/sweets, and sodium) low intake was desirable. Therefore, the lowest quintile received 5 points and the highest was given 1 point. The resulting 8 component scores were summed to create the overall DASH score which could range from 8 to 40.

Assessment of other variables

Data on physical activity were obtained using a validated questionnaire and expressed as metabolic equivalents hour/day (METs-h/day), in which 9 different MET levels were ranged on a scale from sleep/rest (0.9 METs) to high-intensity physical activities (> 6 METs).[19,20] The MET-time was calculated by multiplying time spent on each activity level by the MET value of each level. Additional covariate information regarding age, smoking habits, medical history, and current use of medications was obtained by questionnaires.

Statistical analysis

To determine normality of data distribution, Kolmogorov-Smirnov test was used. Systolic blood pressure and diastolic blood pressure were not normally distributed. Therefore, log transformation was used in statistical tests. Baseline characteristics and components of DASH score were compared between those with and without CHD using t-test for continuous variables and chi-square for dichotomous and categorical variables. DASH score was divided into four ascending categories on an ordinal scale. Mean or prevalence of baseline characteristics was computed for each category. Baseline characteristics were also compared between quartiles of DASH score using ANOVA for continuous variables and chi-square for dichotomous and categorical variables. Dietary intakes of the participants by quartiles of DASH score were analyzed using analysis of covariance (ANCOVA) after being adjusted for age and total energy intake. The relationship between CHD variable and adherence to DASH diet was assessed using multiple regression analysis in different models; controlling for age and energy intake (kcal/day) in model I, for BMI, multivitamin intake, physical activity (METh/day), aspirin use, history of diabetes, hypertension, and hyperlipidemia in model II, and for gender and smoking in model III. Partial correlation was used to assess the relationship between DASH score and blood pressure while controlling for the effects of age and energy intake, BMI, multivitamin use, physical activity, aspirin use, gender, history of diabetes, hypertension, and hyperlipidemia. All the statistical analyses were done in SPSS for Windows (version 19; SPSS Inc., Chicago, IL, USA). Values of $P \leq 0.05$ were considered significant.

Results

In this study, 102 men and 99 women with the mean age of 59.72 ± 10.43 years participated. Characteristics of the participants by status of CHD are summarized in table 1. Patients with CHD were slightly older, smoked more, and were more likely to have a history of hypertension.

Components of DASH score were not different with or without CHD (Table 2).

Basic characteristics and dietary intakes of the studied participants by quartiles of DASH score are shown in table 3. In the higher quartiles of DASH score, there were more women than men, and more non-smokers than smokers. Furthermore, the subjects in the top quartile of DASH score were less likely to have CHD. The calculated DASH scores for all FFQs ranged from 13 to 34 and the mean DASH score of all the participants was 23.99 ± 4.41. The subjects with higher DASH scores tended to consume more protein, potassium, calcium, and magnesium, but less total energy.

In table 4, odds ratio (OR) is presented for CHD across quartile of DASH score. After adjusting for age, energy intake, BMI, multivitamin use, physical activity, aspirin use, history of diabetes, hypertension, and hyperlipidemia, individuals in the highest quartile of the DASH score were less likely to have CHD [OR = 0.38, 95% Confidence interval (CI): 0.15-0.93; P = 0.05 for trend]. However, when analysis was further adjusted for gender and/or smoking, the trend was not significant.

There was a significant negative correlation (r = -0.13, P = 0.05) between DASH score and diastolic blood pressure, but no such relationship was observed for systolic blood pressure (r = -0.09, P = 0.09). The association was still held even after controlling effect of age, energy intake, BMI, aspirin use, multivitamin use, physical activity, and gender, history of diabetes, hypertension, and hyperlipidemia.

Table 1. Coronary heart disease (CHD) Risk Factors of study participants according to the status of CHD†

Variables	Without CHD (n = 99)	With CHD (n = 102)	P††
Age (year)	58.00 ± 9.80	61.39 ± 10.88	0.012
BMI (kg/m^2)	28.71 ± 5.07	27.48 ± 4.33	0.062
Systolic blood pressure (mmHg)	122.71 ± 15.65	127.61 ± 20.35	0.074
Diastolic blood pressure (mmHg)	76.56 ± 10.89	78.42 ± 11.26	0.203
Female	67 (68.3)	29 (28.7)	0.001
Current smokers	10 (10.9)	25 (24.3)	0.015
History of hypertension	58 (57.4)	83 (81.5)	0.001
History of hyperlipidemia	39 (39.6)	52 (50.9)	0.124
History of diabetes	21 (20.8)	30 (29.6)	0.147

† Data are presented as mean ± standard deviation or number (%); †† Independent t-test for quantitative variables and chi-square test for qualitative variables; CHD: Coronary heart disease; BMI: Body mass index

Table 2. Components of dietary approach to stop hypertension (DASH) score according to the status of coronary heart disease (CHD)†

Variables	Without CHD (n = 99)	With CHD (n = 102)	P††
Fruit (servings/d)	2.28 ± 0.09	2.28 ± 0.11	0.960
Vegetables (servings/d)	2.25 ± 0.10	2.23 ± 0.11	0.932
Whole grain (servings/d)	1.94 ± 0.25	1.57 ± 0.20	0.265
Nuts and legumes (servings/d)	1.54 ± 0.08	1.42 ± 0.08	0.344
Low-fat dairy (servings/d)	1.94 ± 0.08	1.80 ± 0.08	0.248
Red/processed meats (servings/d)	0.85 ± 0.04	0.95 ± 0.06	0.152
Soft drinks/sweets (servings/d)	2.60 ± 0.23	2.77 ± 0.27	0.613
Sodium (mg/d)	2174.91 ± 101.42	2308.92 ± 113.18	0.381

† Mean ± SEM (all such values); †† Independent t-test; CHD: Coronary heart disease

Table 3. Basic characteristics and dietary intakes of study participants by quartiles of dietary approach to stop hypertension (DASH) score†

Dietary intake	Quartiles of DASH score				P for trend††
	Q1 (n = 50)	Q2 (n = 60)	Q3 (n = 44)	Q4 (n = 47)	
Female/male	14/36 (28/7)	33/27 (55/4)	23/21 (52.3/47.7)	29/18 (61.7/38.3)	0.005
Current smokers	22 (44.0)	15 (25.0)	9 (20.5)	6 (12.8)	0.004
Coronary heart disease	32 (64.0)	29 (48.3)	22 (50.0)	19 (40.4)	0.051
Multivitamin use	7 (14.0)	6 (10.3)	9 (20.9)	14 (29.8)	0.053
BMI (kg/m^2)	28.31 ± 4.51	28.32 ± 4.81	28.27 ± 4.52	27.41 ± 5.12	0.731
Physical activity††† (MET)	29.87 ± 7.98	30.01 ± 7.89	30.25 ± 7.89	30.35 ± 7.25	0.932
Nutrients‡					
Total energy (kcal/d)	2333.90 ± 98.27	2041.04 ± 89.23	2195.74 ± 104.86	1999.50 ± 103.17	< 0.001
Carbohydrate (% of total energy)	56.00 ± 0.90	55.00 ± 0.87	53.74 ± 1.00	56.81 ± 0.98	0.061
Protein (% of total energy)	13.10 ± 0.34	14.05 ± 0.30	14.51 ± 0.36	14.71 ± 0.33	< 0.001
Fat (% of total energy)	32.20 ± 0.91	33.01 ± 0.82	33.81 ± 1.01	31.48 ± 1.00	0.263
Potassium (mg/d)	2533.14 ± 72.12	2955.12 ± 65.72	3196.22 ± 76.12	3551 ± 75.34	< 0.001
Calcium (mg/d)	951.26 ± 38.36	1043.64 ± 34.13	1064.09 ± 40.44	1191.48 ± 39.63	< 0.001
Magnesium (mg/d)	321.11 ± 10.84	349.27 ± 9.80	367.54 ± 11.42	414.58 ± 11.27	< 0.001
Components of DASH score					
Fruit (servings/d)	1.50 ± 0.12	2.13 ± 0.10	2.57 ± 0.13	3.03 ± 0.13	< 0.001
Vegetables (servings/d)	1.36 ± 0.12	1.92 ± 0.10	2.70 ± 0.12	3.11 ± 0.12	< 0.001
Whole grain (servings/d)	0.80 ± 0.30	1.29 ± 0.27	1.51 ± 0.32	3.67 ± 0.31	<0.001
Nuts and legumes (servings/d)	0.84 ± 0.09	1.40 ± 0.08	1.78 ± 0.10	2.01 ± 0.09	< 0.001
Low-fat dairy (servings/d)	1.40 ± 0.11	1.85 ± 0.10	1.96 ± 0.11	2.29 ± 0.11	< 0.001
Red/processed meats (servings/d)	0.90 ± 0.06	0.90 ± 0.05	0.85 ± 0.06	0.80 ± 0.07	< 0.001
Soft drinks/sweets (servings/d)	3.21 ± 0.30	3.01 ± 0.27	2.26 ± 0.31	2.15 ± 0.31	< 0.001
Sodium (mg/d)	2468.39 ± 85.69	2132.82 ± 78.09	2221.12 ± 90.19	2170.69 ± 88.96	<0.001

† Data are presented as mean ± SEM or number (%); †† ANCOVA for quantitative variables and chi-square test for qualitative variables; ††† MET: metabolic equivalent task; 1 MET: Energy expenditure of sitting quietly or approximately 1 kcal per kg body weight per hour; ‡ Nutrients (except total energy) food intakes were adjusted for age and total energy intake
DASH: Dietary approaches to stop hypertension; BMI: Body mass index

Table 4. Multivariate adjusted odds for coronary heart disease (CHD) across quartiles of dietary approaches to stop hypertension (DASH) score†

CHD	Quartiles of DASH score				P for trend
	Q1 (n = 50)	Q2 (n = 60)	Q3 (n = 44)	Q4 (n = 47)	
Crude	1.00	0.52 (0.24-1.13)	0.56 (0.24-1.18)	0.38 (0.16-0.86)	0.032
Model I††	1.00	0.52 (0.23-1.11)	0.54 (0.23-1.27)	0.36 (0.15-0.86)	0.036
Model II‡	1.00	0.51 (0.22-1.12)	0.55 (0.23-1.13)	0.38 (0.15-0.93)	0.051
Model III‡‡	1.00	0.54 (0.21-1.39)	0.66 (0.24-1.70)	0.42 (0.15-1.20)	0.085

DASH: Dietary approaches to stop hypertension; BMI: Body mass index; † Values are odds ratio (OR) with 95% of confidence interval (CI);
†† Model I: Adjusted for age and energy intake
‡ Model II: Additionally adjusted for BMI, multivitamin use, physical activity and aspirin use, history of diabetes, hypertension and hyperlipidemia
‡‡ Model III: Further adjusted for gender and smoking

Discussion

This study showed that a diet that was more similar to the DASH diet was associated with lower CHD. The rate of coronary heart diseases (CHD) was lower in patients who were at the highest quartile of DASH score (those with a diet most similar to DASH pattern) compared to patients with the lowest quintile score (those with a diet less similar to DASH pattern). However, these relationships were eliminated after adjustment for gender and smoking. Furthermore, DASH score had a reverse correlation with diastolic blood pressure, while no correlation was observed with systolic blood pressure.

A clinical trial showed that DASH diet could reduce systolic blood pressure by 7.7 and diastolic blood pressure by 3.6 mmHg.[21] Furthermore, DASH plan might influence other cardiovascular risk factors such as high LDL cholesterol, metabolic syndrome, and inflammation, which could reduce atherosclerosis and CHD.[7,12,22] Several studies have shown that having a diet similar to DASH diet in which higher intake of whole grains, vegetables, and fruits are emphasized is associated with lower risk of cardiovascular diseases and stroke.[23-25] This decrease in the risk of heart diseases may be related to the micronutrient content of the food recommended in DASH; since higher intake of magnesium, potassium, calcium, and other nutrients like dietary fiber may have a favorable effect on blood pressure, insulin sensitivity, satiety, and BMI.[26,27] However, red meat and processed products, which are not recommended in the DASH diet, contain high sodium and saturated fats and could have adverse effects on blood pressure.[26,28] In addition, the DASH diet may increase intake of antioxidant and plant compounds with estrogenic activity which could play a role in reduction of cardiovascular diseases.[29-32]

In this study, when regression analysis was adjusted for gender or smoking, there no longer was a significant relationship between diet and CHD. This might indicate that a healthy diet is highly related to gender or smoking habit. Generally, adherence to the DASH diet in men was significantly lower than that of women in the current study and frequency of CHD was higher in men compared to women. A similar conclusion could also be made in the case of smoking habit. One clear and potentially important difference between this study and some previous studies was that they have examined DASH scores separately in men or women, but this study was conducted on both genders.[11,12,33] A similar study showed no correlation between DASH diet and incidence of hypertension and CHD mortality after adjustment for risk factors of CHD.[6] However, the results were inconsistent with the study of Fung et al. that observed a significant negative correlation between DASH diet score and incidence of CHD and stroke.[12]

In this study, adherence to DASH diet in men was significantly less than of women. This difference may be related to the fact that women, compared to men, tend to have a healthier diet. In men, intake of vegetables, fruits, low-fat dairy products, beans, and nuts that increase the DASH diet score was lower, while the intake of red meat/processed products, and sweet drinks which decrease the DASH diet score was higher. Another study reported that, even in similar DASH diet scores, men received more sweet drinks and red meat/processed products than women.[33] Another finding was that non-smokers had higher DASH diet scores than smokers. One possible explanation for the low score of smokers might be related to higher consumption of sweets and sweet drinks, since smokers generally consume more sweets than nonsmokers.[34,35] DASH score in diabetics was higher than that of non-diabetics, which was consistent with a national study conducted in the United States of America, showing that, the diet of diabetics had more similarities to the DASH plan

than that of non-diabetics.[36] One limitation of this study was that the present study's FFQ was not designed to precisely estimate sodium intake. Given that the amount of sodium intake cannot be accurately estimated through FFQs, by using the quintile approach the probability of misclassifying the participants' DASH score would decrease.[12] Another limitation of this study was its cross-sectional design, since a cohort design was more appropriate.

In conclusion, following a diet similar to the DASH plan was not independently associated with coronary heart diseases in this study. This might indicate that having a healthy dietary pattern such as DASH pattern is highly related to gender (dietary pattern is healthier in women than men) or smoking habit (non-smokers have healthier dietary pattern compared to smokers). With respect to the role of DASH diet in reducing cardiovascular risk factors and events, it is appropriate to provide approaches for encouraging people with high risk of CHD to take this diet.

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Conflict of Interests

Authors have no conflict of interests.

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Clinical investigation of the acute effects of pomegranate juice on blood pressure and endothelial function in hypertensive individuals

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Original Article

Abstract

BACKGROUND: Pomegranate juice (PJ) is rich in bioactive phytochemicals with antioxidant, and anti-inflammatory and cardioprotective functions. The present trial investigated the acute effects of PJ consumption on blood pressure and markers of endothelial function.

METHODS: In this single-arm study, thirteen hypertensive men aged 39–68 years were recruited. Included subjects were assigned to natural PJ (150 ml/day) following a 12 hour fast. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and flow-mediated dilation (FMD), along with serum concentrations of C-reactive protein (CRP), intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin and interleukin-6 (IL-6) were measured at baseline and 4-6 hours after PJ consumption.

RESULTS: Comparison of pre- vs. post-trial values revealed a significant reduction in both SBP (7%; P = 0.013) and DBP (6%; P < 0.010). However, changes in FMD (20%) as well as circulating levels of CRP, ICAM-1, VCAM-1, E-selectin, and IL-6 did not reach statistical significance (P = 0.172).

CONCLUSION: PJ has promising acute hypotensive properties. Consumption of PJ could be considered in the context of both dietary and pharmacological interventions for hypertension.

Keywords: Punica Granatum L., Cardiovascular Disease, Hypertension, Inflammation, Endothelium-Dependent Dilation

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Introduction

Cardiovascular disorders are among the leading causes of death and disability in the world.[1,2] Hypertension is a major risk factor for cardiovascular and cerebrovascular disease, end stage renal disease, type 2 diabetes, and metabolic syndrome. Hypertension has a high global prevalence of about 15% that is estimated to reach as high as 30% by 2025.[3,4] Controlled studies have indicated that each 5 mmHg decrease in diastolic blood pressure (DBP) is associated with 15% and 40% reductions in the risk of cardiovascular disease and stroke, respectively.[5] In spite the introduction of several classes of anti-hypertensive agents with different mechanisms of action, uncontrolled hypertension resistant to drug therapy still remains a frequent medical problem.

Pomegranate (Punica granatum L.; Family Punicaceae) is a popular edible fruit with wide applications in traditional medicine.[6,7] Several lines of modern scientific evidence have also indicated the therapeutic efficacy of pomegranate against different types of disorders.[8-11] The pomegranate is characterized by considerable amounts of biologically active phytochemicals including flavonoids (e.g. anthocyanins, catechins, quercetin, and rutin), other types of polyphenols, ellagitannins, and antioxidant vitamins.[12-15] Many of these phytochemicals have been shown to possess antioxidant and anti-inflammatory properties plus additional biological activities such as inhibition of angiotensin converting enzyme.[16-20] All these activities of the pomegranate are potentially beneficial for the treatment of hypertension and improvement of

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endothelial function.[11] However, clinical studies investigating the hypotensive and cardioprotective effects of pomegranate have been scarce.[20-26]

According to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7), prehypertension is defined as 120 mmHg ≤ systolic blood pressure (SBP) < 140 mmHg 12 and/or 80 mmHg ≤ DBP < 90 mmHg.[27] Prehypertension is a clinical stage where subjects are at increased risk (2 folds higher) of developing hypertension in the near future.[28] Emerging findings suggest that interventions at the prehypertension stage can prevent or delay the progression of disease into established hypertension and subsequent detrimental outcomes.[29] The present trial investigated the acute effects of pomegranate juice (PJ) on blood pressure and endothelial function in subjects with diagnosed prehypertension.

Materials and Methods

Subjects

Thirteen hypertensive men aged 39–68 years were recruited for this trial.[30] The Ethics Committee at the Shahrekord University of Medical Sciences (Iran) approved the study protocol (code: 92-3-16) and written informed consents were obtained from all participants.

The inclusion criteria were body mass index (BMI) ≤ 30, and diagnosed hypertension defined as SBP > 120 mmHg and/or DBP > 80 mmHg. Exclusion criteria were type 1 or 2 diabetes, chronic pancreatitis, liver cirrhosis, kidney stones, renal failure, use of non-steroidal anti-inflammatory drugs, use of antioxidant or vitamin supplements, intense physical activity (> 5 h/week), smoking habit, being vegetarian or having any restrictive dietary requirements, and pregnancy.

Study design

The present study was designed as a single-arm clinical trial. The included subjects were assigned to natural PJ (150 ml/day) following a 12-h fast. SBP, DBP, and flow-mediated dilation (FMD), along with serum concentrations of C-reactive protein (CRP), intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and interleukin-6 (IL-6) were measured at baseline and 4-6 h after PJ consumption. Participants were asked not to eat or drink anything during the interval between PJ consumption and final measurements.

Total anthocyanin assay

A pH differential method was carried out to determine the total anthocyanin content of PJ. In this method, the difference in the absorbance of sample at pHs 1.0 and 4.5 is proportional to the total anthocyanin content. Details of this method have been published previously.[24,31]

Anthropometric and BP measurements

Measurement of weight and height was carried out using a standard procedure as described previously.[24,32] Body mass index (BMI) was calculated as weight in kg divided by height in meters squared (m^2). BP was measured by a single operator at baseline and 4-6 h after intake of PJ, according to a standard protocol. BP recordings were performed after rest, employing a stethoscope and calibrated sphygmomanometer (Accurtorr 1A; Datascope, Japan). Systolic blood pressure was defined as the appearance of the first sound (Korotkoff phase 1) and diastolic blood pressure was defined as the disappearance of the sound (Korotkoff phase 5) during deflating of the cuff.[24,32]

FMD measurement

Endothelium-dependent FMD was measured on the right brachial artery as described previously.[33]All measurements were carried out by a single operator following a 5-min rest and employing a GE vivid 3 ultrasound apparatus (AtCor Medical, Solingen, Germany). A BP cuff was inflated around the forearm to 200 mmHg for 5 min. Images were recorded at baseline (before inflation), 30 s before cuff release, and then every 15 sec after cuff release for 3 min. Arterial diameter was measured at the end of end-diastolic phase, coinciding with R-wave on the electrocardiogram (ECG). Brachial FMD was expressed as the percentage change in arterial diameter from baseline.[34]

Blood sampling and biochemical analyses

Twelve-hour fasted blood samples were taken from the left antecubital vein. After being allowed to clot for 2-3 h, serum was isolated by centrifugation at 3500-4000 rpm for 10 min. Serum samples were kept at -80°C prior to biochemical analyses. Biochemical analyses were performed using an automated enzymatic assay (Pars Azmoon, Tehran, Iran) on a Hitachi 902 autoanalyzer (for CRP), or enzyme-linked immunosorbent assay (ELISA) with commercial kits (Boster Biological Technology Ltd., Wuhan, China) (for ICAM-1, VCAM-1, E-selectin, and IL-6). Inter-assay coefficients of variation for ICAM-1, VCAM-1, E-selectin and IL-6 were 4.1-6.4%, 6.1-7.7%, 6.6-8.1%, and 3.1-5.5%, respectively. Intra-assay coefficients of variation for ICAM-1, VCAM-1, E-selectin, and IL-6 were 3.4-5.1%, 2.3-3.7%, 5.2-6.9%, and 2.3-4.9%, respectively.

Statistical analysis

All statistical analyses were performed using SPSS for Windows (version 17; SPSS Inc., Chicago, IL, USA). Data wereexpressed as mean ± SD. Group comparisons were made using paired t-test (in case of normally distributed data) or Wilcoxon signed-ranks test (in case of non-normally distributed data). A two-sided P-value of < 0.05 was considered to be statistically significant.

Results

This trial was a comprised of 13 hypertensive male adolescents with a mean age, weight, and BMI of 55.92 ±7.92 yrs, 80.42 ± 11.01 kg, and 27.34 ± 3.82 kg/m^2, respectively. All 13 subjects completed the study. Total anthocyanin content of PJ was determined to be 5.8 mg per 100 ml of the administered juice.

Comparison of pre- vs. post-trial values revealed a significant reduction in both SBP (P = 0.013) and DBP (P = 0.010), amounting to an approximate reduction by 7% and 6%, respectively. However, percentage changes in FMD (20%) was not found to be statistically significant (P = 0.172). In the same manner, there was no significant difference in the circulating concentrations of inflammatory biomarkers namely hsCRP (P = 0.263), ICAM-1 (P = 0.248), VCAM-1 (P = 0.657), E-selectin (P = 0.182), and IL-6 (P = 0.763) following consumption of PJ. Baseline and post-trial values for the evaluated parameters are summarized in table 1.

Discussion

The present pilot trial is one of the few clinical evidences on the acute hypotensive and vascular effects of PJ. The results indicated amelioration of both SBP and DBP following consumption of a single dose of PJ. In a previous study, Aviram and Dornfeld investigated the effects of 2-week supplementation with PJ (50 ml/day) on the SBP of hypertensive patients.[20]The findings revealed a significant decrease in SBP amounting to 5%. In the same study, a 36% decrement in the activity of serum angiotensin converting enzyme (ACE) was reported from PJ.[20]

The same group also investigated the effect of chronic supplementation with PJ (50 ml/day) in patients with carotid artery stenosis. Their results indicated a significant reduction in SBP, but not DBP, starting from 1 month after starting supplementation and generally increasing up to month 12 (equivalent to 12%decrement). However, no further reduction was observed when supplementation was continued for another 2 years.[21] Another study conducted by Lynn et al. indicated a significant reduction in both SBP and DBP following consumption of PJ (330 ml/day) by healthy subjects for 4 weeks.[22] Mathew et al. reported that consumption of pomegranate extract, either during or 15 min before a high-fat meal, can effectively prevent postprandial SBP rise at 2 and 4 h postprandial. Nevertheless, no significant effect was found on DBP.[23]In a recent trial conducted by our group, the effects of a 2-week intake of PJ (from the same source as that used in the present study) were evaluated on BP of hypertensive subjects. Our results implied a significant reduction of both SBP (5%) and DBP (4.5%) compared to the control group who consumed water instead of PJ.[24]Although most of the findings by previous trials infer the hypotensive impact of PJ consumption, there are some contrasting findings that are worth attention. Kelishadi et al. investigated the acute (4 h) and chronic (1 month) effects of PJ (240 ml/day) on BP of adolescents with metabolic syndrome and could not find any significant effect.[25] In another trial, administration of PJ (240 ml/day) for 3 months was not found to significantly affect SBP or DBP in patients with stable coronary heart disease (CHD), as reported by Sumner et al.[26] These negative

Table 1. Acute effects of pomegranate juice on blood pressure, flow-mediated dilation, and biochemical parameters

Parameters	Before	After	P
SBP (mmHg)	125.38 ± 11.80	116.15 ± 7.94	0.013
DBP (mmHg)	82.69 ± 5.25	78.08 ± 3.25	0.010
FMD (%)	0.30 ± 0.17	0.36 ± 0.17	0.172
Hs-CRP (mg/L)	1.39 ± 1.14	1.20 ± 0.94	0.263
ICAM-1 (ng/mL)	297.00 ± 122.30	265.23 ± 125.35	0.248
VCAM-1 (ng/mL)	1008.31 ± 450.99	993.92 ± 447.42	0.657
E-Selectin (ng/mL)	27.77 ± 15.89	25.49 ± 14.19	0.182
IL-6 (ng/mL)	0.45 ± 0.16	0.46 ± 0.15	0.763

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FMD: Flow-mediated dilation; hsCRP: High-sensitivity C-reactive protein; ICAM-1: Intracellular adhesion molecule-1; VCAM-1: Vascular cell adhesion molecule-1; IL-6: Interleukin-6; Group comparisons were made using paired t-test (in case of normally distributed data) or Wilcoxon signed-ranks test (in case of non-normally distributed data).

findings might be attributed to the difference in the inclusion criteria applied by these two latter trials. It appears that hypotensive effects of PJ are more likely to be elicited in hypertensive patients, rather than patients with established CHD or metabolic syndrome. Moreover, the overall findings of the trials conducted so far weigh in favor of the beneficial effect of PJ consumption on BP.

The hypotensive properties of PJ could be ascribed to the promising antioxidant properties of phytochemicals present in this complex juice. Oxidative stress is known to play a key role in the pathogenesis of hypertension.[35] Increased levels of oxidants have been shown in different experimental models of hypertension.[36-39] Detrimental effects of oxidative stress are mainly due to the interaction of reactive oxygen species (ROS) (in particular superoxide anion) with vital cellular components, lipids, and proteins, which leads to endothelial dysfunction and vascular resistance. In addition, ROS interfere with the production and vasodilatory actions of endothelium-derived nitric oxide (NO) via attenuating NO synthase (NOS) activity and enhancing NO breakdown.[40,41] Epidemiological evidence has indicated that diets rich in natural antioxidants are associated with a reduced risk of developing hypertension and cardiovascular events.[42,43] A plethora of studies have confirmed the considerable antioxidant and radical scavenging effects of PJ, which are mainly due to the anthocyanins and hydrolysable tannins present in the fruit. Interestingly, it has been suggested that some 50% of the total antioxidant activity of PJ is exerted by a specific ellagitannin, named punicalagin.[16] Intestinal hydrolysis of punicalagin yields ellagic acid; the latter being a strong antioxidant compound.[44]

Apart from antioxidant properties, PJ may lower BP through a direct interaction with ACE. As referred above, a significant reduction in the activity of serum ACE has been observed in hypertensive patients following PJ consumption.[20]Besides, in an animal study by Mohan et al. PJ administration attenuated angiotensin II-induced hypertension in diabetic rats, and also blocked the effects of different catecholamines on arterial BP and vasoreactivity. Furthermore, chronic administration of PJ counterbalanced the increased ACE activity in diabetic hypertensive rats.[45] In spite of these positive reports, the ACE inhibitory effect of PJ needs to be further explored as Lynn et al. failed to report any change in serum ACE concentration following PJ consumption for 4 weeks.[22]

Another primary outcome measure that was evaluated by the present trial was changes in endothelium-dependent flow-mediated dilation. Our results did not indicate any improvement in FMD and this is in agreement with our recent report on the effects of 2-week PJ intake.[24] However, findings from another trial indicated significant improvement in both endothelium-dependent and –independent (nitroglycerin-induced) dilation after 4 h of PJ consumption. In addition, this increased vasodilation was persisted until the end of supplementation period (1 month).[25] In the present study, biomarkers of endothelial function, namely ICAM-1, VCAM-1, and E-selectin, remained statistically unaltered compared to baseline levels. It should be noticed that alterations in the circulating levels of these biomarkers is more likely to be exerted by chronic, rather than single dose, consumption of PJ and needs a longer term evaluation to allow the turnover of previously released proteins and observation of possible changes in the expression and subsequent release of these markers due to PJ consumption. This notion is corroborated by our previous study which showed a decreasing trend in serum levels of the aforementioned biomarkers following consumption of PJ.[24]

The present study has certain limitations that need to be acknowledged. This study did not include a control group. Therefore, our findings might have been confounded by bias. In addition, the present trial was conducted in pilot scale and with a small population size. This small size could potentially account for lack of detecting significant difference despite the increasing trend in FMD and decreasing trends in serum CRP, ICAM-1, VCAM-1, and E-selectin levels. With respect to these limitations, findings of the present trial may not be generalizable to the general population and should be interpreted with caution.

In conclusion, the key finding to emerge from the present study is the acute hypotensive effect of PJ in hypertensive patients. While this trial is not a substitution for well-designed randomized controlled trials, it generates a hypothesis and motivates further research on this topic. Future large-scale investigations are indeed warranted in order to obtain a mechanistic understanding on this observed hypotensive activity.

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Conflict of Interests

Authors have no conflict of interests.

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Modulation of coronary artery disease risk factors by menopausal status: A population based study among Iranian women (KERCADRStudy)

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Original Article

Abstract

BACKGROUND: Menopause is now viewed as a risk factor for coronary heart diseases (CHD). There is a scarcity of evidence concerning the effects of menopause on coronary artery disease (CAD) risk factors. The present study aimed to evaluate the effects of menopausal status on CAD risk factors.

METHODS: The present study was designed as part of the Kerman coronary artery disease risk study (KERCADRS) that was a population-based study among a cohort of 6000 individuals aged 15 to 75 years in Kerman, Iran. Only women aged 35 to 60 years were enrolled. Participants were categorized according to reproductive age into the three groups of premenopausal, perimenopausal, and postmenopausal states.

RESULTS: The premenopausal status was accompanied with lower levels of triglyceride (TG), cholesterol, fasting plasma glucose (FPG), and blood pressure compared with the other two groups ($P < 0.001$). In addition, women in the postmenopausal group had higher levels of low-density lipoprotein (LDL) in comparison with the other two groups ($P < 0.001$). After adjusting for age, total cholesterol and LDL levels were significantly higher in the postmenopausal group compared with the other two groups ($P < 0.05$). In addition, total cholesterol and LDL levels, and systolic blood pressure were statistically different according to menopausal status after adjustment for both age and body mass index ($P < 0.05$).

CONCLUSION: The increased risk of cardiovascular disease in postmenopausal period can be explained by elevated levels of lipid profile and increased systolic blood pressure, regardless of effects of advanced age or other anthropometric parameters.

Keywords: CAD Risk Factors, Women, Premenopause, Perimenopause, Postmenopause

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Introduction

Menopause is now accepted as a risk factor for coronary heart diseases (CHD) and its occurrence results in increased risk of coronary artery disease (CAD) in women.[1] This increased risk can be caused not only by estrogen deprivation, but also by its effect on lipid profile, which is likely to occur in the perimenopause period.[2] Some studies have suggested a central role for insulin in increasing CAD risk factors.[3] Moreover, menopause can be associated with the aggravation of multiple cardiovascular risk factors. These deleterious factors can be indirectly affected by treatment with estrogen and progestin combination.[4] Besides, the pointed probable mechanisms, an increase in the prevalence of CAD risk factors at the time of menopause has also been shown to be a potential risk factor for increasing CAD risk. Some studies have found high total cholesterol level, high low-density lipoprotein (LDL) cholesterol level, and high triglyceride level to be associated with menopausal status.[4-6] In addition, menopause-

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associated differences in plasma LDL cholesterol levels have been attributed to the effect of sex hormones on LDL metabolism.[7,8] However, little information is available on the effects of menopause on CAD risk factors, especially lipid levels, in large populations. The present study aimed to clarify the effects of menopausal status on various CAD risk factors and determine the situation of these risk factors in different age groups of women around menopause.

Materials and Methods

The present study was designed as a part of The Kerman coronary artery disease risk study (KERCADRS-No. 88/110).[9] The KERCADRS was a population-based, epidemiological research among a cohort of 6000 individuals aged 15 to 75 years and residences of Kerman city, Iran. The study addressed the epidemiological data regarding various coronary artery disease risk factors and menopausal status. All subjects with a history of metabolic disorders or using antilipidemic drugs were not included into the study. A well-validated questionnaire regarding risk profile was administered by trained and certified medical staff. Participants also underwent a clinical examination that included measurement of height, weight, and arterial blood pressure (mean of two measurements performed with a standard sphygmomanometer in a sitting position after a 5-min rest) according to standardized protocols. Furthermore, blood samples were taken after at least 12 h of overnight fasting and hemoglobin A_1c (HbA_1c), fasting plasma glucose (FPG), serum triglyceride (TG), and total cholesterol, and high-density lipoprotein (HDL) cholesterol were measured. LDL cholesterol was also calculated using the Friedewald formula. In this study, only women aged 35 to 60 years were enrolled. Participants were categorized according to their reproductive age into three groups of postmenopausal group (with an amenorrhea for at least 12 months),[2,10] perimenopausal group (with an amenorrhea for 6 to 12 months, or older than 40 years old with irregular bleeding, and/or older than 40 years old with regular bleeding using progesterone medication), premenopausal group (age less than 40 years with regular bleeding, or age more than 40 years with regular bleeding without using progesterone drugs, or age less than 40 years with irregular bleeding).

Lipid-lowering therapy was defined as the daily intake within the previous 15 days of at least one lipid-lowering drug among those defined by the National Guide Drug Prescription used at the time of the study.[11] Similar definitions of treatments were used for diabetes mellitus and antihypertensive treatments.[2] Hormonal treatments were defined as the daily intake of contraceptive drugs or hormone replacement therapy. Women with a history of hysterectomy were excluded. The study protocol was approved by the research and ethics committees of the Kerman University of Medical Sciences, and informed consents were obtained from all participants.

Statistical analysis

Results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by absolute and relative frequencies for categorical variables. Categorical variables were compared using chi-square test or Fisher's exact test. Quantitative variables were also compared using ANOVA, and Tukey's post-hoc analysis was used to elicit pairwise difference between means where significant differences were found. The analysis of factors associated with coronary risk factors was conducted with multivariable logistic modeling. Age and body mass index were considered as potential confounding factors. Statistical significance was determined as a P value of ≤ 0.05. All statistical analysis was performed using SPSS for Windows (version 18; SPSS Inc., Chicago, IL, USA).

Results

The mean age of the whole sample was 49.25 ± 4.61 years. Among the 1538 women, 21.0% were taking daily antihypertensive drugs, 10.9% were taking daily diabetes mellitus medication, and 13.1% were taking daily lipid-lowering drugs. Fifty women (3.3%) were currently taking hormonal replacement therapy. According to study classification, 931 women were allocated in the premenopausal group, 84 women in the perimenopausal groups, and 523 women in postmenopausal group. As presented in table 1, except for the overall prevalence of current smoking that was similar across the three subgroups, other traditional cardiovascular risk factors including family history of coronary diseases, hypertension, hyperlipidemia, and diabetes mellitus were more frequent in perimenopausal and postmenopausal groups compared to others. Systolic and diastolic blood pressures, FPG, TG, total cholesterol, and LDL cholesterol were associated with the menopausal status (Table 2). The premenopausal status was accompanied with lower levels of TG, total cholesterol, FPG, and

blood pressure compared with perimenopausal and postmenopausal statuses (P < 0.001). Moreover, women in the postmenopausal group had higher levels of LDL cholesterol in comparison with perimenopausal and premenopausal statuses (P < 0.001). After adjusting for age variable, only total cholesterol and LDL cholesterol were significantly higher in postmenopausal women compared with the other two groups. However, serum level of TG, FPG, and systolic and diastolic blood pressures were not statistically different in the different menopausal statuses. In addition, serum total cholesterol and LDL levels, and systolic blood pressure did not differ according to menopausal status.

After further adjustment for age and body mass index, postmenopausal women were importantly characterized by higher total cholesterol (P < 0.001), LDL cholesterol (P < 0.001), and systolic blood pressure (P = 0.025) compared with others (Table 1).

Discussion

The incidence of cardiovascular diseases in postmenopausal period has been estimated to be higher than 50% in some observational studies. Different physiological mechanisms are now identified which are related to the increased risk of cardiovascular disease in postmenopausal status. The beneficial effects of hormone replacement therapy on reducing risk of CAD emphasize the role of the impairment of sex hormones in triggering CAD and its-related risk factors. The major part of these deleterious effects appears to be due to an increase in total cholesterol level, LDL cholesterol level, and reduction of HDL cholesterol. Furthermore, deregulation of hormonal systems can result in endothelial dysfunction predisposing to the appearance and progression of CAD.[12,13] Besides, some other studies have shown mechanisms by which estrogen might increase coagulation or

Table 1. Comparison of cardiovascular risk factors according to menopausal status

Characteristics	Premenopausal (n = 931)	Perimenopausal (n = 84)	Postmenopausal (n = 523)	P
Family history of CAD	212 (22.8)*	20 (23.8)	154 (29.4)	0.005
Hypertension	89 (9.6)	15 (17.9)	117 (22.4)	< 0.001
Hyperlipidemia	69 (7.4)	17 (20.2)	138 (26.4)	< 0.001
Diabetes mellitus	14 (1.5)	3 (3.6)	18 (3.4)	0.017
Current smoking	80 (8.6)	9 (10.7)	38 (7.3)	0.384

*N (%); Comparing was performed by the ANOVA test; CAD: Coronary artery disease

Table 2. Comparison of age-adjusted means of coronary heart diseases (CHD) risk factors according to menopausal status

	Premenopausal (n = 931)	Perimenopausal (n = 84)	Postmenopausal (n = 523)	Unadjusted P	Age-adjusted P	Age and BMI adjusted P
BMI (kg/m2)	27.9 ± 4.7*	27.8 ± 4.6	28.5 ± 5.0	0.077	0.458	0.444
Waist circumference (cm)	85.0 ± 11.1	88.6 ± 11.0	89.2 ± 11.7	< 0.001[a,c]	0.578	0.014[c]
Systolic blood pressure (mmHg)	112.7 ± 17.3	126.0 ± 20.8	125.7 ± 20.5	< 0.001[a,c]	0.070	0.025[c]
Diastolic blood pressure (mmHg)	75.8 ± 9.5	80.6 ± 10.7	80.9 ± 10.8	<0.001[a,c]	0.186	0.073
Fasting glycemia (g/dl)	100.4 ± 34.3	113.7 ± 51.6	117.8 ± 55.2	<0.001[a,c]	0.504	0.478
Total cholesterol (mg/dl)	197.2 ± 37.9	209.3 ± 43.6	219.3 ± 47.2	< 0.001[a,c]	< 0.001[a]	< 0.001[a]
LDL cholesterol	129.1 ± 31.7	133.7 ± 32.5	144.3 ± 39.4	< 0.001[a,b]	< 0.001[a]	< 0.001[a]
HDL cholesterol	39.9 ± 10.9	39.6 ± 10.6	40.4 ± 9.2	0.663	0.791	0.788
Triglycerides	143.7 ± 79.1	167.7 ± 79.9	170.5 ± 87.9	< 0.001[a,c]	0.166	0.110

* Mean ± SD, a: P < 0.05 for postmenopausal vs. premenopausal; b: P < 0.05 for postmenopausal vs. perimenopausal; c: P < 0.05 for perimenopausal vs. premenopausal; Comparisons were performed by ANOVA test followed by Tukay's post-hoc analysis; CHD: Coronary heart diseases; BMI: Body Mass Index; LDL: Low density lipoprotein; HDL: High density lipoprotein

inflammation, which trigger coronary events in advanced lesions.[14] Animal studies have also suggested that hormones might retard early atherosclerosis, while both animal studies and human angiographic trials are conclusive that hormones do not retard progression of raised lesions.[15] It seems that these conflicting results can be caused by the different changes in the situations of CAD risk factors in postmenopausal period. Although it has been found that after adjustment for initial potential cofounders, the trend of these changes is discrepant.

In the present study on the situations of CAD risk factors in different menopausal-related periods, most of the CAD risk factors were considerably more prevalent in postmenopausal status than premenopausal and perimenopausal periods, when unadjusted for potential cofounders such as age and body mass index. However, after adjustment for these confounding indicators, the condition of some risk profiles such as anthropometric parameters, fasting blood sugar, and blood pressure changed significantly. On the other hand, regardless of the effects of age or body mass index, postmenopausal status is associated with increased levels of total serum and LDL cholesterol. Some previous studies found similar findings. In the study by Agrinier et al. after adjustment of LDL cholesterol level for age and body mass index, these parameters were still significantly higher in postmenopausal women than in premenopausal women, indicating that other factors, independent of age and BMI, strongly influence LDL cholesterol levels in women.[2] The decrease in plasma estrogen levels after menopause might play a significant role in the reduction of the clearance of LDL particles and subsequent increase in LDL cholesterol level in postmenopausal women. In this regard, estrogen replacement treatment has been shown to markedly decrease LDL cholesterol level in dyslipidemic postmenopausal women. In addition, studies in animal models have indicated that estrogen treatment is followed by a marked increase in the number of hepatic cell surface LDL receptors and a faster clearance of LDL particles.[16] Furthermore, treatment with estrogen has been shown to increase cholesterol excretion in humans, and to decrease the conversion of VLDL-apoB to LDL-apoB in rabbits.[17,18] Moreover, according to our findings, postmenopausal and perimenopausal women suffered from increased systolic blood pressure compared with premenopausal women even after adjustment for confounders. Some studies showed that elevated systolic blood pressure is a potent risk factor in premenopausal women.[19] Recent epidemiologic and experimental evidence indicate that estrogen deficiency may cause increases in systolic blood pressure through impacting endothelial vascular function and/or systemic arterial compliance.[20-23] It can be certainly intensified in postmenopausal period because of its related hormonal disturbances. However, these hypotheses should be further investigated in future studies.

Conclusion

In summary, the increased risk of cardiovascular disease in postmenopausal period can be explained by elevated levels of lipid profile and increased systolic blood pressure regardless of the effects of advanced age or anthropometric parameters.

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Conflict of Interests

Authors have no conflict of interests.

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Impact of the components of Mediterranean nutrition regimen on long-term prognosis of diabetic patients with coronary artery disease

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Original Article

Abstract

BACKGROUND: The impact of different nutritional regimens on long-term prognosis and outcome in diabetic patients with coronary artery disease (CAD) has been questioned. Therefore, the objective of the present study was to determine the effects of different nutritional components of Mediterranean regimen on long-term cardiovascular events in diabetic patients with CAD in the Iranian population.

METHODS: In a prospective cohort study, we recruited 233 consecutive patients with the diagnosis of type 2 diabetes mellitus and with at least 6 months of documented CAD. Nutritional assessment was obtained by a validated semi-quantitative food frequency questionnaire (FFQ) and the diet score was calculated on the basis of the Mediterranean diet quality index (Med-DQI). For Assessing long-term CAD prognosis, the patients were followed by telephone for one year. The study endpoint was long-term major adverse cardiac and cerebrovascular event (MACCE).

RESULTS: Death was observed in 19 patients (8.2%) during the one-year follow-up. Two patients (0.9%) suffered non-fatal myocardial infarction and 14 (6.0%) needed revascularization within 1 year after discharge from hospital. Overall MACCE within one year in the study population was 12.4%. There were significant differences between number of deaths and dietary scores of saturated fatty acid, cholesterol, meats, fish, and fruit and vegetables ($P < 0.05$). Moreover, significant differences were found between MACCE rate and dietary scores of saturated fatty acid, cholesterol, and fruit and vegetables ($P < 0.05$). Using multivariate logistic regression models, Mediterranean dietary regimen could effectively predict long-term death as well as MACCE adjusted for gender and age variables.

CONCLUSION: Mediterranean dietary regimens, including low level of cholesterol and saturated fatty acid, can effectively improve long-term outcome including death and MACCE in diabetic patients with CAD.

Keywords: Diabetes Mellitus, Coronary Artery Disease, Nutrition

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Introduction

According to the results of recent epidemiological surveys, diabetes mellitus confers an increased risk for coronary artery disease (CAD) and leads to cardiac mortality and morbidity that accounts currently for almost 32% of all deaths among diabetic patients.[1,2] The largest increase in mortality and morbidity due to CAD in diabetic patients is expected to occur in developing countries. In Iran, as a developing country, the prevalence of CAD among diabetic patients was estimated to be 28.0%, and among patients with CAD who died in hospital 75.6% were diabetics.[3,4]

Previous studies have emphasized the role of some risk factors, such as advanced age, fasting glucose levels, smoking, hypertension, and triglyceride levels, as independent risk factors for development of CAD events in diabetic patients.[5,6] However, the role of different nutritional regimens on severity of CAD in these patients is questioned. It has been shown that the optimal nutrition therapy is associated with a 2.0% decrease in glycated

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haemoglobin (HbA_1C) in patients with newly diagnosed type 2 diabetes mellitus.[7]

Some studies showed the possibility of comprehensive lifestyle changes, and improvements in coronary risk factors and quality of life by an optimal nutritional program in patients with concomitant CAD and diabetes mellitus in comparison to those without diabetes.[8-9] Furthermore, some others suggested that the combination of dietary change and physical conditioning is associated with improved glucose tolerance for diabetics and can improve patients' quality of life.[10] However, fewer evidences are available regarding the role of nutritional habits, especially Mediterranean regimen, as a predictor of CAD prognosis among diabetic patients. In the present study, we planned to determine the effects of different nutritional components of Mediterranean regimen on long-term cardiovascular events in Iranian diabetic patients with CAD.

Materials and Methods

In a prospective cohort study, we recruited 233 consecutive patients with the diagnosis of type 2 diabetes mellitus for at least 6 months with documented CAD; they were diagnosed and hospitalized at the Tehran Heart Center, Tehran, Iran, in 2012. In this study, CAD was considered significant if there was a 75% or greater stenosis in the cross-sectional diameter and 50% or greater stenosis in the luminal view.[11] The data included for analysis were demographic characteristics, preoperative risk factors, paraclinical data, and cardiac status.[12]

Diabetes mellitus was defined on the basis of the American Diabetes Association (ADA) criteria as the presence of diabetes symptoms plus plasma glucose concentration ≥ 11.1 mmol/l or fasting plasma glucose ≥ 5.6 mmol/l or 2-hp ≥ 11.1 mmol/l or using anti-diabetic drugs.[13] Studied patients were also interviewed on admission and asked to report how often they consumed each of the food items listed as the number of times per day, per month, or per year during the previous year. Nutritional assessment was obtained by a validated semi-quantitative food frequency questionnaire (FFQ), with 48 items which was previously validated in Iran, and a 24-hour dietary recall questionnaire to record the types, amounts, and frequencies of foods consumed.[14] We used the sum of the consumption of each of several food items to estimate the overall consumption of the food group to which each item belonged.[15,16]

The Mediterranean diet is define as a dietary pattern usually used among the populations around the Mediterranean Sea, and it is reported as a model for healthy eating and better quality of life. The diet score was calculated on the basis of the Mediterranean diet quality index (Med-DQI); the construction of the score for this index is mentioned in table 1.[17] To calculate the dietary intake we divided the consumption amount by the frequency of consumption. The index assigns a score of 0, 1, or 2 according to the daily intake of each of the seven components and then final score is reported as a summation of all nutrient (saturated fatty acids, cholesterol, meats, olive oil, fish, cereals, and vegetable and fruits) scores ranging between 0 and 14. A lower score on this index indicates a better nutrition quality.

For Assessing long-term CAD prognosis, the patients were followed by telephone for one year. The study endpoint was long-term major adverse cardiac and cerebrovascular event (MACCE) (defined as occurrence of one of these morbidities including death, non-fatal myocardial infarction, or need to revascularization).

Statistical analysis

Results were reported as mean ± standard deviation (SD) for quantitative variables, and number (percentages) for categorical variables. Categorical variables were compared using chi-square test or Fisher's exact test if required. P-values of 0.05 or less were considered statistically significant. All the statistical analyses were performed using SPSS for Windows (version 16.0; SPSS Inc., Chicago, IL, USA).

Results

Demographic characteristics and clinical data of studied patients are summarized in table 2. Mean age of studied patients was 59.00 ± 8.39 (ranging from 38 to 75 years) and almost two thirds of them were male. The most common general risk factors for CAD included hypercholesterolemia (76.4%), hypertension (58.4%), and family history of CAD (50.2%). Mean left ventricular ejection fraction was 49.37 ± 10.05, and 82.4% of cases had functional class I-II. In the review of angiographic reports, it was found that the majority of patients (79.0%) suffered from three coronary vessels disease.

One-year death was revealed in 19 (8.2%). Two patients (0.9%) suffered non-fatal myocardial infarction and 14 (6.0%) needed to revascularization within 1 year after discharge from hospital. Overall, one-year MACCE in the study population was 12.4%. One year death and MCCE rates in different dietary groups in men and women are shown in tables 3. There were significant relationships

between death rate and dietary scores of saturated fatty acid, cholesterol, meats, fish, and fruit and vegetables. Moreover, significant relationships were found between MACCE rate and dietary scores of saturated fatty acid, cholesterol, and fruit and vegetables. These differences were independent to gender variable for both one-year mortality and MACCE rates. Using multivariate logistic regression models, Mediterranean dietary regimen could effectively predict long-term mortality and MACCE adjusted for gender and age variables (Tables 4 and 5). A significant relationship was found between total score of Mediterranean regimen and MACCE after adjustment by sex and age (P = 0.039).

Table 1. Construction of the score for the Mediterranean Dietary Quality Index

Scoring	0	1	2
Saturated fatty acids (% energy)	< 10	10-13	> 13
Cholesterol (mg)	< 300	300-400	> 400
Meats (g)	< 25	25-125	> 125
Olive oil (ml)	> 15	5-15	< 5
Fish (g)	> 60	30-60	< 30
Cereals (g)	> 300	100-300	< 100
Vegetables + fruits (g)	> 700	400-700	< 400

Table 2. Demographic characteristics and clinical data of studied patients (n = 233)

Characteristic	Mean ± SD	n (%)
Age (year)	59.00 ± 8.39	
Body mass index (kg/m^2)	28.31 ± 4.19	
NYHA score	2.11 ± 0.78	
Ejection fraction (%)	49.37 ± 10.05	
Euroscore	2.46 ± 2.27	
Laboratory indices		
Fasting blood sugar (mg/dl)	126.14 ± 45.97	
Creatinine (mg/dl)	1.26 ± 0.30	
Triglyceride (mg/dl)	175.40 ± 79.21	
Cholesterol (mg/dl)	158.55 ± 47.66	
High density lipoprotein (mg/dl)	40.04 ± 8.57	
Low density lipoprotein (mg/dl)	83.52 ± 34.82	
Hemoglobin A_1C (%)	6.90 ± 1.59	
Albumin (g/dl)	4.64 ± 3.34	
Men		146 (62.7)
Family history of CAD		117 (50.2)
Current cigarette smoking		69 (29.6)
Opium addiction		26 (11.2)
Hypercholesterolemia		178 (76.4)
Hypertension		136 (58.4)
Cerebrovascular disease		14 (6.0)
Peripheral vascular disease		88 (37.8)
Recent myocardial infarction		107 (45.9)
Congestive heart failure		33 (14.2)
Functional class		
I		78 (33.5)
II		114 (48.9)
III		41 (17.6)
Education level		
Primary		137 (58.8)
Secondary		62 (26.6)
Higher		34 (14.6)
Coronary vessels involvement		
Single-vessel disease		6 (2.6)
Two-vessel disease		43 (18.5)
Three-vessel disease		184 (79.0)

CAD: Coronary artery disease; NYHA: New York Heart Association

Table 3. The number of death and major adverse cardiac and cerebrovascular event (MACCE) according to the nutrition components

Dietary group	Death	MACCE
Saturated fatty acid		
0 (n = 85)	0 (0.0)	1 (1.1)
1 (n = 64)	4 (6.3)	8 (12.5)
2 (n = 33)	15 (45.6)	20 (60.6)
P	< 0.001	< 0.001
Cholesterol		
0 (n = 134)	1 (7.5)	5 (3.7)
1 (n = 24)	5 (20.8)	8 (33.3)
2 (n = 24)	13 (54.2)	16 (66.7)
P	< 0.001	< 0.001
Meats		
0 (n = 66)	2 (3.0)	7 (10.6)
1 (n = 108)	14 (13.0)	19 (9.2)
2 (n = 8)	3 (37.5)	3 (37.5)
P	0.006	0.106
Olive		
0 (n = 19)	1 (4.5)	2 (9.9)
1 (n = 52)	6 (11.5)	9 (17.3)
2 (n = 111)	12 (10.8)	18 (16.2)
P	0.650	0.731
Fish		
0 (n = 24)	0 (0.0)	2 (8.3)
1 (n = 45)	3 (6.7)	7 (15.6)
2 (n = 113)	16 (14.2)	20 (17.7)
P	0.037	0.351
Cereal		
0 (n = 121)	11 (9.1)	13 (10.7)
1 (n = 57)	7 (12.3)	15 (26.3)
2 (n = 4)	1 (25.0)	1 (25.0)
P	0.364	0.079
Fruits and vegetables		
0 (n = 147)	11 (7.5)	10 (6.8)
1 (n = 28)	4 (14.3)	15 (53.6)
2 (n = 7)	4 (57.1)	4 (57.1)
P	0.003	< 0.001

MACCE: Major adverse cardiac and cerebrovascular event

Table 4. Multivariate analysis of the effects of nutrition components on death adjusted for sex and age

Variables	Univariate analysis				Multivariate analysis			
	Odds ratio	95% Confidence interval		P	Odds ratio	95% Confidence interval		P
		Lower	Upper			Lower	Upper	
Age	1.031	1.009	1.053	0.005	1.021	0.999	1.044	0.067
Male gender	1.650	1.105	2.463	0.014	1.534	1.019	2.309	0.040
Total score of Mediterranean regimen	2.411	1.102	2.998	0.037	1.990	1.091	2.022	0.042

Hosmer-Lemeshow goodness of fit test; $\chi^2 = 8.190$; Degree of freedom = 8; P = 0.415

Table 5. Multivariate analysis of the effects of nutrition components on major adverse cardiac and cerebrovascular event (MACCE) adjusted for sex and age

Variables	Univariate analysis				Multivariate analysis			
	Odds ratio	95% Confidence interval		P	Odds ratio	95% Confidence interval		P
		Lower	Upper			Lower	Upper	
Age	1.142	1.021	1.191	0.021	1.110	1.099	1.104	0.041
Male gender	1.424	1.078	2.217	0.042	1.987	1.178	2.190	0.034
Total score of Mediterranean regimen	1.664	1.148	2.098	0.021	1.987	1.056	2.088	0.039

Hosmer-Lemeshow goodness of fit test; $\chi^2 = 7.655$; Degree of freedom = 8, P = 0.428; MACCE: Major adverse cardiac and cerebrovascular event

Discussion

Improving the contents of nutrients for diabetic patients facilitates health care in these patients, and this critical issue can influence medical and clinical outcomes and patient quality of life. It has been confirmed that promoting healthy food choices can lead to decrease in the risk of diabetes and cardiovascular complications.[18]

In the present study, we tried to consider the impact of different Mediterranean dietary components on long-term outcome of diabetes in patients suffering from CAD. We found that one-year death in these patients was dependent on the consumption of some nutrient components such as saturated fatty acids and cholesterol. Epidemiological studies strongly support the suggestion that low intakes of cholesterol and free fatty acids prevent the development of type II diabetes mellitus and people who consume foods with the lowest levels of these nutritional agents are less likely to develop diabetes and its-related complications than higher level consumers.[19,20] The effect of low-fat diets in the prevention of CAD has also been demonstrated. It has been concluded that a relationship between cereal intake and CAD was seen with considerable reduction in risk for those who eat this food habitually versus those who eat them rarely.[21-23] Some studies also showed that the consumption of rich-cholesterol foods increases the effect of plasma lipoprotein risk factors in cardiovascular disease. Total plasma and low density lipoprotein cholesterol concentrations were significantly lowered, and the ratios of plasma high-density-lipoprotein cholesterol to total cholesterol and of apolipoprotein A-I to B were significantly increased with the consumption of this dietary group.[24] Therefore, it seems that low consumption of Mediterranean diet, including low consumption of saturated fatty acids and cholesterol, can improve one-year survival in diabetic patients with CAD via regulation of lipids metabolism and loss of body weight.

Our study also showed a relationship between the consumption of saturated fatty acids and cholesterol, and mid-term MACCE rate. It has been confirmed that the consumption of saturated fatty acids induces hyperlipidemia and obesity, causing progression of atherosclerosis especially in diabetic patients.[25] Furthermore, the increase of adipose tissue stores can disturb insulin-mediated regulation of lipolysis and increase circulating fatty acid concentrations which may promote insulin resistance and cardiovascular complications; therefore, these pathways can predispose diabetic CAD patients to poor prognosis.[26,27]

In the present study, we finally showed that the Mediterranean diet in diabetic patients with CAD, including low cholesterol, and high cereal, fruits and vegetables, and olive and fish intake, can be an acceptable regimen for these patients leading to favorable outcome. Besides, it seems that overall dietary patterns in various populations are dependent upon socioeconomic status, demographic characteristics, and patients' lifestyle.[28] It is recommended that the impact of these factors on dietary patterns be investigated in different populations, especially diabetic patients.

Limitation

A limitation of this study was the relatively small sample size. For this reason, these findings cannot be generalized to the broader community based on this study alone.

Conclusion

It can be concluded that nutritional pattern of Mediterranean regimen, particularly consumption of lower level of cholesterol and saturated fatty acids, can effectively improve one-year death and MACCE in diabetic patients with CAD.

Conflict of Interests

Authors have no conflict of interests.

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Amiodarone versus lidocaine for the prevention of reperfusion ventricular fibrillation: A randomized clinical trial

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Original Article

Abstract

BACKGROUND: Reperfusion ventricular fibrillation after aortic cross clamp is one of the important complications of open cardiac surgery and its prevention could reduce myocardial injuries. This study aimed to evaluate the efficacy of single dose of amiodarone or lidocaine by the way of pump circuit three minutes before aortic cross clamp release and compare the results with normal saline as placebo in a randomized double blinded controlled trial.

METHODS: One hundred fifty patients scheduled for first time elective coronary artery bypass graft surgery were randomly assigned to receive either single dose of amiodarone (150 mg), lidocaine (100 mg), or normal saline (5 ml) three minutes before aortic cross clamp release. The incidence of ventricular fibrillation and the need for reuse of drug were compared between these groups by chi-square, Student's t-test, Mann-Whitney test, and One-way ANOVA. SPSS software was used for statistical analysis.

RESULTS: The incidence of ventricular fibrillation is higher in the placebo group (15.9%) compare to lidocaine (11.8%) and amiodarone (8.9%) groups; however, there was no statistical difference among the three groups (P = 0.41). Moreover, the reuse of amiodarone (22.7%) was statistically higher (P < 0.05) than lidocaine (5.9%).

CONCLUSION: This study showed no difference among lidocaine, amiodarone, and placebo in preventing ventricular fibrillation after aortic cross clamp release.

Keywords: Amiodarone, Lidocaine, Ischemia Reperfusion Injury, Ventricular Fibrilation, Randomized Controlled Trial

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Introduction

Reperfusion arrhythmia is one of the important complications after pump coronary artery bypass graft (CABG) surgery. It has been shown that the incidence of postoperative supraventricular arrhythmias is 11–54% while the incidence of ventricular arrhythmias is 1.8–13%.[1] Although a large majority of such arrhythmias can be controlled by electrical cardioversions, the metabolic demands of such fibrillation or its treatment by means of direct current (DC) shock may contribute to myocardial injury.[2] Therefore, avoiding reperfusion ventricular fibrillation (RVF) after aortic cross clamp (ACC) release would reduce myocardial dysfunction during cardiopulmonary bypass (CPB).[3]

Several studies have evaluated the efficacy of amiodarone (class III antiarrhythmic agent) and lidocaine (class IB antiarrhythmic agent) in preventing postoperative RVF. Most of trials demonstrated that intravenous amiodarone is superior to other antiarrhythmics in preventing arrhythmias that may occur after coronary artery bypass operations.[4-6] However, in a recent study,

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amiodarone did not reduce the incidence of RVF compared to the placebo group while lidocaine did.[7] Therefore, the superiority of either amiodarone or lidocaine still remain a matter of controversy.

This study aimed to evaluate the efficacy of a single dose of amiodarone or lidocaine by the way of pump circuit 3 minutes before ACC release and compared the results with normal saline as placebo in a randomized, double-blinded, controlled trial.

Materials and Methods

Between September 2010 and September 2011, 150 patients scheduled for first time elective CABG were enrolled in this prospective randomized double-blinded controlled trial. On the basis of the available literature, the expected overall incidence of ventricular fibrillation (VF) after removal of the aortic cross clamp is approximately 70%. By using a chi-square test with 80% power and an alpha of 0.0167 to adjust for multiple comparisons (given the total of 3 comparisons, 0.05/3 = 0.0167 was used in the calculation), we estimated that we would need 44 patients in each group. Given the potential for patient dropouts, we planned to enroll 50 patients in each group for a total study population of 150 patients.

The protocol of this study was approved by the local ethical committee of the School of Medicine, Tehran University of Medical Sciences, Tehran, Iran (IRCT201205198860N2). A written informed consent was obtained from every patient and they were randomly assigned to three groups using balanced block randomization (block of six pieces). Exclusion criteria were any history of treatment with digoxin, amiodarone, or lidocaine (including cardiopulmonary resuscitation), contraindications to amiodarone (sick sinus syndrome, atrioventricular conduction abnormalities, thyroid disease, interstitial lung disorders, renal or liver disease, and known allergic or toxic reactions to amiodarone), combined cardiac surgery, and emergent operation.

Patients' baseline characteristics included age, sex, weight, height, body surface area (BSA), history of treatment with beta blocker, any history of MI. The patients' echocardiographic information included concomitant valve disease, left ventricular aneurysm, left ventricular ejection fraction (LVEF), and left ventricular end diastolic diameter (LVEDD).

Standard clinical protocol was used for all patients. Complete blood count, a standard coagulation profile, electrolytes, and cardiac enzyme (CPK-MB and Troponin I) were performed a day before surgery. All surgeries were performed by a single experienced surgeon at Rajaie Cardiovascular Medical and Research Center, Tehran, Iran.

All patients were monitored with pulsoxymeter, invasive blood pressure (IBP) device, central venous pressure (CVP), and electrocardiography (lead II to V5). Premedication included lorazepam, 1 mg orally the night before surgery and 1 mg one hour before surgery, plus morphine, 1 mg/kg intramuscular one hour before beginning the operation. General anesthesia was induced with sufenta 1 μg/kg, etomidate 0.2 mg/kg, and atracurium 0.5 mg/kg, and anesthesia was maintained using sufenta 1 μg/kg/hour, midazolam 1 μg/kg/min, and atracurium 4-12 μg/kg/min.

The operations were performed through a standard median sternotomy with cardiopulmonary bypass (CPB) with a flow rate of 2.4 l/m^2 and mild hypothermia at 34°C. CPB was instituted with a standard kit and a hollow-fiber membrane oxygenator (Dideco Simplex D708, Dideco). The CPB circuit was primed with Ringer's acetate and carefully de-aired. Standard cannulation consisted of arterial cannulation in the distal part of the ascending aorta and a 2-stage venous cannula inserted into the right atrium and the inferior vena cava. Myocardial preservation consisted of either anterograde or intermittent antegrade and retrograde St. Thomas solution cardioplegia. Cardioplegia was repeated every 20 to 30 minutes or on the return of electrical activity of the heart. In all the patients, the distal anastomoses were constructed first, and the proximal anastomoses were created after ACC.

Patients were randomly assigned to three groups. Group A received 150 mg (3 ml) of amiodarone diluted in 2 ml distilled water, group B received 5 ml of lidocaine 2% (100 mg), and group C (control group) received 5 ml normal saline by the way of pump circuit, 3 minutes before aortic cross clamp release.

Intraoperative variables included ACC time, CPB time, cardioplegic volume, and two samples for electrolyte and arterial blood gas (ABG) values. Patients were weaned from CPB when rewarmed to core temperature of at least 37°C and were hemodynamically stable. Electrolyte and ABG values were tested once more after weaning from CPB. Whenever the patient's rhythm was VF after ACC release, the antiarrhthmic drug was reused and the patient was treated with internal biphasic truncated exponential direct current (DC) shock with stepwise increasing energy at the frequency of 10, 10, and 20 J.

Furthermore, in spite of normal ABG and serum level of electrolytes, if this did not lead to a stable rhythm, a 30-J shock was given after the administration of another dose of antiarrhthmic drug. Reuse of antiarrythmic drug means another single dose of lidocaine in group A, amiodarone in group B, and lidocaine in group C. It should be mentioned that surgeons were blinded to the type of drugs during this study.

The primary outcome was incidence of VF requiring defibrillation during 30 minutes after ACC release. The secondary outcomes of this study were incidence of VF afterIntensive care unit(ICU) and ward admission, and reuse of antiarrhythmic drug after ACC release. The electrolyte, ABG, CPK-MB, and troponin I values were also evaluated three times (1, 6, and 24 hours) after entering the ICU in order to compare hemodynamic status of patients.

Statistical analysis

Continuous variables are summarized as mean ± SD and categorical variables are expressed as proportion (%). Univariate analyses were performed by chi-square, student's t-test, Mann-Whitney test and One-way ANOVA (where appropriate). SPSS for Windows (version 17; SPSS Inc., Chicago, IL., USA) was used for statistical analysis. Results were considered significant if P values were less than 0.05.

Results

One hundred fifty patients were randomized into three groups. Group A (n = 50) received amiodarone, group B (n = 50) lidocaine, and group C (n = 50) received placebo (normal saline) (Figure 1). The mean age for all patients was 58.69 ± 12.48. The patients' characteristics, and perioperative and postoperative variables were reported in table 1, table 2, and table 3, respectively. It has been shown that there was no statistically significant difference between the three groups in terms of patients' characteristics, preoperative data, and postoperative findings (serum electrolyte and cardiac biomarkers, medications,myocardial infarction (MI) history, left ventricular end diastolic volume, concomitant valve disease, left ventricular aneurysm, and operation condition). The detailed characteristics of outcomes such as incidence of VF and the rate of DC shock during post ACC, ICU, and ward stay, reuse of drugs after removal of ACC, and need for antiarrhythmic drugs during ICU stay are demonstrated in table 4.

One patient in the amiodarone group and another one in the placebo group demonstrated postoperative myocardial infarction.[8] The patients' rhythm and need for defibrillation were monitored at three time points including after removal of ACC, and after admission to ICU and ward. Table 5 demonstrates the results. Although the incidence of VF and the need for defibrillation was higher in the placebo group compared to the two other groups and it was higher in the lidocaine group compared to the amiodarone group, these differences did not achieve statistical significance.

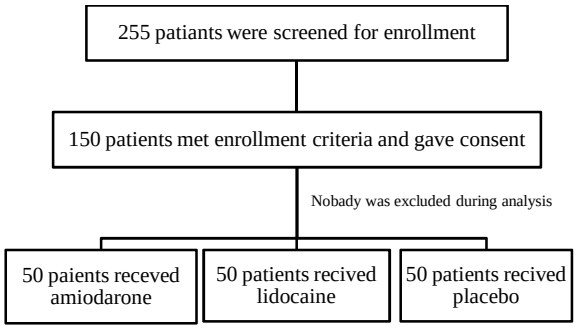

Figure1. Flow diagram of patient enrollment

Table 1. Demographic characteristics of patients

	Total (%)	Group A (%)	Group B (%)	Group C (%)	P
Sex					
Male	122 (81.40)	39 (78.40)	40 (80.0)	43 (86.40)	0.585
Female	28 (18.60)	11 (21.60)	10 (20.0)	7 (13.60)	
MI history	44 (29.30)	18 (23.50)	12 (22.7)	22 (43.20)	0.055
Beta blocker					
Propranolol	23 (15.00)	8 (15.70)	6 (11.10)	9 (18.20)	0.629
Metoral	115 (76.40)	39 (78.40)	38 (75.60)	38 (75.00)	
Carvedilol	13 (8.60)	3 (5.90)	7 (13.30)	3 (6.80)	
LV aneurysm	0	0	0	0	-
Cardioplegia					
Antrograde	91 (60.40)	28 (56.90)	32 (63.60)	31 (61.40)	0.788
Antrograde + retrograde	59 (39.60)	22 (43.10)	18 (36.40)	19 (38.60)	
Re-exploration after operation	0	0	0	0	-

MI: Myocardial infarction; LV: Left ventricular

Table 2. Preoperative continuous characteristics of patients

	Total	Group A	Group B	Group C	P
Age	58.69 ± 12.47	58.06 ± 10.47	60.64 ± 15.62	57.43 ± 10.97	0.43
Weight	72.34 ± 13.02	70.71 ± 10.72	75.38 ± 16.06	71.14 ± 11.64	0.16
Height	164.07 ± 17.80	162.29 ± 23.77	164.36 ± 16.32	165.84 ± 9.35	0.62
BSA	1.82 ± 0.25	1.83 ± 0.25	1.82 ± 0.32	1.88 ± 0.29	0.35
CPK-MB	27.68 ± 9.53	25.54 ± 7.24	24.50 ± 7.39	30.47 ± 11.53	0.21
Na	140.59 ± 3.26	140.78 ± 3.24	140.64 ± 3.27	140.32 ± 3.36	0.78
K	4.15 ± 0.44	4.10 ± 0.44	4.11 ± 0.42	4.24 ± 0.47	0.24
Ca	7.98 ± 1.54	7.98 ± 1.72	7.76 ± 1.52	8.21 ± 1.33	0.39
Mg	2.43 ± 0.40	2.50 ± 0.40	2.36 ± 0.37	2.42 ± 0.44	0.24
Cr	1.19 ± 0.23	1.16 ± 0.22	1.22 ± 0.24	1.18 ± 0.23	0.41
EF	43.24 ± 6.99	42.65 ± 6.80	43.64 ± 6.93	43.52 ± 7.36	0.75
LVEDD	4.64 ± 0.71	4.78 ± 0.77	4.61 ± 0.70	4.49 ± 0.64	0.14

BSA: Body Surface Area; CPK-MB: Creatinin Phosphokinase–MB; EF: Ejection fraction; LVEDD: Left ventricular end diastolic diameter

Table 3. Continuous intra-operative characteristics of patients

		Total	Group A	Group B	Group C	P
Cross Clamp (min)		36.34 ± 15.89	38.20 ± 19.63	35.64 ± 12.62	34.89 ± 13.98	0.56
CPB time (min)		70.14 ± 27.10	72.80 ± 29.16	72.09 ± 21.24	65.11 ± 29.67	0.33
Cardio. Volume		904.60 ± 424.18	913.53 ± 421.11	931.82 ± 419.45	867.05 ± 439.34	0.76
†	Na	135.86 ± 5.37	136.43 ± 5.83	135.13 ± 4.93	135.93 ± 5.28	0.49
	K	4.54 ± 0.52	4.67 ± 0.50	4.48 ± 0.53	4.46 ± 0.52	0.09
	pH	7.35 ± 0.66	7.35 ± 0.07	7.34 ± 0.06	7.36 ± 0.06	0.62
	HCO_3	18.35 ± 2.04	18.29 ± 2.06	18.51 ± 1.98	18.25 ± 2.11	0.81
‡	Na	135.69 ± 5.11	136.06 ± 5.25	135.31 ± 4.91	135.64 ± 5.25	0.77
	K	4.55 ± 0.59	4.54 ± 0.60	4.49 ± 0.48	4.61 ± 0.69	0.65
	pH	7.348 ± 0.07	7.35 ± 0.07	7.34 ± 0.06	7.35 ± 0.07	0.43
	HCO_3	18.54 ± 2.03	18.39 ± 2.00	18.64 ± 2.13	18.59 ± 2.00	0.81
$	Na	136.74 ± 5.54	137.47 ± 5.60	136.96 ± 5.46	135.68 ± 5.51	0.28
	K	4.52 ± 0.61	4.46 ± 0.59	4.59 ± 0.62	4.51 ± 0.62	0.58
	pH	7.36 ± 0.07	7.36 ± 0.07	7.36 ± 0.07	7.34 ± 0.07	0.60
	HCO_3	18.74 ± 2.18	18.24 ± 2.20	19.00 ± 1.99	19.07 ± 2.29	0.11

† The first laboratory test during cardiopulmonary bypass machine; ‡ The last laboratory test before weaning from CPB
$ The first laboratory test after weaning from CPB; CPB: cardiopulmonary bypass; Cardio. Volume: Cardioplegic volume

Reuse of drug means using another single dose of lidocaine in group A, another single dose of amiodarone in group B, and a single dose of lidocaine in the placebo group after removal of aortic cross clamp. Table 5 shows that the reuse of drug incidence was statistically higher in the amiodarone group than the lidocaine group.

Discussion

Reperfusion after myocardial ischemia induces ventricular arrhythmias such as ventricular tachycardia and ventricular fibrillation.[9-10] Reperfusion ventricular fibrillation is considered to be caused by a re-entry resulting from decreased conduction velocity and increased inhomogeneity in the refractory periods of cardiomyocytes.[11]

It has been shown that agents that have sodium channel blockade are capable of preventing reperfusion-induced arrhythmias.[12] Lidocaine, a class IB antiarrhythmic drug, binds to sodium channels, decreases the slope of phase 4 depolarization, and increases the diastolic electric current threshold in Purkinje fibers. Rinne and Kaukinen studied the effect of an intravenous bolus of lidocaine given before clamping the aorta followed by a continuous infusion for 20 hours.[13] They reported neither an increase in cardiac protection nor a decrease in the incidence of RVF. Baraka et al. showed that the administration of a bolus of 100 mg of lidocaine by the way of the pump 2 min before releasing the ACC can significantly decrease the incidence of RVF, without increasing the incidence of atrioventricular block.[14] In our study, a single dose of 100 mg

Table 4. Postoperative laboratory results duringintensive care unit (ICU) stay

		Total	Group A	Group B	Group C	P
†	Na ICU	136.31 ± 5.18	135.43 ± 4.77	136.82 ± 5.55	136.82 ± 5.24	0.31
	K ICU	4.533 ± 0.64	4.61 ± 0.70	4.40 ± 0.57	4.58 ± 0.62	0.24
	Mg ICU	2.439 ± 0.39	2.40 ± 0.39	2.43 ± 0.45	2.49 ± 0.34	0.50
	Ca ICU	8.03 ± 1.45	8.09 ± 1.20	8.25 ± 1.26	7.75 ± 1.82	0.25
	pH ICU	7.317 ± 0.36	7.29 ± 0.42	7.37 ± 0.07	7.30 ± 0.45	0.52
	HCO_3 ICU	18.46 ± 2.09	18.43 ± 2.08	18.33 ± 2.04	18.64 ± 2.17	0.78
	CPK MB ICU 1	57.09 ± 34.81	57.22 ± 28.84	54.91 ± 30.51	59.18 ± 44.57	0.84
‡	Na ICU	136.93 ± 5.68	136.71 ± 6.22	137.24 ± 5.23	136.86 ± 5.58	0.89
	K ICU	4.47 ± 0.58	4.42 ± 0.59	4.45 ± 0.45	4.55 ± 0.68	0.54
	pH ICU	7.36 ± 0.07	7.36 ± 0.07	7.36 ± 0.067	7.35 ± 0.06	0.88
	HCO_3 ICU	18.46 ± 2.09	18.33 ± 2.15	18.78 ± 2.11	18.28 ± 2.02	0.46
	CPK MB ICU	53.31 ± 29.03	57.27 ± 31.14	46.09 ± 23.12	55.93 ± 31.03	0.61
	Troponin ICU	1.25 ± 0.89	1.34 ± 1.05	1.23 ± 0.75	1.14 ± 0.80	0.52
*	Na ICU	136.83 ± 5.79	136.65 ± 5.72	137.67 ± 6.13	136.18 ± 5.52	0.66
	K ICU	4.54 ± 0.60	4.51 ± 0.53	4.61 ± 0.65	4.49 ± 0.62	0.61
	Ca ICU	8.30 ± 0.84	8.25 ± 0.89	8.39 ± 0.82	8.28 ± 0.79	0.66
	CPK Mb ICU	58.41± 34.71	55.51 ± 28.71	64.98 ± 44.86	55.20 ± 28.84	0.31
	Cr ICU	1.21 ± 0.25	1.19 ± 0.26	1.24 ± 0.28	1.21 ± 0.20	0.65
	Postoperative LVEF	44.64 ± 6.75	45.00 ± 7.14	44.89 ± 6.96	43.98 ± 6.15	0.73

† The first test at ICU; ‡Six hours after admitting to the ICU; * Twenty four hours after admitting to the ICU
CPK Mb: Creatinin Phosphokinase–Mb; ICU: Intensive care unit; LVEF: Left ventricular ejection fraction

Table 5. Final results for the three groups

	Group A (%)	Group B (%)	Group C (%)	P
VF after removal of ACC	6 (11.8)	5 (8.9)	8 (15.9)	0.562
VF during ICU stay	0	1 (2.3)	0	0.595
VF during ward stay	0	0	0	-
Defibrillation after removal of ACC	6 (11.8)	4 (6.8)	8 (15.9)	0.410
Defibrillation during ICU stay	0	0	0	-
Defibrillation during ward stay	0	0	0	-
Reuse of drug	3 (5.9)	11 (22.7)	-	0.048

VF:Ventricular fibrillation; ACC: Aorticcross clamp; ICU: Intensive care unit

lidocaine was administered 3 minutes before removal of ACC. The results did not show any statistical difference between the lidocaine group and the placebo group for incidence of ventricular fibrillation (0.56).

Amiodarone [2-butyl, 3-(4-diethylaminoethoxy, 3, 5-diiodo, benzoyl) benzofuran hydrochloride] is a class III antiarrhythmic agent which displays a wide cellular electrophysiologic spectrum, inhibiting the potassium currents, sodium currents, and L-type calcium currents in isolated cardiomyocytes.[15,16] Animal studies confirmed that amiodarone use not only improved the cardiac metabolic efficiency after ischemic reperfusion period but also decreased the transmural dispersion of repolarization of the heart, which is closely associated with the development of ventricular arrhythmias.[17-20] Bigdelian and Gharipour demonstrated that postoperative course of amiodarone administration is an effective, possibly safe, well-tolerated, and widely applicable therapy for the prevention of postoperative atrial tachyarrhythmia after cardiac surgery.[21] In our study, 150 mg amiodarone was administered 3 minutes before removal of aortic cross clamp. Although amiodarone could reduce the incidence of VF from 15.9% to 11.8%, it was not statistically significant (P = 0.18). Ayoub et al. demonstrated that although amiodarone could not reduce the frequency of VF compared to the control group, it did reduce the requirements of direct current shocks energy in the amiodarone group (16.7 J) as compared to the control group (25.8 J).[7] In contrast, Samantaray et al. showed that amiodarone administration could reduce the incidence of VF. However, they considered only 17 patients in each group which was significantly lower than our study and the difference in the results might be related to this fact.[22]

Furthermore, the results showed that the incidence of VF was lower in the amiodarone group

(8.9%) than the lidocaine group (11.8%) which was not statistically significant (0.41). This is not in agreement with the results of Ayoub et al. in which the incidence of VF in the lidocaine group was significantly lower than both amiodarone and control groups.[7] Recently, Mauermann et al. reported that neither amiodarone nor lidocaine reduced the incidence of ventricular fibrillation in patients undergoing a variety of cardiac surgical procedures.[23] However, amiodarone decreased the number of shocks required to terminate ventricular fibrillation. These results were compatible with the results of our study.

Many factors might contribute to the incidence of VF such as preoperative and intraoperative acidosis, hypoxia, and potassium level. In this study we evaluated these metabolic abnormalities for all three groups before cross clamp, two times during CPB, after removal of ACC, and three times during ICU stay. Moreover, any history of MI, concomitant valve disease, use of beta blocker, type of cardioplegia, and aortic cross clamp time might influence the incidence of VF. In our study there was no statistical difference in these predisposing factors among the three groups. Therefore, the incidence of RVF could be attributed to the administration of amiodarone, lidocaine, and placebo, respectively.

The current trial has some limitations. First, the mean left ventricular ejection fraction was normal. Thus, it is unclear whether our results are applicable to patients with decreased left ventricular function. Second, the dose of administered amiodarone may not have been high enough to achieve therapeutic tissue levels given the added circulatory volume of the CPB circuit.

Conclusion

In conclusion, the present study demonstrated lower incidence of VF after ACC release in amiodarone and lidocaine groups compared to the placebo group and in amiodarone group compared to lidocaine group; these results did not achieve statistical significance (P = 0.41).

Conflict of Interests

Authors have no conflict of interests.

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Designing and standardizing a questionnaire for evaluating knowledge, attitude, and practice of Iranian adults with cardiovascular diseases about oral health

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Original Article

Abstract

BACKGROUND: Cardiovascular diseases are the most common cause of death in Iran. Moreover, periodontal diseases are very common in our country. In this study, we have designed a standardized questionnaire for evaluating knowledge, attitude, and practice (KAP) of Iranian adult patients with cardiovascular diseases about oral health.

METHODS: For designing and standardizing a self-administered questionnaire, we performed a cross-sectional pilot study on 51 cases with periodontal complaints. A dentist carried out the physical examination to determine oral health indicators. Twelve experts and ten lay people of the target population answered questions about validity. Cronbach's alpha, factor analysis, and Pearson correlation coefficients were used in the analysis.

RESULTS: The cases of this pilot study were middle aged, with moderate financial and health status, but low oral health and educational level. Debris score was correlated with all other physical exam findings except decay, missing, and filled (DMF). Reliability was 0.826 according to Cronbach's alpha score. Face validity was higher than 80%. Content validities of the whole of the questionnaire were 85.98% for clarity, 78.05% for relevancy, 85.16% for simplicity, and 82.32% for consistency of each question with the question set. Factor analysis showed that 15 components explain 74% of the total variance.

CONCLUSION: This questionnaire is culturally adjusted and appropriate for our community, valid and reliable, and sufficiently estimates the variance of the oral health status. It can be used as a standard tool in further studies in adult population of the Iranian middle aged patients with low level of education and moderate socioeconomic status.

Keywords: Questionnaires, Reproducibility of Results, Validation Studies, Validity, Reliability, Oral Health, Periodontal Diseases

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Introduction

Cardiovascular diseases are the most common cause of death in Iran.[1] Periodontal diseases are also very common in our country.[2-3] A recent systematic review showed that there are limited studies on the efficacy of oral health promotion activities in patients with cardiovascular diseases. Moreover, it stated that efforts related to oral health promotion seem to improve periodontal health and change endothelial function in the short term. However, it is not clear whether these effects can result in reducing the risk of secondary cardiovascular events yet.[4]

It is also undetermined whether the association between oral and cardiovascular diseases is real (causal) or confounded by unknown/unmeasured factors. A recent prospective genetic study among

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twins showed evidence of shared genetic factors between cardiovascular disease and dental/periodontal disease. It stated that common pathogenetic mechanisms exist between poor oral health and cardiovascular disease.[5] However, it seems that dental health behavior and cardiovascular diseases share a common behavioral background.[6] Therefore, this association is mostly explained by confounders, particularly those relating to health behavior.[7]

There are some questionnaires about knowledge, attitudes, and practice of different persons (healthy appearing or patients) in different subgroups like school children, pregnant women, diabetics, hypertensive and obese patients, health care workers, and even dentists about oral health.[8-13] However, in this study we designed an Iranian version of this type of questionnaire in adult Iranian patients with cardiovascular diseases and standardized it.

Even if there is no association between oral health and cardiovascular disease, both have common risk factors. Therefore, policy makers' awareness of the patients' knowledge, attitude, and practice (KAP) will enable them to plan health programs for disease prevention and raise the health status of the patients as much as possible in a better way (by understanding the exact weak points of the patients according to KAP study).

Within the KAP model we are searching to understand in which subjects the knowledge of people is not sufficient and which interventions can help us rapidly improve the attitudes and practice of our target population to increase their oral health situation.

By this study, we have developed and standardized a questionnaire for evaluating knowledge, attitude, and practice of Iranian adult patients with cardiovascular diseases about oral health which can be used as a standard tool in further studies in this field. Before this study, there was no Iranian questionnaire with psychometrics properties for this population.

Materials and Methods

Study population

This project consists of different steps for producing and standardizing a questionnaire about KAP of patients with cardiovascular diseases about oral health. After designing the questionnaire, in the second step, 12 experts were involved to ensure the validity of the questionnaire. In the third step, as a cross-sectional study, 51 cases (39-73 years old, 32 males and 19 females) with cardiovascular diseases, 29 hospitalized and 21 outpatient cases, answered the questions in a self administered manner. This step was followed by a reliability analysis to determine internal consistency of the items with each other and reduce the items which do not have consistency with other items. Finally, a factor analysis was done on 150 cases separately for confirming the grouping of items.

Designing and standardizing the questionnaire

Item generation

After a thorough search in the literature, we planned two focus group discussions and one expert panel in order to design our flowchart for characterizing main domains of our KAP survey. Then, we detailed our main domains to some questions. Participants of the focus groups were 16 experts in related fields, which consisted of dental diseases, cardiovascular disease, epidemiology, community medicine, psychology, and psychiatry. Sessions were held at the Dental Implants Research Center of Tehran University of Medical Sciences, Tehran, Iran, between May 2011 and July 2011. They were selected based on a small assessment by researchers of this study.

Item modification

According to expert opinions, we changed the face and content of some questions. A pilot study on 51 persons was done to help us modify the structure and content of the primary questionnaire. Choices of each question were also revised according to the different answers to questions in the pilot study.

Item reduction (Factor analysis)

Factor analysis provides a better understanding of which variables form a "relatively coherent subset, independent of others".[6] We performed this analysis to see whether our main domains (knowledge, attitude, and practice), which were categorized by this analysis, were consistent with our primary pattern in which we first categorized them. We wanted to confirm our primary flowchart of the questionnaire in this way.

Item standardization

Reliability: Internal consistency reliability (Cronbach's alpha), which measures the extent that the questions both in each domain and also in all three main parts (knowledge, attitude, and practice) tap a particular concept, was determined according to the pilot study.[14]

Face validity: A separate sample of 10 lay cases of cardiovascular diseases from the target population of interest and 12 experts in the field of dental diseases and/or designing the questionnaire reviewed the questionnaire and answered the question: "How well do you think the questionnaire

measures knowledge, attitude, and practice of a patient with cardiovascular diseases about oral health status"? They responded using a 5-point Likert scale from 1 (not at all) to 5 (very well).

Content validity: The content validity of the final questionnaire was determined according to the clarity, relevancy, simplicity, and consistency of each question with the questions set from 12 experts in the field of dental diseases (6 persons) and methodologists (6 persons). They evaluated the instrument for important deletions or inappropriate choices of items.

For decreasing the prestige bias, demographic variables were inserted at the end of the questionnaire. For quality assurance, there was also a guidance form for the examiner to know how everybody should be examined.

Data collection

Subjects answered the questionnaire of knowledge, attitudes, and practice about oral health and its association with cardiovascular diseases. Each questionnaire was evaluated for missing data as soon as getting them from the patients. In case of need, the questionnaire was returned in order to be completed by the interviewees.

Then, an expert dentist carried out the physical examination to determine oral health indicators.[15] He defined the indices oral hygiene, debris, calculus, periodontal disease, and decay, missing, and filled (DMF) in addition to exam for presence and extent/severity of gingivitis, periodontitis, plaque, artificial teeth, loosed teeth, and gingival bleeding. One assistant helped assessment of the files of the hospitalized patients for completing demographic variables.

Demographic variables

Age, gender, height, weight, marital status, education level, job, financial status, dental insurance, living area (rural/urban), smoking behavior, and diet were among our demographic variables.

Physical examination

After oral examination of each case by an expert dentist, the dentist recorded the below mentioned indices.

The Oral Hygiene Index is composed of the combined Debris Index and Calculus Index, each of these indices is in turn based on 12 numerical determinations representing the amount of debris or calculus found on the buccal and lingual surfaces of each of three segments of each dental arch.[16]

Debris Index = (The total of the upper and lower buccal scores) + (The total of the upper and lower lingual scores) / (The number of segments scored).[16]

Calculus Index = (The total of the upper and lower buccal scores) + (The total of the upper and lower lingual scores) / (The number of segments scored).[16]

The average individual or group debris and calculus scores are combined to obtain Oral Hygiene Index.[16]

Periodontal disease index (Ramfjord's Periodontal Index) is a thorough clinical examination of the periodontal status of six teeth, with an evaluation of the gingival condition, pocket depth, calculus, and plaque deposits, attrition, mobility, and lack of contact. Individuals with clinically normal gingiva have an index of 0 to 0.2. The index reaches a maximum of 8.0 in persons with severe terminal destructive periodontitis.[16]

DMF index (decayed, missing, filled) is a technique for managing statistically the number of decayed, missing, or filled teeth in the oral cavity. Analysis may be based on the average number of DMF teeth (sometimes called DMFT) per person or the average number of DMF tooth surfaces (DMFS).[16]

Data analysis

We calculated the internal consistency of the questionnaire using Cronbach's alpha coefficient. Factor analysis was done for data reduction and grouping the related variables in conceptually similar and statistically related groups. Extraction method was the principal component analysis. Varimax rotation method was used, and we extracted factors based on Eigenvalue greater than 1. Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were used, and cut off point for loading on each factor was 0.3.

We used mean ± SD for expressing quantitative variables, and correlation test with Pearson coefficient for assessing the relation between these variables.

For calculating Item Content Validity Index (I-CVI), the average number of experts who believed that item was desirable/completely desirable was calculated and expressed as percentage. Scale Content Validity Index (S-CVI) for each one of clarity, relevancy, simplicity, and consistency was calculated as the average of items which our experts believed are desirable/completely desirable.

Ethics

All subjects signed an informed written consent before entering the study. This project is reviewed and accepted by the Ethics Committee of the Dental Implant Research Center with the code number: 90-03-104-17668.

Results

The final questionnaire consisted of five parts (Appendix 1):

1. Nine questions about knowledge,
2. Eleven questions about attitudes,
3. Twenty-one questions about practice,
4. Demographic variables
5. Physical examination part

Demographic characteristics of study participants

Fifty one subjects were assessed in the pilot study. They were middle aged and most of them had moderate physical activity, moderate financial status, moderate health status, but low educational level (Table 1).

Table 1. Demographic characteristics of participants in item reduction part of the study

Characteristic	Mean ± SD	n (%)
Age (year)	54.20 ± 8.10	
Weight (kg)	78.40 ± 12.60	
Height (cm)	166.20 ± 9.10	
BMI (kg/m^2)	28.50 ± 4.50	
Home area (m^2)	116.40 ± 53.50	
Number of rooms	2.26 ± 1.05	
Number of family members	4.30 ± 1.80	
Monthly income (Rials)*	5250000 ± 2760000	
Marital status†		
Married		46 (90.2)
Not married		5 (9.8)
Occupation status		
Retired		8 (15.7)
Unemployed (free work)		11 (21.6)
Household		16 (31.4)
Education		
Illiterate		14 (27.5)
Primary		19 (37.3)
Secondary		9 (17.7)
University		6 (11.8)
Financial status		
Good		4 (7.8)
Moderate		35 (68.6)
Poor		11 (21.6)
Dental insurance coverage		
Yes		5 (9.8)
No		46 (90.2)
Smoking		
Current smoker		10 (19.6)
Ceased		9 (17.6)
Not at all		28 (54.9)
Hookah smoker		2 (3.9)
On medication		
Yes		41 (80.4)
No		10 (19.6)
General health status		
Good		4 (7.8)
Moderate		40 (78.4)
Poor		3 (5.9)
Living area		
Urban		45 (88.2)
Rural		6 (11.8)
Diet†		
Traditional		33 (64.7)
Mediterranean		11 (21.6)
Others		7 (13.7)
Physical activity		
Good		6 (11.8)
Moderate		26 (51)
Poor		18 (35.3)

* At the time of data collection each dollar was equivalent with 12260 Rials; † due to missing values, percentages do not reach 100%; In addition, in some variables, only important choices are mentioned; BMI: Body mass index

Psychometric properties

Reliability

Cronbach's alpha score, measuring the internal consistency of questions, was 0.826. Its value in each domain is shown in table 2.

Face validity

All experts rated the option (answer) 4 or higher except one person, producing an overall mean of 4.0 (from total score of 5). All lay experts also rated 4 or higher except two experts and the average was 4.0 in these 10 lay persons.

Content validity

The characteristics of the content validity of the whole questionnaire were clarity 85.98%, relevancy 78.05%, simplicity 85.16%, and consistency of each question with the questions' set 82.32% (Table 3).

Factor analysis

KMO was 0.39 and Bartlett's test of sphericity was significant ($P < 0.001$) which shows that our variables are related and therefore suitable for structure detection. Extraction communalities are estimates of the variance in each variable accounted for by the components. Our communalities were all above 0.62 and most of them above 0.75. Therefore, all are high, which indicates that the extracted components represent the variables well and we do not need to extract another component. Exploratory varimax rotations and discarding of redundant items resulted in 15 component solutions explaining 74% of the variance in KAP of the subjects.

Findings of the pilot study about main outcomes

Table 4 shows that the situation of both KAP status and oral health is not good.

As table 5 shows, Debris Index was correlated with all other physical exam findings except DMF and all KAP parts and DMF were not related with any other index except practice of participants.

Table 2. Internal consistency of the questionnaire and its different domains

Domains	Cronbach's alpha
Knowledge	0.762
Practice	0.729
Attitude	0.700
All together	0.826

Table 3. Percentage of content validity according to different domains

Domains	Clarity	Relevancy	simplicity	Consistency
Knowledge	85.61	78.79	82.58	75.76
Practice	85.71	76.98	87.30	83.73
Attitude	87.04	79.63	83.33	87.04

Table 4. Results of physical findings and knowledge, attitude, and practice (KAP) questionnaire

Variables	Score (Mean ± SD)
DMFS	48.6 ± 29.7
PPI	4.1 ± 3.5
Debris Index	2.2 ± 1.0
Calculus Index	2.6 ± 1.1
OHI	4.6 ± 1.8
Knowledge score*	57.8 ± 27.8
Attitude score*	46.4 ± 22.7
Practice score*	43.3 ± 18.1
Total score*	46.8 ± 17.3

* Maximum possible score for knowledge, attitude, practice, and total score were 100; DMFS: Decayed, missing, and filled tooth surfaces in a person's mouth; PPI: Periodontal disease index; OHI: Oral hygiene index

Discussion

The questionnaire of KAP study in patients with cardiovascular diseases which was designed in this study had acceptable indices of a standard questionnaire.

About I-CVI and S-CVI, all percentages higher than 80% are considered as the minimum acceptable for a new tool.[17-20] Therefore, most of them are acceptable and such a questionnaire can be used in similar Iranian patients with cardiovascular diseases. It may also be used in other Iranian subjects who do not have significant demographic, clinical, and other characteristic differences which affect the validity of this questionnaire or force us to re-validate this questionnaire for that group.

As table 4 shows, mean knowledge score is higher than 50% (57.8%) of maximum possible score. However, attitude is lower than 50% (46.4%) and practice is the worst (43.3%) in comparison with knowledge and attitude. This finding is compatible with what we expect when comparing these domains in different studies.[21-24] This is also in line with the results of table 5 which shows that practice score is more highly correlated with the total score. As we expected, the subjects' knowledge and attitude are far from their practice, and practice is a more suitable index showing the total situation of each subject.

Debris Index, as the only one which is correlated with all other physical exam findings and all parts of KAP, can be evaluated when we do not have sufficient time or budget to evaluate similar group of subjects for KAP and/or oral health status. It can also be of interest as a proxy for different parts of oral health and KAP of subjects when there is an obstacle for evaluation of each of these variables. On the other hand, DMF is not a good indicator of the situation of the tooth, and knowledge and

Table 5. Correlation between different parts of knowledge, attitude, and practice (KAP) and physical exam findings

	DMFS	Debris Index	Calculus Index	OHI	Knowledge score	Attitude score	Practice score
Debris Index							
Calculus Index		r = 0.561*					
OHI		r = 0.851*	r = 0.618*				
Knowledge score		r = -0.285‡					
Attitude score		r = -0.400§			r = 0.487*		
Practice score	r = -0.262§	r = -0.440*		r = -0.281‡	r = 0.392§	r = 0.382§	
Total score		r = -0.503*		r = -0.324‡	r = 0.750*	r = 0.740*	r = 0.838*

DMFS: Decayed, missing, and filled tooth surfaces in a person's mouth; OHI: Oral hygiene index
* P < 0.001; § 0.001 < P < 0.01; ‡ 0.05 < P < 0.01

attitude of the patients about oral health, but a good indicator of the practice of the patients. Those with higher score of DMFS have a lower attitude score.

Factor analysis confirmed that there are no limited numbers of questions that can explain most of variance of the questionnaire. Fifteen factors explained 74% of variance; this shows that we have truly split the different parts of KAP into subtitles with least overlap. Factors were related to the same parts of KAP. In our study, the low value of KMO shows factor analysis may not be so useful for our data; however, significance level of Bartlett's test was less than 0.05 which indicates that a factor analysis may be useful with our data. It means that, although our sample size is not sufficient for factor analysis, factor analysis can run for such data. Maybe, by higher sample of the patients, indices of factor analysis improve to a higher degree.

Reliability of subscales was higher than 0.7 and of the total questionnaire higher than 0.8 which shows high internal consistency of the items.

In comparison with a similar study we performed for standardizing KAP of Iranian medical specialists about viral hepatitis, this study has higher reliability, comparable face validity, and lower content validity.[25] The results of factor analysis were also better in the present study.

Conclusion

We believe that this questionnaire which is culturally adjusted and appropriate for our community, valid and reliable, and sufficiently estimates much of the variance of oral health status, can be used as a standard tool in further studies in Iranian middle aged patients with cardiovascular diseases who have low level of education and moderate socioeconomic status.

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Conflict of Interests

Authors have no conflict of interests.

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Impacts of fresh lime juice and peel on atherosclerosis progression in an animal model

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Original Article

Abstract

BACKGROUND: The main protective role of antioxidants in the progression of atherosclerosis has been shown in some studies. Therefore, this project evaluated the effects of Citrus aurantifolia (Christm) juice and peel on antioxidant activity and atherosclerosis progression in rabbits receiving a hypercholesterolemic diet.

METHODS: Forty white New Zealand male rabbits were randomly allocated to four groups. All groups were on hypercholesterolemic diet for two months. While the first group was considered as the hypercholesterolemic control, groups 2 and 3 (intervention groups) received 5 ml/day lime juice and 1 g/day dried lime peel powder, respectively. Group 4 was fed a normal diet (normal control). Before and after the study, weight was measured and a fasting blood specimen was taken from the rabbits. Serum lipids analyses and antioxidant activity evaluations were then performed. The rabbits' aorta and coronary arteries were separated and the presence of fatty streaks was studied.

RESULTS: Comparing to the hypercholesterolemic control group (-25.2 ± 7.0), only the plasma total antioxidant capacity change was significantly more in rabbits supplemented with lime juice (16.3 ± 14.7) and peel (8.6 ± 7.1) ($P = 0.008$). The presence of fatty streaks in coronary arteries and aorta of the intervention groups [juice (0.2 ± 0.01); peel (0.0 ± 0.00)] was significantly decreased compared to the hypercholesterolemic control group (1.2 ± 0.4) ($P < 0.001$).

CONCLUSION: Based on our findings, Citrus aurantifolia peel and juice increase plasma antioxidant capacity in rabbits, and can thus prevent or decelerate the process of atherogenesis. However, lime peel is more effective than lime juice.

Keywords: Animal, Atherosclerosis, Atherogenic Diet, Fatty Streak, Intervention, Lime

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Introduction

Atherosclerosis is a leading cause of mortality and morbidity worldwide.[1] Hypercholesterolemic diet, on the other hand, is a main factor in initiation and progression of atherogenesis.[2] Moreover, the etiology of several chronic diseases, including coronary artery disease and stroke is thought to be associated with oxidative stress.[3] Accordingly, the protective role of antioxidants in progression of atherosclerosis has been shown in some in vitro and in vivo studies.[4]

Scientists have been long seeking effective components to prevent the atherosclerotic process. Although, research on the anti-atherogenic effects of fruits and vegetables has found that their content of various bioactive compounds with high antioxidant capacity seems to protect the body from the harmful effects of oxidative stress.[5-9]

Despite the proven benefits of some fruits and vegetables in this field, evidence for the impact of some others, like citrus fruits, is less consistent.[10]

As rich sources of dietary fiber, vitamin C, phenolic components, and flavonoids, citrus fruits are believed to have potential health-promoting properties.[11] Limonoids, the major cause of bitterness in citrus juice, have been reported to

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possess substantial antioxidant and anticancer activities.[12] In addition to individual actions of citrus antioxidants, a large number of studies have indicated the existence of cooperative/synergistic interactions among antioxidants in plasma.[13,14]

Citrus aurantifolia (Christm) is one of the most popular citrus fruits throughout the world whose anti-atherogenic effect has not yet been ascertained. On the other hand, the antioxidant-rich lime peel is wasted and only lime juice is consumed. Therefore, in this project, using an animal model, the effects of fresh Citrus aurantifolia (Christm) juice and peel on antioxidant activity and atherosclerosis progression were studied.

Materials and Methods

Animals

Forty white male New Zealand rabbits (mean body weight: 2.0 ± 0.3 kg) were purchased from the Pasteur Institute (Karaj, Iran). Before the experiment, the animals were kept in a laboratory for three weeks to allow them to adapt to laboratory conditions. They were then randomly allocated into four equal groups. Animals in group 1 (hypercholesterolemic control group) received only a hypercholesterolemic diet. Groups 2 and 3 were fed a hypercholesterolemic diet supplemented with 5 ml of fresh lime juice and 1 g of dried lime peel powder, respectively. Rabbits in group 4 were normal controls that received a normal diet. The study lasted for 60 days. The study was approved by the ethics committee of Isfahan Cardiovascular Research Center (Isfahan, Iran) which is a member of the Office for Human Research Protections, US Department of Health and Human Services. The animals were handled according to the guidelines of Isfahan University of Medical Sciences for Laboratory Animal Sciences (Isfahan, Iran).

Fruit collection

Fruits of Citrus aurantifolia were collected from Shiraz gardens (Fars Province, Iran) during the fruiting period in 2007. The fruits were authenticated by a botanist at the Department of Biology, School of Sciences, Isfahan University (Isfahan, Iran). A voucher specimen of the fruits is available at the herbarium of Isfahan University (ID: 5527).

Fruit peels were separated and dried at room temperature (in the shade) for four days. The dried peels were ground by an electric blender. The fruit juice was prepared by squeezing fruits exactly before consumption.

Blood analyses

Before and after the interventions, fasting blood samples were obtained from the rabbits' hearts for serum lipid analyses and plasma total antioxidant capacity measurement. Serum lipids were measured by an auto-analyzer (Hitachi 902) using Pars-Azmoon kits (Iran).

In order to determine plasma total antioxidant status, red blood cells (RBCs) were acquired from healthy voluntary donors. After removal of plasma and buffy coat, the RBCs were washed with phosphate buffered saline (PBS) (150.0 mM NaCl + 1.9 mM NaH_2PO_4 + 8.1 mM Na_2HPO_4; pH = 7.4) three times. They were then kept in PBS for subsequent analyses. 20 µl RBC suspension was incubated in a shaking bath for 10 min with 5 µl plasma objected for total antioxidant capacity measurement. After adding 2,2'-azobis (2-amidinopropane) dihydrochloride (APPH) (70 mM in PBS) and two hours of incubation, the suspension was centrifuged at 2500 rounds per minute for 10 minutes. The extent of hemolysis was spectrophotometrically evaluated (Shimadzu UV 3100, Japan) at 540 nm as hemoglobin (Hb) released from cells in the supernatant.[15]

The radical-scavenging activities of the plasmas, represented as inhibition percent of APPH, were calculated according to the following formula:

Inhibition percent = [1-(ODT/OD)] × 100

Where, ODT and OD were the absorbance values of the tested plasma and control after two hours of incubation, respectively.

The rabbits were sacrificed with pentobarbital (60 mg/kg) after two months and their aorta, coronary arteries, liver, and stomach were removed and preserved in formalin solution (10%) until pathologic investigations. Coronary artery and aortic specimens were sectioned and prepared using particular histological methods. After staining with hematoxylin, they were assessed for the presence of fatty streaks by a pathologist. Atherosclerotic thickness was evaluated on a scale of 1-4 as described by Chekanov.[15]

Statistical analysis

All analyses were performed using SPSS for Windows (version 15.5; SPSS Inc., Chicago, IL, USA). Results were expressed as the mean ± SD for fatty streak grade, and plasma antioxidant capacity and serum lipids changes. After the intervention, concentration of serum biomarkers was compared with before the intervention in each group by Wilcoxon test. Moreover, comparison of serum biomarker mean levels of other three study groups with hypercholesterolemic control group was done by the Mann-Whitney U test. Kruskall-Wallis test

was used to compare fatty streak percentage and also serum biomarkers between study groups. P values of less than 0.05 were considered significant.

Results

Table 1 shows comparison of serum lipids and plasma total antioxidant capacity mean level between before and after the intervention in each group.

Except for the normal diet group, for other groups, a significant increase was observed in serum lipids after the interventions (P < 0.02). However, plasma antioxidant capacity significantly decreased after the intervention only in the hypercholesterolemic group (P = 0.017), and in other groups it increased but not significantly.

The comparison of serum lipids and plasma total antioxidant capacity change during the study period between the groups is presented in table 2. There were no significant differences in the change of serum total cholesterol (TC) (P > 0.05), high density lipoprotein cholesterol (HDL-C) (P > 0.55), and triglyceride (TG) (P > 0.14) levels between the hypercholesterolemic group and the lime peel or juice consumers. However, hypercholesterolemic controls and rabbits supplemented with lime juice were significantly different in the mean changes in serum low density lipoprotein cholesterol (LDL-C) levels (P = 0.04). Compared to the hypercholesterolemic group (group 1), the plasma total antioxidant capacity increase was significantly higher in the lime juice and lime peel administrated rabbits (groups 2 and 3) (P < 0.05). Serum LDL-C levels had significantly higher increments in rabbits receiving lime juice than in those receiving peel (P = 0.043). The opposite was true in the case of plasma antioxidant capacity increase (P < 0.05).

The stage of fatty streak in coronary arteries and aorta of groups 2 and 3 was significantly decreased compared to the hypercholesterolemic group (P < 0.001) (Table 3).

Table 1. Comparison of serum lipids and plasma total antioxidant capacity mean level between before and after the intervention in each group

Serum marker	Hypercholesterolemic diet		Hypercholesterolemic diet + lime juice		Hypercholesterolemic diet + lime peel		Normal diet	
	Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
TC (mg/dl)	45.9 ± 10.2	987.0 ± 227.2*	49.6 ± 5.8	931.6 ± 197.7*	48.5 ± 9.9	999.5 ± 106.1*	68.6 ± 40.9	69.4 ± 38.2
LDL-C (mg/dl)	15.0 ± 5.7	279.9 ± 167.2*	17.6 ± 3.9	454.1 ± 112.3*	16.0 ± 7.4	299.4 ± 121.7*	33.2 ± 35.8	29.2 ± 36.4
HDL-C (mg/dl)	14.2 ± 5.1	110.0 ± 19.7*	15.1 ± 5.2	114.4 ± 28.8*	15.6 ± 5.1	117.6 ± 26.3*	17.6 ± 4.0	23.8 ± 7.4
TG (mg/dl)	59.2 ± 25.4	216.1 ± 267.8*	54.3 ± 13.8	96.7 ± 23.8*	39.6 ± 6.8	138.6 ± 162.7*	67.0 ± 33.7	81.8 ± 18.5
Antioxidant capacity (%)	62.3 ± 19.1	35.0 ± 15.2*	31.7 ± 8.3	40.2 ± 25.0	57.9 ± 10.9	67.1 ± 16.1	48.9 ± 4.2	47.8 ± 5.1

* Significant difference between before and after the intervention (P value of Wilcoxon test < 0.02); TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; TG: Triglyceride

Table 2. Comparison of the mean change in serum lipids and plasma total antioxidant capacity during the study period between the groups

Parameter	Hypercholesterolemic diet	Hypercholesterolemic diet + lime juice	Hypercholesterolemic diet + lime peel	Normal diet	P∫
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
TG (mg/dl)	156.9 ± 246.6	42.1 ± 28.7	99.0 ± 162.9	14.8 ± 35*	0.110
TC (mg/dl)	941.1 ± 224.6	882.0 ± 194.4	951.0 ± 101.7	0.8 ± 12.6*	0.002
LDL-C (mg/dl)	264.9 ± 165.1	435.1 ± 114.2	283.4 ± 121.2Ł	-4.0 ± 10.8*	0.001
HDL-C (mg/dl)	95.8 ± 18.3	99.3 ± 26.0	102.0 ± 23.0	6.2 ± 7.9*	0.006
Total antioxidant capacity (%)	-25.2 ± 19.8	16.3 ± 32.8*	8.6 ± 21.3*	1.5 ± 1.2*	0.011

∫ P value of Kruskal-Wallis test; * Significant difference with hypercholesterolemic group (P < 0.05) (P value of Mann-Whitney U test); Ł Significant difference with lime juice users (P < 0.05) (P value of Mann-Whitney U test); TG: Triglyceride; TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol

Table 3. Comparison of mean fatty streak grade between the study groups

Group	Fatty streak stage Mean ± SD
Hypercholesterolemic diet	1.2 ± 0.040
Hypercholesterolemic diet + lime juice	0.2 ± 0.001
Hypercholesterolemic diet + lime peel	0.0 ± 0.000
Normal diet	0.0 ± 0.000
P*	< 0.001

* P value of Kruskal-Wallis test

No abnormality was observed in the stomach tissue or liver of rabbits who consumed lime peel or juice.

Discussion

The results of this study showed that consumption of lime (Citrus aurantifolia) peel or fresh juice could inhibit the process of atherogenesis. Meanwhile, the peel had better effects than the juice.

Hypercholesterolemic diet is considered to play a main role in atherosclerosis progression as it acts through increasing lipid profile, especially LDL-C.[16,17] Such an effect was also observed in our study. On the other hand, the etiology of aging and some diseases like cancer, atherosclerosis, coronary artery disease, stroke, ischemic injury, and inflammation has been reported to associate with reactive oxygen species.[18] Therefore, antioxidant compounds should improve them. Antioxidant vitamins, enzymes, and some poly-phenolic compounds comprise the human's total antioxidant defense system.[19] Fruits and vegetables are the main sources of bioactive and antioxidant compounds.

Flavonoids, one of the important bioactive components of vegetables and fruits, may contribute to protection against the diseases through enhancing the human immune system.[20] Several in vivo and in vitro studies have revealed the preventive effects of flavonoid contents of fruits and vegetables on oxidative stress.[21] Based on our findings, changes in the total serum antioxidant capacity were significantly higher in rabbits consuming lime juice or peel than in controls. This increase was significantly more in the juice users than in the peel consumers. Phenolic compounds (i.e. flavonoid content of lime peel and juice) should be responsible for the observed differences. In other words, lime peel and juice might have different types of flavonoids. Research has indicated that hesperidin, naringenin and eriocitrin exist in lime juice.[22] The peel, on the other hand, contains polymethoxylated flavones (PMF), limonoid, and diosmin.[23-25] The high ascorbic acid content of fresh lime juice can justify the significantly higher serum antioxidant capacity among rabbits supplemented with lime juice than those with lime peel.

Diets rich in fruits and vegetables have been suggested to be inversely related with the risk of various diseases.[26] For instance, seven-day consumption of red orange juice by non-diabetic patients with cardiovascular diseases could increase endothelial function and decrease inflammation.[27] We found that consumption of lime peel or fresh lime juice prevented atherosclerosis in rabbits with atherogenic diet. Although lime juice induced greater changes in serum antioxidant capacity than did lime peel, the latter caused significantly more reductions in fatty streak grade. This difference can again be related to the effects and absorption of each type of flavonoid. Unfortunately, we have no information on the type and amount of absorbed flavonoids.

Numerous studies have assessed the effects of separate bioactive components of lime juice and peel. Some have also investigated how the compounds play their atherogenic role. Yen et al. concluded that 5-demethyl-nobiletin (a flavonoid in citrus fruits) has antiatherogenic properties and inhibits monocyte-to-macrophage differentiation and foam cell formation. This flavonoid, and nobiletin may increase the expression of LDL-C receptor gene and simultaneously decrease diacylglycerol acyltransferase 2 (an acyl coenzyme A) expression.[28] A previous study reported a 70%-reduction in atherosclerosis among mice treated with naringenin.[29] Similarly, Lee et al. showed that naringin could prevent atherosclerosis through Phosphatidylinositol 3-kinase (PI3K)/Akt and mammalian target of rapamycin (mTOR)/p70S6K pathways (PI3K/AKT/mTOR/p70S6K pathway) repression, invasion, and migration, and the subsequent suppression of matrix metalloproteinase-9 (MMP-9) expression.[30] Eguchi et al. found nobiletin to suppress the activator protein-1 transcriptional activity and hence regulate atherosclerosis.[31] In another animal study, citrus juice inhibited atherosclerosis and decreased serum

TCho and TG. Ascorbic acid in a dose similar to citrus juice showed the same effect on atherosclerosis.[32] In a research on male New Zealand white rabbits receiving a hypercholesterolemic diet for eight weeks, Choe et al. suggested naringin to cause antiatherogenic effects as it decreased fatty streak formation and intercellular adhesion molecule 1 expression in endothelial cells.[33]

Another component of lime peel is pectin. Grapefruit pectin has been shown to inhibit hypercholesterolemia and atherogenesis. Such antiatherogenic effect may be related to a mechanism independent of cholesterol levels.[34] A research on pigs receiving grapefruit pectin indicated the same results.[35]

A limitation of the present study was not measuring cellular and molecular pathways that could cause such results. In addition, it was better to assess the specific effects of each flavonoid separately.

Conclusion

Although both fresh lime (Citrus aurantifolia) juice and peel could prevent atherogenesis through increasing serum antioxidant capacity, the antiatherogenic effect of the latter was significantly stronger. It is thought that the type and amounts of flavonoids in lime peel and juice are responsible for this difference.

Further research is warranted to clarify the effects of each flavonoid in lime juice and peel on atherosclerosis and cellular and molecular pathways.

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Conflict of Interests

Authors have no conflict of interests.

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Comparison of effects of soft margarine, blended, ghee, and unhydrogenated oil with hydrogenated oil on serum lipids: A randomized clinical trail

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Original Article

Abstract

BACKGROUND: Trans fatty acids (TFAs) are known as the most harmful type of dietary fats. Therefore, this study was done to compare the effects of some different oils including unhydrogenated, blended, ghee, and soft magazine with hydrogenated oil on serum lipid profile of healthy adults.

METHODS: This study was a randomized clinical trial conducted on 206 healthy participants of 20 to 60 years of age. Subjects were randomly divided into 5 groups and each of them was treated with a diet containing unhydrogenated oil, ghee, blended oil, soft margarine, or hydrogenated oil for 40 days. Fasting serum lipids were measured before and after the study.

RESULTS: Compared to hydrogenated oil, total cholesterol (TC) and triglyceride (TG) had a significant reduction in all groups, LDL-C declined in unhydrogenated oil and soft margarine groups, and apolipoprotein (Apo) B only in unhydrogenated oil group (all $P < 0.05$). However, there was a significant enhancement in ApoA of ghee oil ($P < 0.001$).

CONCLUSION: Consuming unhydrogenated oil, ghee, soft margarine, and blended oil had some beneficial effects on serum lipids.

Keywords: Clinical Trial, Dietary Fat, Commercial Oil, Lipid

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Introduction

Diets affect the occurrence, progress, and prevention of non-communicable diseases, including cardiovascular diseases (CVD), cancers, diabetes, and hypertension.[1,2] In the last few decades, the reduction of fat intake has been the major recommendation for decreasing CVD risk.[3] There is a great amount of evidence confirming that the type of dietary fat is more determinant in CVD development than its amount.[4] Saturated fatty acids (SFAs) cause an increase in serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels.[5]

In the past, the effects of fats on increasing plasma TC levels were estimated by their saturation degree.[4,6] However, the evidence obtained during this past decade indicates that the trans fatty acid (TFA) existing in hydrogenated oils not only increases LDL-C, TC, and apolipoprotein (Apo) B levels, but also decreases high-density lipoprotein cholesterol (HDL-C) and ApoA levels.[7] Therefore, TFAs are more harmful than SFAs.[8] Scientists at the Public Health School of Harvard University estimated that in the US in 2001, about 30,000 people died of CVD events caused by TFAs.[9] It was reported that the mean TFA content of hydrogenated oils produced in Iran was 34.6 ± 6.6% (Range: 22.5-46.2%), which is much higher than the World Health Organization recommendation.[10,11]

Thus, now TFA substitutions are needed to preserve the originality of and offer an appealing many packed food textures. Blending is one alternative method to partial hydrogenation for modifying the physical and functional characteristics of edible fats and oils.[12] This process can provide nutritional needs with improved oxidative stability for domestic cooking and deep-frying, while, unlike partial hydrogenation, it will not produce TFAs with low content of saturated fatty acids.[13]

Furthermore, ghee is produced from milk by traditional methods and usually called "yellow oil" or "Kermanshahi oil" in Iran. Although it is produced

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from animal fat and contains high amounts of SFAs and cholesterol, a few studies reported it was useful for decreasing LDL-C and increasing HDL-C.[14-16] In an animal study which was conducted in Iran, it has been observed that ghee oil consumption significantly increased HDL-C level, but did not have any significant effect on other serum lipids.[17] However, there is no precise scientific information on this issue in human subjects. Thus, there are some controversies about how ghee consumption and serum lipid profile are linked.[18,19] As TFAs are considered as the most harmful dietary fats, this study was conducted aiming to compare the effects of soft margarine, unhydrogenated, blended, and ghee oils with hydrogenated oil as a main source of TFA on serum lipids of Iranian adults.

Materials and Methods

Study design and sampling

This randomized clinical trial has been conducted on 249 healthy subjects aged 20-60 years in 2009. They were chosen from the Emam-Zaman Beneficiary Organization and consumed only hydrogenated oil in their diet. Normotensive, non-diabetic participants without cardiovascular diseases were invited to the study center. According to the sample size which was calculated 40 in each group and considering the dropout rate, at the beginning we invited 265 volunteers. After overnight fasting, venous blood samples were drawn at 7:00 to 10:00 am. Subjects with TC ≥ 240 mg/dl, triglyceride (TG) ≥ 400 mg/dl, LDL-C ≥ 160 mg/dl, or HDL-C ≤ 40 mg/dl, who also had body mass index (BMI) ≥ 35 were excluded. However, 16 subjects were excluded because of not meeting the inclusion criteria. Then, the remaining participants were divided into five groups of soft margarine, hydrogenated, unhydrogenated, ghee, and blended oils by simple randomization. Moreover, 43 participants were excluded from the study due to traveling, sickness, unwillingness to participate in the next sampling, or not complying with dietary recommendations. Therefore, 206 healthy subjects were included in the study. The flow chart of the study is presented in figure 1.

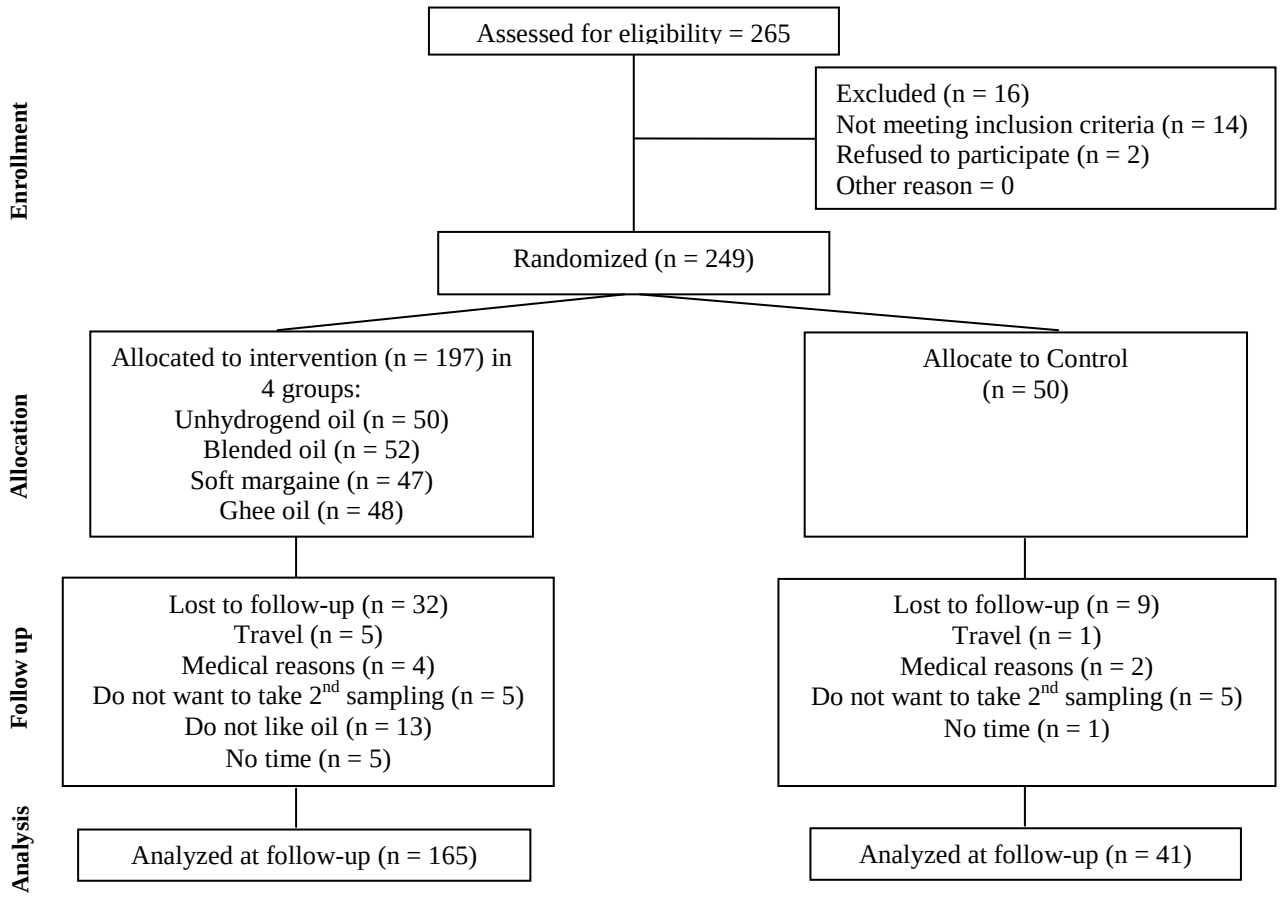

Figure 1. Flow chart showing number of eligible and excluded participants, number of participants allocated to intervention and control groups, and number of participants lost to post-text, as well as reasons for loss to follow-up

After signing informed written consents, the subjects were referred to the trained nutritionist to obtain socio-demographic characteristics, past medical history, and food habits by 24-hour recall questionnaire. Anthropometrical measurements were taken with shoes removed and the participants wearing light clothing. BMI was calculated by dividing the weight in kilograms to the square of height in meters. Eligible subjects who consumed hydrogenated oil, based on obtained food habits, were randomly divided into 5 groups. They took a diet consultation with the same amount of oil containing soft margarine, cooking and frying unhydrogenated oil, ghee, and blended or hydrogenated oils for 40 days.[20] In order to keep the type of oils similar among each group, oils were given to the subjects (every 10 days) by the project conductor. Ghee was provided from Bakhtiari nomads. Soft margarine and blended oil were provided from companies which were their only producers in Iran at that time. The commonest brands of unhydrogenated and hydrogenated oils were bought from the supermarket. Dietary recommendations were given to the subjects by the same dietitian, so that the only difference among the 5 groups was the kind of dietary oil. Oils were provided for all family members, about 20-30 gr per person, even if one of them was chosen in the study. Subjects were followed by phone every two weeks or during their referral to the study center for taking their oil.

Biochemical measurements

Blood samples were taken after the subject had been fasting for 14 hours. Serum lipid levels, including TC, TG, and HDL-C levels, were measured. TC and TG were determined by standard enzymatic method using special kits in Hitachi 902 autoanalyzer and using special kits (Diasys Diagnosis Inc., Holzheim, Germany) performed by Pars-Azmun (Tehran, Iran). HDL-C was measured enzymatically after precipitating the other lipoproteins with dextran sulphate magnesium chloride.[21] LDL-C was calculated by using the Friedewald formula. ApoA1 and ApoB100 were assayed by immunoturbidimetric methods (Diasys Diagnosis Inc., Holzheim, Germany) performed by Pars-Azmun. Direct measurement of LDL-C was performed with a turbidimetric method for those with TG ≥ 400 mg/dl.[22] Apolipoproteins A and B levels were determined by Merk kits. Blood samples were collected before and after the study, at Isfahan Cardiovascular Research Center laboratory, a WHO-collaborating center which meets the criteria of the National Reference Laboratory. The lipid profile changes were the primary endpoint of the study.

Ethics

This study was approved by the Research Council of Isfahan Cardiovascular Research Center and registered in the Iranian Randomized Clinical Trail Center by ID number of IRCT138905124497N1.

Statistical analysis

In the beginning of the study, the mean of age, BMI and serum lipids levels among the three groups were compared by one-way analysis of variance (ANOVA). Comparison of the frequency distribution was conducted using chi-square test based on gender and education level. Mean of serum lipid levels before and after the study were compared by paired t-test in each group. The comparison of changes in serum lipids and ApoA and B levels between 5 groups was done with two-way ANOVA test by adjusting for age, gender, and education level. The post-study mean serum lipid was compared with analysis of co-variances test by adjusting with age, gender, education, and before study serum lipids. P value less than 0.05 was considered significant.

Results

The study was performed on 206 subjects including 41, 43, 39, 42, and 41 subjects in hydrogenated, unhydrogenated, ghee, blended oil, and soft margarine groups, respectively. However, 43 subjects were excluded from the study due to traveling, sickness, unwillingness to participate in the next sampling, or not complying with dietary recommendations. Therefore, 206 healthy subjects were included in the study. Thus, the participation rate was about 82.7%. They included 88 men and 118 women with the mean age of 34.8 ± 11.4 years. As shown in table 1, there is no significant difference in mean of age, serum lipids, including TC, TG, LDL-C, HDL-C, ApoA, and ApoB levels, and also gender, educational, and marital status distribution between 5 groups in the beginning of the study. Table 2 demonstrates the comparison between mean of serum lipids and ApoA and ApoB levels before and after the study in each group.

In the hydrogenated oil group, TC increased and ApoA decreased significantly (P = 0.039 and P = 0.031, respectively). Unhydrogenated oil group had a significant reduction in TC, TG (P < 0.001), and ApoB (P = 0.003) and in the ghee group, ApoA significantly increased (P < 0001). Blended and soft margarine groups had a significant decline in TG (P = 0.010 and P < 0.001, respectively).

Except for LDL-C, and ApoA and ApoB levels, the comparison of the mean and percentage of

Table 1. Basic characteristics and serum lipids in the beginning of the study

Oil	Hydrogenated	Unhydrogenated	Ghee	Soft margarine	Blended	P
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
	n = 41	n = 43	n = 39	n = 41	n = 42	
Age (year)	32.8 ± 11.1	33.9 ± 11.6	35.4 ± 10.9	36.5 ± 12.2	36.6 ± 11.6	0.203
Body mass index (kg/m^2)	26.5 ± 4.4	26.1 ± 5.3	25.7 ± 4.6	26.4 ± 4.13	26.8 ± 6.8	0.821
Total cholesterol (mg/dl)	174.9 ± 23.4	176.7 ± 25.4	183.8 ± 30.9	174.0 ± 31.5	175.8 ± 29.4	0.734
Triglyceride (mg/dl)	134.1 ± 52.8	127.4 ± 42.1	125.0 ± 52.0	114. 9 ± 33.7	109.5 ± 26.1	0.268
HDL-C (mg/dl)	43.3 ± 7.5	45.3 ± 6.9	44.0 ± 5.6	45.3 ± 6.7	47.2 ± 6. 8	0.510
LDL-C (mg/dl)	104.7 ± 19.5	105.9 ± 22.6	114.8 ± 27.2	105.8 ± 28.7	108.6 ± 23.8	0.392
Apolipoprotein A (mg/dl)	129.9 ± 17.3	126.5 ± 17.4	122.0 ± 14.0	111.1 ± 150.0	123.4 ± 12.9	0.009
Apolipoprotein B (mg/dl)	92.3 ± 17.9	99.4 ± 20.2	98.4 ± 20.1	94.2 ± 19.9	91.5 ± 18.6	0.605
Gender (male) [n (%)]	17.0 ± 41.5	18.0 ± 41.9	16.0 ± 41.0	19.0 ± 46.0	18.0 ± 42.9	0.677
Illiterate [n (%)]	17.0 ± 41.4	15.0 ± 34.9	14.0 ± 35.9	16.0 ± 39.0	16.0 ± 38.2	0.042

HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

Table 2. Comparison of serum lipid before and after the study

Oil	Before	After	P
	Mean ± SD	Mean ± SD	
Hydrogenated			
Total cholesterol (mg/dl)	174.9 ± 23.4	178.6 ± 25.3	0.039
Triglyceride (mg/dl)	134.1 ± 52.8	137.5 ± 51.8	0.124
HDL-C (mg/dl)	43.3 ± 7.5	44.1 ± 7.0	0.233
LDL-C (mg/dl)	104.7 ± 19.5	107.1 ± 21.2	0.141
Apolipoprotein A (mg/dl)	129.9 ± 17.3	125.8 ± 13.3	0.031
Apolipoprotein B (mg/dl)	92.3 ± 17.9	95.7 ± 21.8	0.222
Unhydrogenated			
Total cholesterol (mg/dl)	176.7 ± 25.4	173.1 ± 26.2	< 0.001
Triglyceride (mg/dl)	127.4 ± 42.1	122.8 ± 41.6	< 0.001
HDL-C (mg/dl)	45.3 ± 6.9	44.3 ± 6.8	0.324
LDL-C (mg/dl)	105.9 ± 22.6	104.3 ± 24.4	0.387
Apolipoprotein A (mg/dl)	126.5 ± 17.4	127.8 ± 14.8	0.352
Apolipoprotein B (mg/dl)	99.4 ± 20.2	93.3 ± 20.5	0.003
Ghee			
Total cholesterol (mg/dl)	183.8 ± 30.9	183.5 ± 28.6	0.703
Triglyceride (mg/dl)	125.0 ± 52.0	122.6 ± 49.3	0.512
HDL-C (mg/dl)	44.0 ± 5.6	45.3 ± 7.6	0.244
LDL-C (mg/dl)	114.8 ± 27.2	113.7 ± 23.9	0.126
Apolipoprotein A (mg/dl)	122.0 ± 14.0	125.4 ± 13	< 0.001
Apolipoprotein B (mg/dl)	98.4 ± 20.1	98.7 ± 17.3	0.788
Blended			
Total cholesterol (mg/dl)	175.8 ± 29.4	173.5 ± 27.6	0.225
Triglyceride (mg/dl)	109.5 ± 26.1	95.5 ± 24.9	0.010
HDL-C (mg/dl)	47.2 ± 6. 8	47.4 ± 7.3	0.438
LDL-C (mg/dl)	108.6 ± 23.8	107.1 ± 23.5	0.451
Apolipoprotein A (mg/dl)	123.4 ± 12.9	120 ± 13.4	0.237
Apolipoprotein B (mg/dl)	91.5 ± 18.6	92.5 ± 15.4	0.507
Soft margarine			
Total cholesterol (mg/dl)	174 ± 31.5	169.4 ± 25.6	0.128
Triglyceride (mg/dl)	114.9 ± 33.7	108.2 ± 31.1	< 0.001
HDL-C (mg/dl)	45.3 ± 6.8	46.0 ± 6.0	0.345
LDL-C (mg/dl)	105.8 ± 28.7	101.8 ± 25.4	0.109
Apolipoprotein A (mg/dl)	111.1 ± 14.6	115.8 ± 21.2	0.099
Apolipoprotein B (mg/dl)	94.2 ± 20.0	94.1 ± 22.4	0.723

HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

serum lipids, changes with age and gender adjustment, revealed a significant difference among the three studied groups (Figures 2-3). TC, TG, and ApoB levels had a significant reduction in the unhydrogenated oil group when compared with the hydrogenated oil group ($P < 0.001$). In the ghee oil group, TG was significantly decreased, while ApoA had a significant increase ($P < 0.001$). Comparing with the ghee group, the unhydrogenated oil group had a significant reduction in HDL-C ($P < 0.05$).

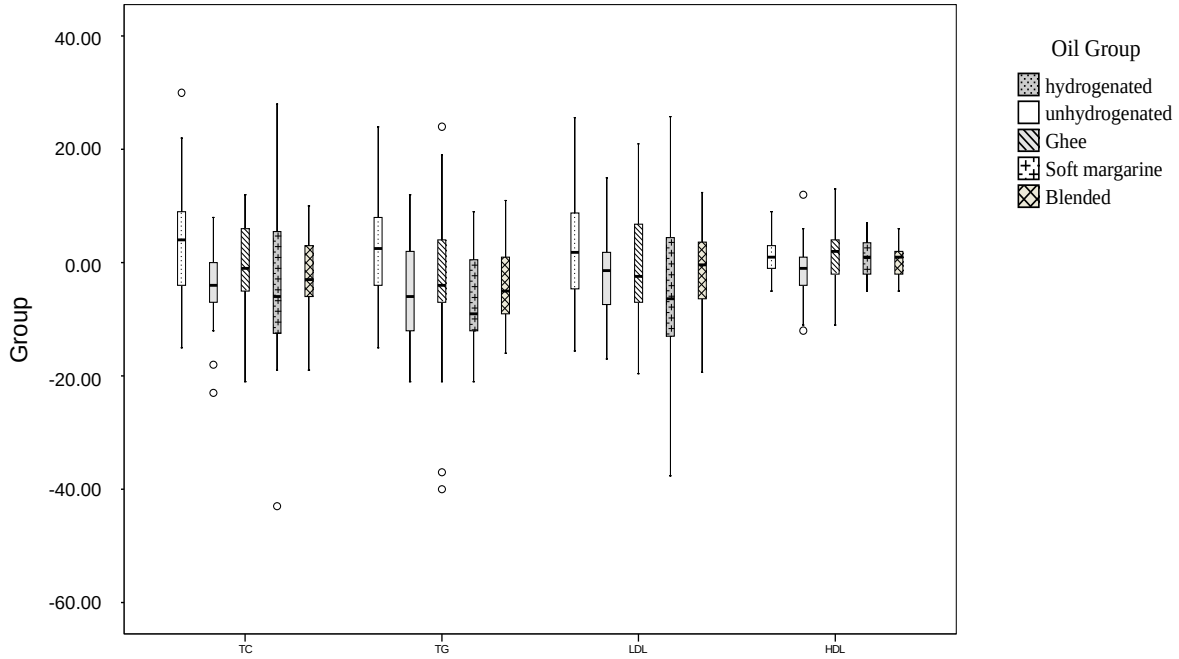

Figure 2. Comparison of the mean of serum lipid differences between unhydrogenated, ghee, soft margarine, blended, and hydrogenated oil groups
TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

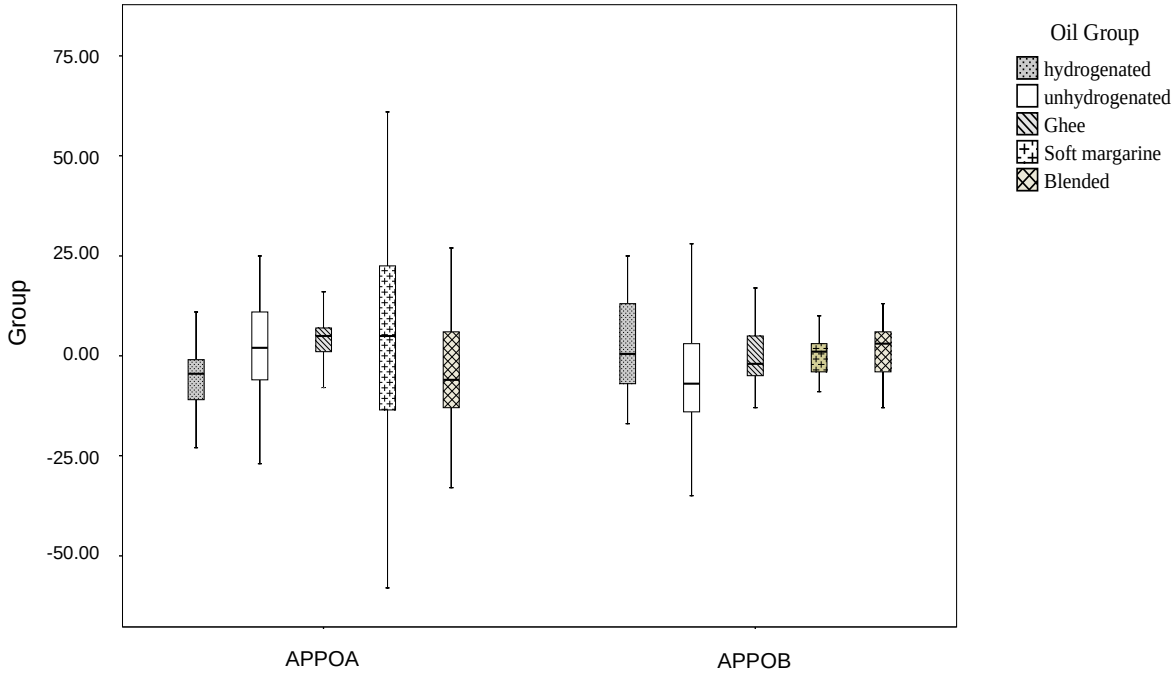

Figure 3. Comparison of the mean of apolipoprotein differences between unhydrogenated, ghee, soft margarine, blended, and hydrogenated oil groups
APPOA: Apolipoprotein A; APPOB: Apolipoprotein B

Table 3. Comparison of the adjusted mean of serum lipids and changes in intervention groups vs reference group after the intervention

Group	Post-study	Change	
	Mean ± SE	β*	95% (CI)**
Total cholesterol (mg/dl)			
Unhydrogenated	173.9 ± 1.2	-7.1[c]	-10.8-(-3.5)
Blended	175.2 ± 1.6	-5.9[b]	-10.1-(-1.7)
Ghee	177.8 ± 1.3	-3.3[c]	-7.1-0.5
Soft margarine	172.7 ± 1.6	-8.4[c]	-12.7-(-4.1)
Hydrogenated	181.1 ± 1.5	R†	-
Triglyceride (mg/dl)			
Unhydrogenated	117.7 ± 1.3	-8.4[c]	-12.6-(-4.1)
Blended	116.5 ± 1.8	-9.6[c]	-14.6-(-4.6)
Ghee	119.8 ± 1.5	-6.3[b]	-10.7-(-2.0)
Soft margarine	114.8 ± 1.9	-11.2[c]	-16.2-(-6.3)
Hydrogenated	126.1 ± 1.7	R	-
LDL-Cholesterol (mg/dl)			
Unhydrogenated	166.4 ± 1.3	-3.8 (2.0)[c]	-7.8-(-0.1)
Blended	106.7 ± 1.7	-3.5 (2.3)	-8.1-1.1
Ghee	107.7 ± 1.4	-2.5 (2.1)	-6.6-1.7
Soft margarine	104.0 ± 1.8	-2.5 (2.3)[a]	-10.8-(-1.5)
Hydrogenated	110.2 ± 1.6	0	-
HDL-Cholesterol (mg/dl)			
Unhydrogenated	43.6 ± 0.5	-1.5 (0.9)	-3.2-0.1
Blended	45.4 ± 0.7	-0.07 (0.9)	-2-1.9
Ghee	46.1 ± 0.6	0.6 (0.8)	-1.1-2.3
Soft margarine	45.7 ± 0.7	0.2 (1)	-1.7-2.2
Hydrogenated	45.5 ± 0.7	0	-
Apolipoprotein A (mg/dl)			
Unhydrogenated	126.3 ± 1.8	3.7 (2.9)	-1.9-9.4
Blended	120 ± 2.4	-2.6 (3.3)	-9.1-4
Ghee	120.1 ± 1.9	3.5 (2.9)[c]	2.4-9.4
Soft margarine	121.8 ± 2.6	-0.8 (3.5)	-7.8-6.2
Hydrogenated	122.6 ± 2.3	0	-
Apolipoprotein B (mg/dl)			
Unhydrogenated	90.5 ± 1.6	-8.2 (2.3)[b]	-13.2-(-3.2)
Blended	96.2 ± 2.1	-2.5 (2.9)	-8.2-3.3
Ghee	96.7 ± 1.7	-1.9 (2.6)	-7.1-3.2
Soft margarine	95.5 ± 2.2	-3.2 (2.9)	-8.9-2.7
Hydrogenated	98.6 ± 2.0	0	-

* ß: Regression coefficient of baseline serum lipids; ** 95% confidence interval; † Hydrogenated was considered as reference group; a: P < 0.05; b: P < 0.01; c: P < 0.001
SE: Standard Error; CI: Confidence interval; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

However TC and TG declined in blended oil and soft margarine groups significantly (P < 0.001 and P < 0.05, respectively).

The adjusted mean level of serum lipids after the intervention and the mean changes of serum lipids in each intervention group versus hydrogenated oil group are presented in table 3. As it shows the soft margarine group had the most significant reduction

in TC and TG (β ± SE: -8.4 ± 2.2; β ± SE: -11.2 ± 2.5, respectively) (P < 0.001). LDL-C had a significant reduction in unhydrogenated oil and soft margarine (β ± SE: -3.8 ± 2, P < 0.001; β ± SE: -2.5 ± 2.3, P < 0.05, respectively). ApoA had a significant increase only in the ghee group and ApoB declined significantly only in the unhydrogenated oil group (β ± SE: 3.5 ± 2.9, P < 0.001; β ± SE: -8.2 ± 2.3, P < 0.001, respectively).

Discussion

This study indicated that unhydrogenated oil can generally reduce serum lipid levels when compared with hydrogenated oil. However, changes in serum lipids, except for TG reduction and ApoA enhancement, were not significant when the ghee oil group was compared with the hydrogenated oil group (serum HDL-C levels had an insignificant increase). Moreover, blended oil and soft margarine as 2 new products of oil in Iran could reduce TC and TG.

Several studies have indicated that hydrogenated fat and/or TFAs could increase TC, TG, and LDL-C, decrease HDL-C, and enhance the LDL-C: HDL-C ratio.[16,23,24] However, the responsible mechanisms for these changes are complicated. It has been proposed that the serum lipid-raising effect of hydrogenated fat is due to either delayed LDL-C clearance or enhanced LDL-C production.[25] Matthan et al. have reported that hydrogenated oil decreased HDL-C, and raised LDL-C by increasing ApoA-I and decreased LDL ApoB-100 catabolism. Thus, it was indicated that damaging the cholesterol catabolism is responsible to a greater degree than decreasing its synthesis for the higher serum TC seen by intake of high hydrogenated and saturated fat diets.[26] However, Kelley et al. showed that a diet containing cotton seed oil could not modify serum lipids including TC, TG, LDL-C, HDL-C, ApoA and ApoB in comparison with a normal diet.[27]

The study by Al-Amoudi and Abu Araki indicated that a blend of the various specific vegetable oils improved serum lipid profiles due to a synergistic effect of various blending oils.[28] Enhancement oxidative stability and the synergistic effect of different vegetable oils might cause the serum lipid improvement in the blended oil group of the current study.

According to the study by Asgary et al. the average TFA contents in hydrogenated oils, and unhydrogenated cooking and frying oils produced in Iran were 35.2 ± 4.8%, 0.9 ± 0.3%, and 772.6 ± 0.8%, respectively.[29] Therefore, serum lipid modification by unhydrogenated oils seems reasonable in this study.

Ghee oil is an important dietary fat used in India and other South Asian countries, which contains high amounts of SFAs (about 59% of its whole fatty acids).[14,30] SFAs, except for stearic acid, increase serum TC.[8] Therefore, ghee oil, that is high in cholesterol and SFAs, is considered as harmful. On the other hand, ghee is a good source of oleic acid which is capable of protecting LDL-C particles from oxidation and prevents atherosclerosis.[14] Furthermore, according to Asgary et al. the average TFA content in ghee produced by Bakhtiari nomads (the kind of ghee that was used in this study) is 8.3 ± 0.7 which is 1.4 times less than the amount of existing TFA in hydrogenated oils.[29]

Kumar et al. study indicated that consumption of ghee in the diet, even with high intakes, does not increase serum lipids.[15] This animal study did not show any linking between ghee consumption and hypercholesterolemia and hyperlipidemia, which are considered to be risk factors for heart diseases. Interestingly, consuming increased levels of ghee reduced serum TC and TG levels.[15] Another idea was that there is a link between the consumption of anhydrous milk fat, such as ghee, and increased risk of heart diseases.[31] However, use of excess intake of ghee as a means for lowering serum TC is not recommended, but the study indicates that there is no reason for apprehension for consuming ghee in the diet, which is an age-old practice that is relished in Indian cuisine.[15] Mozaffarian et al. stated that substituting 8% of energy intake from TFA with SFA cause a decrease in CVD by modifying TC:HDL-C ratio.[32] Therefore, it confirms the suitable effect of ghee on serum lipid profile.

Limitation

As the subjects used the oils for cooking at home, blinding was not applicable. Thus, it was the limitation of this study.

Conclusion

Blended and soft margarine as two new kinds of oils in Iran had some beneficial effects on serum lipids. Furthermore, ghee was useful in modifying serum, including TG and HDL-C, and unhydrogenated oil and frying oil consumption resulted in a general reduction in serum lipids. Therefore, it can be said that ghee might be effective on serum lipid modification in metabolic syndrome, but it should not be forgotten that ghee, which is traditionally made from milk fat,

has high amounts of SFAs, and also its production method should be carefully supervised.

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

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Pivotal role of microRNA-33 in metabolic syndrome: A systematic review

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Review Article

Abstract

Metabolic syndrome (MetS) is a major public health concerns and increase in the incidence of MetS caused a rise in the rates of global morbidity, and mortality due to cardiovascular disease and diabetes. Lifestyle modification, a healthy diet, and pharmacological treatment and bariatric surgery are recommended in order to control this syndrome. Molecular mechanisms of metabolic disorders are essential in order to develop novel, valid therapeutic strategies. MicroRNA-33 plays imperative regulatory roles in a variety of biological processes including collaboration with sterol regulatory element-binding protein (SREBP) to maintain cholesterol homeostasis, high-density lipoprotein formation, fatty acid oxidation, and insulin signaling. Investigation of these molecules and their genetic targets may potentially identify new pathways involved in complex metabolic disease processes, improve our understanding of metabolic disorders, and influence future approaches to the treatment of obesity. This article reviews the role of miRNA-33 in metabolic syndrome, and highlights the potential of using miRNA-33 as a novel biomarker and therapeutic target for this syndrome.

Keywords: MicroRNA-33, Insulin Resistance Syndrome X, Regulatory Role

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Introduction

Metabolic syndrome (MetS) is characterized by the clustering of several risk factors such as dyslipidemia, hypertension, insulin resistance, and central obesity. Moreover, this condition increases the risk of cardiovascular disease (CVD) and type 2 diabetes mellitus.[1-5] MetS is considered as a major health problem, and presents an impressive therapeutic challenge.[1,3,5-10] The prevalence of MetS, based on the National Cholesterol Education Program (NCEP) definition, is high and has recently been estimated in adults around the world to be 15.5%, in the U.S. 23.7%, Russia 17.6%, and in Finland 13.7%.[11] Published data showed that the prevalence of MetS is relatively higher in the Middle East regain than other parts; for example, it is 66% in Oman, and 29.9% in Turkey, and in Iran it was reported from 24.1% to 38.9%.[5,12-14] Increase in the incidence of MetS has caused a rise in the rate of global morbidity, and mortality due to cardiovascular disease and diabetes.[15] Lifestyle modification, a healthy diet, and pharmacological treatment are recommended for controlling this syndrome.[1] However, the evidences showed that greater role of genetic determinates than environmental factors on incidence of MetS.[16-18] It is well documented that failure of function in up and down regulation of regulatory genes and enzymes lead to MetS.[19] However, there is controversy about the role of genes in up and down regulation; for example, genome-wide association studies have demonstrated numerous genes and regions, which are susceptible to individual MetS risk factors such as hypertension, obesity, and diabetes.[18,20-22] However, family studies did not find any genetic loci mediate clustering of MetS components.[16,23-25]

The most recent studies have discovered new molecules named microRNA, which play a crucial role in controlling metabolic and homeostasis pathways. Latest findings have demonstrated the remarkable role of small non-coding RNAs (microRNAs; 19–22 nucleotides) in the post-transcriptional regulation of a number of genes and their involvement in many pathological states, such as diabetes, atherosclerosis, and cancer.[26] MicroRNAs (miRs) have been indicated as potential novel biomarkers for many pathological states, consequent to their tissue specific expression and

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association with clinic pathologic variables.[27] Investigation of these tiny molecules and their genetic targets may potentially identify new pathways involved in complex metabolic disease processes, improving our understanding of metabolic disorders and influencing future approaches to the treatment of MetS.

Materials and Methods

We reviewed English-language MEDLINE publications from 2007 through 2013 for experimental, observational, and clinical studies on the relation between metabolic syndrome and microRNA. Search terms of metabolic syndrome included insulin resistance syndrome X, syndrome X, dysmetabolic Syndrome X, Reaven syndrome X, syndrome X, Reaven, metabolic cardiovascular syndrome, cardiovascular syndromes, and metabolic cardiovascular. In addition, search terms of microRNA-33 included miRNAs-33, miRNA-33, micro RNA-33, RNA Micro, MicroRNA-33, primary microRNA-33, microRNA, pri-miRNA-33 Temporal RNA-33, pre-miRNA-33. Approximately 47 papers were reviewed. Based on the relevance, strength, and quality of the design and methods, 37 publications were selected for inclusion in the study.

As this topic is very novel, we reviewed all studies that were done on animals, cell culture, or humans. We reviewed all observational studies as we did not find any randomized trials of either parallel or crossover design. For overall objective evaluation, the design and quality of individual studies, the consistency of findings across studies, and the biologic plausibility of possible mechanisms evaluated the strength of the evidence.

Results

Studies were done to find the role of microRNA were not homogeny; therefore, we were not able to perform meta-analysis.

MicroRNA-33(miR-33)

The most recent investigations demonstrated that miR-33 plays a key regulatory role in the initiation and progression of atherosclerosis.[28,29] MiR-33 mediated regulation in the metabolic pathways such as lipid metabolism (cholesterol homeostasis, HDL biogenesis, and fatty acid, phospholipids, and triglyceride, and bile acid metabolism), inflammatory response, insulin signaling, and glucose homeostasis.[28]

MiR-33, as an intronic microRNA located within the sterol regulatory element-binding protein (SREBP) genes, is one of the master regulators of cholesterol and fatty acid metabolism. Recently, Moore et al.[30] and Fernández-Hernando and Moore,[31] in their studies, showed that miR-33 regulates cholesterol efflux and high-density lipoprotein (HDL) formation, fatty acid oxidation, and insulin signaling. These results describe a model in which miR-33 works in concert with its host genes to ensure that the cell's metabolic state is balanced, thus highlighting the clinical potential of microRNAs as novel therapeutic targets for treating MetS.[26]

Mir-33a and Mir-33 b in humans

Other researchers provided identification within the intronic sequences of the SREBP genes in organisms ranging from drosophila to humans.[32-34]

MiR-33a and miR-33b differ in only 2 nucleotides in the mature form and have the same targets; they differ in their patterns of evolutionary conservation. MiR-33a is encoded within intron 16 of the human SREBP-2 gene and is conserved in many animal species. In humans two miR-33 genes are present as miR-33b, which is located in intron 17 of the SREBP-1 gene on chromosome 17, and miR-33a, which is presented in intron 16 of the SREBP-2 gene on chromosome 22.

Fatty acid oxidation and insulin signaling in hepatic cell lines inhibits by over expression of miR-33a and –b. While inhibition of endogenous miR-33a and -b increases these two metabolic pathways. Therefore, they interestingly showed that miR-33a and -b regulate pathways controlling three of the risk factors of metabolic syndrome, namely levels of HDL, triglycerides, and insulin signaling, and suggest that inhibitors of miR-33a and -b may be useful in the treatment of this growing health concern.

Role of miR-33a and miR-33 b in MetS

In the hepatocytes, lipoprotein uptake increased in conditions of low intracellular cholesterol or in presence of statins. Inducing SREBP-2, increases endogenous cholesterol biosynthesis. SREBP-1, induced by insulin resistance or hyperinsulinemia, leads to increased fatty acid and triglycerides synthesis. The activation of SREBPs induces miR-33a and -b expression, leading to decreased HDL cholesterol levels by targeting ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), reduced insulin signaling by targeting insulin receptor substrate 2 (IRS2), and reduced cellular β-oxidation by targeting different fatty acid oxidation enzymes. Therapeutic inhibition of miR-33 might result in increased plasma HDL cholesterol levels, reduced very low density lipoprotein (VLDL) secretion, and increased insulin signaling, thus improving the prognosis of patients with metabolic syndrome.

MiR-33 and Sterol Regulatory

Fernández-Hernando et al. demonstrated that inhibitors of miR-33 in vitro and in vivo relieve

repression of these genes resulting in up-regulation of the associated metabolic pathways.[35] It is well known that hypertriglycemia in MetS is caused by the insulin-induced increase in sterol regulatory element-binding protein 2 (SREBP-2) mRNA and protein levels.[31,35,36] For the first time, Horie et al. showed that miR-33 modulates the expression of genes involved in cellular cholesterol transport in mice lacking miR-33.[37] They showed that miR-33 is a key regulator of HDL synthesis by mediating cholesterol efflux from cells to apolipoprotein A (ApoA)-I.[37,38] Horie et al.[37] Rayner et al.[39] showed that antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. Conversely, silencing of miR-33 in vivo increases hepatic expression of ABCA1and plasma HDL levels. Thus, miR-33 appears to regulate both HDL biogenesis in the liver and cellular cholesterol efflux. Therefore, an effective strategy for increasing plasma HDL cholesterol and fatty acid oxidation, and prevention of atherosclerosis is necessary.[31]

MiR-33 and fatty acid metabolism

MiR-33a and -b target key enzymes involved in the regulation of fatty acid oxidation, including Carnitine O-acetyltransferase, carnitine palmitoyltransferase 1A, hydroxyacyl-CoA-dehydrogenase, Sirtuin-6 (SIRT6), and AMP kinase subunit-α. Additionally, miR-33a and -b target the insulin receptor substrate 2, an essential component of the insulin-signaling pathway in the liver. Over expression of miR-33a and -b reduces both fatty acid oxidation and insulin signaling in hepatic cell lines, whereas inhibition of endogenous miR-33a and -b increases these two metabolic pathways. Notably, miR-33 also inhibits translation of several transcript encoding proteins involved in fatty acid oxidation including carnitine palmitoyltransferase 1a (CPT1A), Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), beta subunit (HADHB), and Carnitine O-octanoyltransferase (CROT), thereby reducing fatty acid degradation. Ramírez et al. demonstrated that increase in SREBP activity leads to cholesterol and fatty acid accumulation and the down regulation of their own processing pathway.[26]

MiR-33 regulates fatty acid oxidation and insulin signaling

Gerin et al. showed that overexpression of miR-33a and -b reduces fatty acid oxidation and leads to the accumulation of triglycerides in human hepatic cells and in the fat body of miR-33 transgenic flies.[32] Previous works revealed an attractive responsibility for miR-33a and -b in glucose metabolism; as miR-33a and –b overexpression reduces IRS2 levels and inhibits the activation of downstream messenger cascades. Consistent with these findings is the finding that miR-33b over expression reduces insulin-induced 2-deoxyglucose uptakes in hepatic cells, suggesting that miR-33 plays a key role in regulating insulin signaling.[32]

Conclusion

The documented involvement of microRNAs in glucose and lipid metabolism has provided strong evidence in support of their role as key players in the regulation of complex metabolic pathways. Additionally, miR-33 represents an ideal target for future therapies. Although much remains to be learned concerning the role of miRNAs in regulating lipid homeostasis and insulin signaling, these results highlight the potential of miRNAs in the treatment of diseases.

Conflict of Interests

Authors have no conflict of interests.

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Selection of best door-to-cardiac regeneration (D2CR) time

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Short Communication

Abstract

In spite of great progress in the treatment of acute coronary syndrome (ACS) events in reperfusion era, patients are still at risk for development of heart failure due to negative remodeling. Thus, the importance of regenerative therapies in parallel with reperfusion strategies is fundamental. A key feature in this case is obtaining the most appropriate door-to-cardiac regeneration (D2CR) time. This golden time in which fresh stem cells can invade scare-prone tissue could be defined as door-to-cardiac stem cell (D2CSC) plus door-to-cardiac regeneration (D2CR) time. Application of stem cells in this golden time allows comprehensive regeneration and reconstruction. Therefore, the aim of this study was to plan the outlines of simultaneous application of cellular and vascular reconstruction strategies.

Keywords: Cardiac Regeneration, Stem Cell, Golden Time

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Introduction

Despite the huge progress made in the treatment of patients suffering from acute coronary syndrome (ACS) in reperfusion era, patients are still at high risk for development of heart failure due to the progressive negative remodeling: "myodegeneratio cordis".[1] Thus, adjunct therapies should run with a pace similar to the advanced reperfusion strategies. In this case, earlier stem cell therapies at stages before the need for regenerative therapies have been paid more attention in recent decades. Stem cell therapy in ACS is still in its infancy and more light must be shed on this state-of-science field.

By improvement of health care systems, the goal of ACS care must change from prompt cell survival to restoration of wild type cardiac cells, a battle against initiation of negative remodeling. The goal is prevention from progression to heart failure, which is a growing epidemic in this age. Indeed, appropriate cell therapy results in prolonged enhanced mobilization of endogenous reparative stem cells from bone marrow.[2] What needs to be known is the best time cells can be applied after ACS. Appropriate door-to-cardiac regeneration (D2CR) time should be defined as the golden time which fresh stem cells can invade scar-prone breath lost tissue and facilitate cardiomyocyte survival by escaping from post-infarct cardiosclerosis. It might not be the same as door-to-balloon (D2B) or door-to-needle (D2N) time, since the goals are not truly the same. The former aims to restore functionally and anatomically dead cardiomyocytes, but the latter seeks prompt reperfusion and prohibition of ongoing cell death. This time might actually be divided into temporal intervals as adjunctive to early or late reperfusion strategies. Since activation, mobilization, homing, differentiation, and integration of endogenous and resident cardiac stem cells are additive approaches for cardiac repair, D2CR time includes subdivisions of door-to-cardiac stem cells (D2CSC) or door-to-recruit endogenous progenitor cells (D2REPC). However, total door-to-cardiomyoplasty (D2C) time contains door-to-reperfusion time (D2N+D2B times) plus D2CR time (Figure 1). This is the golden time during which reconstruction should be established for comprehensive regeneration (vascular, muscular, electrical, metabolic, and genomic). The exact time is still inconspicuous, but obviously it cannot be defined in its net number as reperfusion strategies and the goals should be understood as the longest time interval that can be considered acceptable for a system. It refers to the diversity of stem cells and their natural cell cycle. As an example, based on the available data of bone-marrow derived mononuclear cells it is 17.5 ± 0.8 d for trans-epicardial and 7 days for trans-coronary delivery rout.[3,4] This time is reported to be 10-14 hrs for intra-coronary application of bone-marrow derived CD133 cells.[5]

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Discussion

Evidently, not all patients are candidate for reception of such cardiomyocyte accretion procedures. Patients suffering from ACS with lower baseline ejection fraction or no-option ACS cases with markers of infarct expansion and poor rehabilitation seem to be benefited more from "cellular bypass". Individually, adjunctive, facilitated, or rescue cellular interventions might be the only possible care for 5-10% of ACS cases. Strictly speaking, to define this time for indicated cases various options should be abstracted into principal instructive keys as the time for preparation and delivery of chosen cells, and the time for delivered cells to get committed for appropriate actions in cardiac milieu. The former depends on the applied cell characteristics in terms of cell type, dosage, and its bio-distribution properties. The latter, depends on the cell delivery technique, the location of cell delivery, and cell trans-differentiation. Often, a lag phase after ACS allows better cell compliance. In these circumstances, the time is obligatory immediately after harnessed inflammatory flood of plaque rupture/erosion and before initiation of scar expansion. Ultimately, these factors are totally inter-related as a chain. Clearly, the individualized application of appropriate stem cell which seeks its own administration method is the principal part of the therapeutic cascade. As an example, for scar strengthening purposes, direct injection of myoblasts or pre-programmed adult stem cells meets the needs rather than mesenchymal stem cells. Selection of eligible cells to combat the hostile milieu of cardiac tissue after ACS is just one side of the coin, but it is not within the scope of this article. Cellular cardiomyoplasty is a gradually growing field. Menasché et al. opened new horizons to cardiac therapeutics by performing the world's first clinical cellular neomyocardial formation and revitalization of scar tissue.[6] I tried to scheme the dream of simultaneous application of cellular and vascular reconstruction strategies. The idea of pre-occurrence cell reservation banks seems to be developed to bypass cell cultivation and processing time. By a small biopsy taken from vastus laterialis or even usage of other cell resources, like adipose tissue-derived stem cells or umbilical cords at the time of birth, each person can have reserved his or her own cell ready to use. Finally, the best applied time is the time during which cell application is able to thicken infarct walls and prohibit deterioration of ejection fraction and negative remodeling.

Conflict of Interests

Authors have no conflict of interests.

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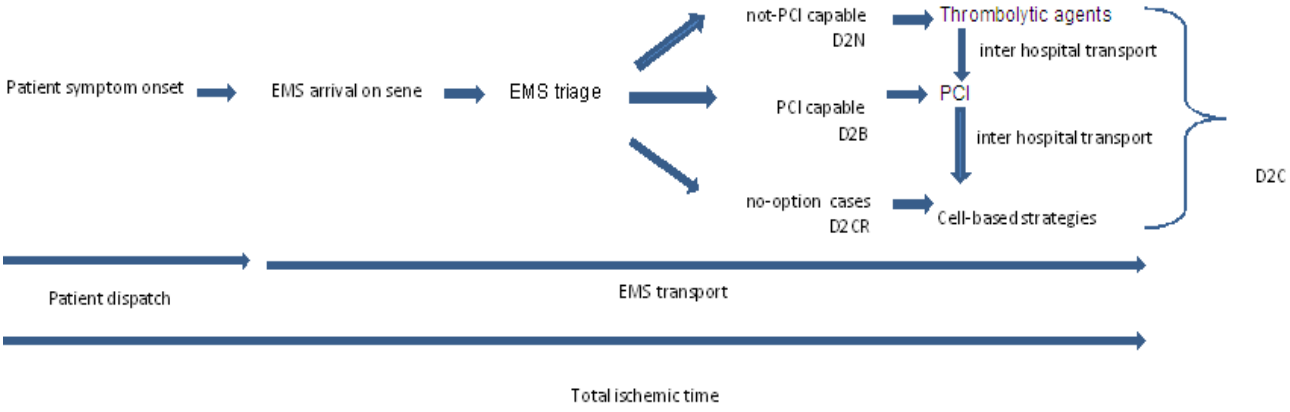

Figure 1. Options for transporting acute coronary syndrome (ACS) cases and recommended strategies for ideal cardiomyoplasty
EMS: Emergency medical services; PCI: Percutaneous coronary intervention
D2B: Door-to-balloon; D2N: Door-to-needle; D2CR: Door-to-cardiac regeneration; D2C: Door-to-cardiomyoplasty

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