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ARYA Atherosclerosis

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2. Bonow RO, Mann DL, Zipes DP, Libby P. Braunwald's Heart Disease E-Book: A Textbook of Cardiovascular Medicine. 7th ed. Philadelphia, PA: Elsevier Health Sciences; 2007. p. 1976, 1981, 1982.

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* All the words of the article containing the references; each table is considered as 300 words.

There will be a 50% discount of publication fee if both the first and the corresponding author are affiliated to Isfahan University of Medical Sciences (IUMS).

Table of Contents

Comparison of the prevalence of enteroviruses in blood samples of patients with and without unstable angina

Aida Gholoobi[(1)], Zahra Meshkat[(2)], Akram Baghani[(3)], Maryam Sadat Alavi[(4)], Toktam Mohammadpoor[(5)], Mastoureh Momen-Heravi[(5)], Mohsen Mouhebati[(6)], Samaneh Sepahi[(7)], Sina Rostami[(8)], Mojtaba Meshkat[(9)], Arash Gholoobi[(10)]

Original Article

Abstract

BACKGROUND: Although the role of enteroviruses has been proved in heart diseases, extensive information is not available on the association between enteroviruses and unstable angina. In the present study, the authors compared the prevalence of enteroviruses in patients with and without unstable angina.

METHODS: Blood samples were taken from 51 patients with unstable angina and 55 patients without unstable angina or myocardial infarction that were admitted to Imam Reza and Ghaem hospitals (Mashhad, northeast of Iran). Reverse transcription polymerase chain reaction (RT-PCR) was performed using specific primers for the detection of the enteroviruses in blood samples of study subjects.

RESULTS: Patients with and without unstable angina were similar in age with mean ± standard deviation of 62.6 ± 12.8 and 59.7 ± 12.7 years, respectively (P = 0.243) and there were no differences in gender in these two groups (P = 0.174). Prevalence of the enteroviruses in patients with unstable angina was higher only in 66-80 years age group compared to the control group (patients without unstable angina, P = 0.032). There was a higher prevalence of enterovirus RNA positivity in the blood samples of women with unstable angina (75.9%) than those without unstable angina (41.7%, P = 0.011), however, no significant difference was observed in men (P = 0.983).

CONCLUSION: Our data showed that enteroviral RNA positivity was higher in patients with unstable angina compared to those without unstable angina. However, the differences between the two groups were not statistically significant.

Keywords: Unstable Angina, Enterovirus, Reverse Transcriptase PCR

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Introduction

Ischemic heart disease is the number one cause of mortality all around the world. Coronary artery disease may be manifested clinically as either stable ischemic heart disease (SIHD) or an acute coronary syndrome (ACS). The latter can be subdivided into two groups. The first sub-group is patients with acute ST-elevation myocardial infarction (STEMI),

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and the second sub-group is patients with unstable angina (UA) and non-ST-segment elevation myocardial infarction (NSTEMI).[1]

Compared to STEMI, the prevalence of UA/NSTEMI is increasing. Almost half of the patients with UA/NSTEMI are women, while more than three-fourths of patients with STEMI are men. Patients with UA/NSTEMI may face a wide range of early risks (first 30 days) that varies from 2 to 10 percent.[2]

UA stems from imbalance between the myocardial oxygen supply and demand. Rupture of unstable atheromatous plaque with superimposed non-occlusive thrombi may be the main etiology in patients with UA. Inflammation and/or infection may play a role for thrombogenesis in these patients.[3] There is some evidence indicating a relationship between bacteria or viruses [such as herpes simplex virus (HSV), cytomegalovirus (CMV), and Helicobacter pylori (HP)] and atherosclerosis or myocardial infarction (MI).[4,5]

It has been found that the enteroviruses infections are related to myocarditis. Furthermore, the experimental animal models have provided evidence that enterovirus infections may cause atrial fibrillation.[6] The enterovirus myocarditis in infancy leads to greater mortality and increases the risk of coronary heart disease in the future.[7] Enteroviruses are endemic. Most people are exposed to various serotypes of the enteroviruses during their lifetime. Enteroviruses are the most common cause of acute and chronic viral myocarditis.[8]

Transmission of the enterovirus infection is through fecal-oral route and respiratory system. They are non-enveloped positive sense RNA virus belonging to Picornaviridae family.[9] Enteroviruses include five subgroups including polioviruses, coxsackieviruses (A and B), echoviruses, and novel types of the enteroviruses. Over 70 serotypes of the enteroviruses have been isolated from human. Some types of human enteroviruses are associated with heart diseases.[10,11] Enteroviruses may cause asymptomatic disease and they probably appear in healthy people as well.

A few studies have evaluated the relationship between MI and enteroviruses. However, the knowledge in this regard is not yet comprehensive. In the current study, the frequency of the enteroviruses was determined in blood samples of UA patients compared to patients without UA.

Materials and Methods

This prospective cross-sectional study was conducted at the Mashhad University of Medical Sciences, Iran and was confirmed by the clinical ethics committee (approval number 89165). Between March to September 2013, 106 consecutive patients (age range, 35 to 80 years) were enrolled in this study who were admitted to the cardiology ward and other departments of Imam Reza and Ghaem hospitals.

In this research, patients were divided into two groups: patients with UA (n = 51) and patients without UA (n = 55). Inclusion criteria for the first group (UA patients) included women and men between 35-80 years of age and diagnosis of UA, based on the criteria of having ischemic type chest discomfort, electrocardiographic (ECG) changes, evaluation of serum markers and coronary artery involvement in angiography.[3]

We excluded patients with signs of myocardial ischemia in the absence of coronary artery disease, such as high-output heart failure (thyrotoxicosis, anemia, beriberi), vasculitis or inflammatory diseases, inherited metabolic disease, congenital anomalies of the coronary arteries, blood diseases (polycythemia vera, thrombocytosis, hypercoagulable disorders), substance abuse and coronary vascular injury.

The second group (patients without UA) consisted of 55 patients who were hospitalized in other departments of hospitals during the same period. These patients were examined by a cardiologist and those who had cardiovascular risk factors were excluded. Moreover, subjects with UA and MI were excluded.

After obtaining informed consent from the study subjects, 2 ml blood sample from the brachial vein of each subject were collected in Ethylenediaminetetraacetic acid (EDTA) tube, and the tubes were kept on ice. Sera were separated immediately and kept frozen at -70 °C.

RNA extraction was done according to the kit protocol (CinnaGen Co, Tehran, Iran). Complimentary DNA (cDNA) synthesis was performed using reverse transcription method according to the recommendations of Easy cDNA Synthesis Kit (Pars Toos Co, Mashhad, Iran) manufacturers. Poliovirus (Picornaviridae family) vaccine was used as a positive control.

For semi-nested PCR, the following primers were used: F1 (5'- CAAGCACTTCTGTTTCCCCGG-3'), F2 (5'- TCCTCCGGCCCCTGAATGCG-3'), R (5'- ATTGTCACCATAAGCAGCCA-3').[12] PCR method was performed as described previously:[9] PCR mixture consisted of 20 pmol of each primer, 3.5 mM $MgCl_2$, 0.2 mM each dNTP, 4U Taq DNA polythermase (Cinagen, Iran) in a total reaction volume of 25 µl.

Table 1. Demographic information of patients with and without unstable angina

Variables	With unstable angina (n = 51)	Without unstable angina (n = 55)	P
Sex [n (%)]			
Men	22 (43.1)	31 (56.4)	0.174*
Women	29 (56.9)	24 (43.6)	
Age (mean ± SD)	62.6 ± 12.8	59.7 ± 12.8	0.243**

* Pearson chi-square test; ** Student's t-test
SD: Standard deviation

Amplification was performed for 40 cycles: 94 °C for 1 min, 42 °C for 1 min, 72 °C for 2 min and an initial denaturation step in 94 °C for 5 min and a final extension step of 72 °C for 5 min was used. The PCR products were analyzed on 1.5% agarose gel.

Student's t-test was used to compare differences for the quantitative variables such as age. Nominal qualitative variables [(sex and reverse transcription polymerase chain reaction (RT-PCR)] were compared using Pearson's chi-square test. The variables were reported as number (percentage) or mean (± standard deviation) as appropriate. The Fisher's exact test was also used for cases in which 20% of expected frequencies of tables were fewer than five. For statistical analysis, SPSS (version 18, SPSS Inc., Chicago, IL, USA) was used, and P-value less than 0.05 was considered significant.

Results

Table 1 summarizes demographic information of patients with and without unstable angina. The differences between the two groups were not statistically significant, in terms of gender (P = 0.174). The comparison of the mean age of subjects with and without unstable angina was performed with the Student's t-test and no significant difference were found between the two groups (P = 0.243).

It can be seen in figure 1 that in the positive control sample (poliovirus vaccine) and selected enterovirus-positive samples using F1/R and F2/R primers, the sizes of the bands were 440 bp and 155 bp, respectively.

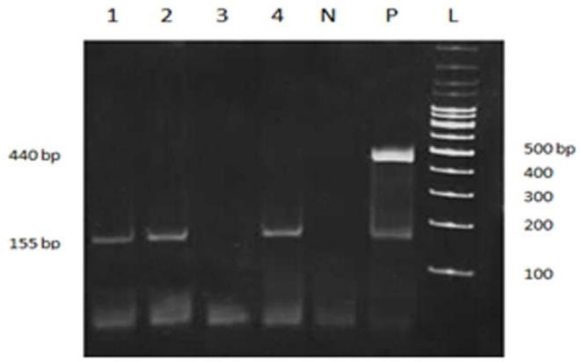

Figure 1. Electrophoresis of polymerase chain reaction (PCR) products
Semi-nested reverse transcription polymerase chain reaction (RT-PCR) was used for amplification of 440 bp and 155 bp fragments, within the 5' transcribed domain of enteroviruses genome, lanes 2, 1 and 4 represent enteroviruses-positive samples and lane 3 represents enterovirus-negative sample
N: Negative control; P: Positive control; L = 100 bp DNA ladder

Table 2 shows the percentages of enterovirus infection in individuals with and without UA, regardless of their age and sex. Among patients with UA, 34 samples were found RT-PCR positive. These results indicate that the rate of enterovirus infection in patients with unstable angina was higher than subjects without unstable angina; however, there was no significant difference between the two groups (P = 0.067).

RT-PCR analysis in men demonstrated that the differences between those with and without UA were not statistically significant (P-value = 0.983). However, significant differences were observed between the two groups of women (P = 0.011, Table 3).

Table 2. Distribution of enterovirus infection in patients with and without unstable angina using reverse transcription polymerase chain reaction (RT- PCR)

Enterovirus infection	With unstable angina [n (%)]	Without unstable angina [n (%)]	Total [n (%)]
RT-PCR-positive	34 (66.7)	27 (49.1)	61 (57.5)
RT-PCR-negative	17 (33.3)	28 (50.9)	45 (42.5)
Statistic test		Pearson chi-square = 3.35	
P		0.067	

RT-PCR: Reverse transcription polymerase chain reaction

Table 3. Detection of enteroviruses in patients with and without unstable angina according to the gender

Sex	Enterovirus RT-PCR	With unstable angina [n (%)]	Without unstable angina [n (%)]	P
Men	Positive	12 (54.5)	17 (54.8)	0.983*
	Negative	10 (45.5)	14 (45.2)	
Women	Positive	22 (75.9)	10 (41.7)	0.011*
	Negative	7 (24.1)	14 (58.3)	

* Pearson chi-square test; RT-PCR: Reverse transcription polymerase chain reaction

Table 4 compares the rate of enterovirus infection among three age groups of patients with and without UA. RT-PCR analysis were statistically significant between the two groups in the range of 66-80 years old (P = 0.032), however it did not reach statistical significance in those in the age range of 35-50 years (P = 0.703) and 51-65 years (P = 0.795).

Discussion

Many studies have considered the association of microorganisms, especially viruses, and heart diseases.[4,11,13] The aim of this study was to compare the prevalence of enterovirus genome in blood samples of patients with UA and a control group.

In the present study, 51 patients with diagnosis of UA and 55 controls without signs and symptoms of UA were screened for the detection of the enterovirus RNA in their blood samples. Patients with and without UA had mean age of 62.6 ± 12.8 and 59.7 ± 12.7 years old, respectively. We have shown in this study that the number of women with UA who had enterovirus infection was significantly higher than women without UA. Moreover, the data has shown that in the age group of 66-80 years old, the number of patients with UA who had enterovirus RNA in their blood samples was significantly higher than the control group. In our study, the enteroviruses were detected in 66.7% of patients with UA and 49.1% of the control group (P-value = 0.067); considering that the P-value is close to 0.05, more extensive research is needed to better clarify the significance of difference.

Compared with other similar studies, the incidence of enterovirus infection in patients with heart diseases was proved to be considerably higher than that of other viruses. In a study in patients with MI, the high levels of antibodies against the enteroviruses, mycoplasma and chlamydia was associated with a higher risk of developing coronary heart disease, however, the study was conducted only in men without baseline history of heart diseases.[5] Particularly, infection with coxsackie B2 virus has been shown to have a great impact on heart disease (55.9%).[11] In another study, it was reported that the enteroviruses and herpes simplex viruses had the highest impact on the coronary artery disease.[14] Pesonen et al. performed a retrospective study to evaluate the role of viruses on heart diseases. They reported that the enteroviruses can greatly increase the risk of developing UA and acute MI.[15] In another study, the enteroviruses were reported in 49% of patients with ACS and 57.3% of patients with MI. This observation suggested that enterovirus infection is a risk factor for developing ACS and heart attack.[16] Evaluation of enterovirus infections and genetic polymorphism (at locus MMP3, MMP1, TNFα) among 208 patients with heart problems showed that 7% of blood samples were related to UA patients and 42% were related to those with MI. Prevalence of the enteroviruses in MI group were noticeably higher than UA group.[17] Our results are in accordance with the above findings and reinforce the hypothesis that enterovirus infections may play a role in the pathogenesis of UA.

Table 4. Detection of enteroviruses in patients with and without unstable angina according to the age groups

Age groups (year)	Enterovirus infection (RT-PCR)	With unstable angina (n = 51)	Without unstable angina (n = 55)	P
35-50	Positive	8 (66.7)	9 (52.9)	0.703*
	Negative	4 (33.3)	8 (47.1)	
51-65	Positive	7 (46.7)	5 (41.7)	0.795**
	Negative	8 (53.3)	7 (58.3)	
66-80	Positive	19 (79.2)	13 (50.0)	0.032**
	Negative	5 (20.8)	13 (50.0)	

* Fisher's exact test; ** Pearson chi-square test
RT-PCR: Reverse transcription polymerase chain reaction

In a study in Mashhad, only 2.6% of serum samples of patients with MI were positive for enterovirus RNA. This study did not find statistically significant results among different age groups or between men and women.[9] In contrast, we found a significant association between prevalence of enteroviruses infection and the incidence of UA in 66 to 80 years old patients. Furthermore, it was found that women with UA had a significantly higher prevalence of enteroviruses RNA in their blood samples compared to the control group. One explanation could be the fact that the other study was conducted in patients with acute MI.

The main advantage of our study was the use of PCR-based detection method which reveals the presence of the virus genome in blood samples of study subjects, instead of using serum antibodies which may show the past infection. The high prevalence of the enteroviruses could be due to the presence of various types of the enteroviruses.

In our work, the pattern of prevalence of the enteroviruses was not determined based on the genus and species and only the total prevalence was reported. Further investigations will be encouraged to categorize the enteroviruses and their prevalence in patients with UA. Besides, it would be of interest to gather more demographic information about the participants. Last but not the least, regarding the various prevalence of the enteroviruses in different seasons, further studies are encouraged to be performed in different time periods throughout the year.

Conclusion

Prevalence of the enteroviruses in patients with UA was significantly higher than those without UA in the elderly and women. There was no significant difference in other age groups and men. This study reports the link between enterovirus infection and UA but further studies with more power will be needed for better understanding of this relation.

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Conflict of Interests

Authors have no conflict of interests.

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Using rats as a research model to investigate the effect of human adenovirus 36 on weight gain

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Original Article

Abstract

BACKGROUND: Recent evidence has shown a positive correlation between obesity and viral infections with a particular emphasis on the human adenovirus-36 (Ad-36). Ad-36 is the first human virus that may increase adiposity in animals, and it is considered as a possible risk factor for obesity in humans; however, the results were not consistent across all the studies. The present study was conducted to examine the influence of Ad-36 infection on obesity in a rat model.

METHODS: Eight-week-old male Wistar rats weighing 170-240 gram (g), were randomly divided into two groups, infection group (48 rats) and a control group (12 rats). The rats in the infection group were infected with human Ad-36. All rats were given free access to a normal chow diet and water. They were weighed weekly.

RESULTS: The mean ± standard deviation (SD) body weights were 229.0 ± 25.9 g and 232.3 ± 16.6 g in the infection and control groups, respectively at the time of infection. The mean ± SD body weight of the infection group (304.0 ± 39.0 g) was higher than the control group (301.0 ± 36.5 g) at 12 weeks post-infection (P = 0.82). Although two groups had approximately same food intakes, the mean change in body weight was greater in the infection group than the control group (75.8 ± 27.9 g vs. 70.8 ± 24.5 g) but it was not significant (P = 0.57).

CONCLUSION: We did not find a statistically significant association between weight gain and Ad-36 infection in the rat model. It seems that longer follow-up duration is needed to develop a significant weight gain in the infected rats. Rats can be used as a good animal model for further investigations about Ad-36-induced obesity, provided not to rely merely on weight measurements. Evaluating body composition or histopathological assessments are suggested.

Keywords: Adenovirus Infections, Weight Gain, Wistar Rats

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Introduction

Obesity is defined as excessive fat accumulation in the adipose tissue that may be associated with increased risk of chronic diseases, and impose a significant economic burden to the health care system. Over 600 million adults were obese in 2014, and it is predicted that 51% of the world's population will be obese by 2030.[1-3]

Obesity is a multifactorial disease that develops from a complex of interactions among genetic, metabolic, behavioral, as well as environmental factors.[1-4] Moreover, infection by certain pathogens may be considered as possible causes of obesity.[4,5] Dhurandhar coined the term infectobesity -obesity of infectious origin- in 2001.[6] Infectobesity is receiving a considerable attention. Several pathogens have been identified as the causes of obesity in animal models.[5-8]

Recent evidence has shown a positive correlation between obesity and adenoviruses, with a particular emphasis on the human adenovirus-36 (Ad-36) that has a direct effect on adipose tissue.[9-12]

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Human adenoviruses are implicated in infections of the upper respiratory and gastrointestinal tracts and in conjunctivitis.[13-16] The first study of Ad-36 infection in adult humans in the United States by Atkinson et al. showed that the antibody-positive obese or nonobese subjects were heavier compared with their antibody negative counterparts. About 30% of obese and 11% of non-obese had been infected with Ad-36. In addition, the obese adults had a higher prevalence of serum neutralizing antibodies to Ad-36 than the lean adults.[17]

Similarly, antibody-positive twins were heavier [body mass index: 26.1 vs 24.5 kg/m^2 ($P < 0.04$)] and fatter [body fat percentage was 29.6 vs. 27.5 ($P < 0.04$)] than their co-twins who did not have Ad-36 antibodies.[17] These results have been confirmed by other studies, while some studies failed to confirm these findings.[18-19] Pasarica et al. have reported that rats infected with Ad-36 by 30 weeks post-inoculation, had a significant increase in body weight and body fat.[20]

Although the link between Ad-36 infection and obesity has been investigated in animal models, only one study has examined the relationship between Ad-36 and obesity in rats.[20] Obviously, rat is a good animal model for investigating human infectobesity and the metabolic and biochemical modulations induced by Ad-36 in adipose tissue. Rat provides a higher body weight and adequate quantities of adipose tissue for use in various mechanistic pathways representing the obesity process.[20,21]

Finding a therapeutic agent for Ad-36-induced obesity may help to treat obesity more effectively at least in a subgroup of population.[10,15] To the best of our knowledge, there is no Food and Drug Administration (FDA) approved vaccine or clinical drug for Ad-36-induced obesity. Therefore, a research project was designed and carried out to investigate "the effects of the alcoholic green tea extract and conjugated linoleic acid on body weight, metabolic indices and inflammatory markers in Ad-36 infected rats". The purpose of the present study was to describe study design and to report the preliminary results.

Materials and Methods

Eight-week-old male Wistar rats from Laboratory Animal Unit of Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran) were used for the experiment weighing 170-240 g. Six rats per cage were housed in a temperature-controlled (20–22 °C) animal room with a 12-hour light-dark cycle. After one-week acclimatization period, rats were randomly divided into two groups, infection group (48 rats) and control group (12 rats). Infection and control groups were housed in separate rooms under biosafety level 2 containment. They had free access to drinking water and standard rat chow for 1 week while adjusting to their new environment. During the experimental period, the rats had free access to a normal chow diet and water. Food disappearance was recorded for each of the cages, but we were not able to measure the amount of food spillage. However, visual inspection indicated that spillage patterns were similar in all groups. At the end of the study, the average food intake per day per rat was calculated. All rats were weighed weekly by means of a digital scale (Sartorius 1413 MP 8/8-1, USA).

A549 cells, a human lung carcinoma tissue culture line purchased from the Razi Vaccine and Serum Research Institute (Tehran-Iran) were used to grow Ad-36. Human Ad-36 was obtained from the American Type Culture Collection (Catalog no. VR-1610; American Type Culture Collection, Manassas, VA 20108 USA). In order to confirm the hexon gene, polymerase chain reaction (PCR) was performed and subsequently, sequencing was carried out by chain termination, using ABI 3730XL DNA Analyzers (Bioneer, Korea).

The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) supplemented with 10% of heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml of penicillin, 100 µg/ml of streptomycin and incubated at 37 °C, 5% CO_2. In preparation of virus seeding, culture of Ad-36 with multiplicity of infection (MOI) 0.01 in confluent A549 cells was performed and 72-hour post-infection cytopathic effect was completed. Infection cells were frozen, thawed, and then centrifuged into 5000 g and supernatant were collected. Virus titration was performed by 50% cell culture infective dose ($CCID_{50}$) assay. 5×10^5 $CCID_{50}$ of Ad-36 virus suspension was injected in the left hind paw of the experimental group rats. On the 14th day after virus injection, blood sera of rats were collected and infection was confirmed by neutralization assay.

This experiment was performed in accordance with the National Research Council (US) Committee for Guide for the Care and Use of Laboratory Animals, 8th edition, and the guidelines provided by the ethical committee of experimental animal care at Ahvaz Jundishapur University of Medical Sciences.

The data were analyzed using SPSS Software (version 20, IBM Corporation, Armonk, NY, USA). One-sample Kolmogorov-Smirnov test was used to

test normal distribution of the variables. Paired t-test was used to compare the baseline with the endpoint values. Data from the infection and control groups were compared with independent samples t-test. The values are presented as means ± standard deviation (SD). P-value of 5% or lower was considered statistically significant.

Results

At the beginning of the study, the mean ± SD of bodyweight for infection group was 192.8 ± 16.3 g and in the control group was 195.3 ± 9.0 g (P = 0.48). At the time of infection, the mean body weights were 229.0 ± 25.9 g and 232.3 ± 16.6 g in the infection and the control groups, respectively (P = 0.60). Obviously, a significant weight increase was observed in the infection (from 36.3 ± 17.7 g to 75.8 ± 27.9 g) and control (from 37.0 ± 12.8 to 70.8 ± 24.5 g) groups after 12 weeks (P < 0.01). At 12 weeks after infection, while the two study groups had approximately equal food intakes and food disappearance, the mean body weight of the infection group was higher than the control group (304.0 ± 39.0 g vs. 301.0 ± 36.5 g, P = 0.82, Table 1). In addition, the mean change in body weight was greater in the infection group than the control group; but the between groups difference were not statistically significant (75.8 ± 27.9 g vs. 70.8 ± 24.5 g, P = 0.57).

Discussion

In this experimental study, no statistically significant association was found between weight gain and Ad-36 infection in male Wistar rats at 12 weeks after Ad-36 infection. Pasarica et al. performed a similar study on Ad-36 infected rats.[20] which, to our knowledge, is the only study that had monitored infected Wistar rats for a long time (12 and 30 weeks). This study showed that body weight and adiposity were significantly increased in the infected vs. control rats at 30 weeks after Ad-36 infection.[20] Ad-36-induced adiposity and significant weight gain had been documented in mice, rats, and marmosets.[18-20] In agreement with Pasarica et al.,[20] the body weights did not differ significantly at 12 weeks after infection in our experiment. It seems that longer follow-up duration, approximately six months, is required to increase significant weight gain in Ad-36 infected rats.

Obesity is a major modifiable risk factor for many chronic diseases and as a critical medical condition that can lead to serious health consequences, including shortened life span and increased morbidity, as well as health care costs. Many people are trying to lose weight by dietary restraint, increased physical activity and healthy lifestyle behaviors.[22-24] These approaches may be difficult, ineffective or inappropriate in the vast majority of overweight and obese individuals. In addition, these weight loss strategies are often ineffective in the long term and may have unintended consequences, particularly higher risk of repeated cycles of weight loss and weight regain.[24-25] Obviously, few individuals successfully achieve long-term results from weight loss strategies. Therefore, prevention strategies or cause-specific treatment could be determined according to various contributing factors to obesity. Although a combination of several factors may lead to obesity, a subtype of obesity may be caused by infections.[10, 22-27] Over the past thirty years, ten pathogens have been reported to induce obesity in animals, but their contribution to human obesity has not yet been fully understood.[28] Overall, viral infections have been implicated as a possible cause of obesity in human. Adenoviruses are the first adipogenic agents that may cause obesity in both animal models and naturally infected humans.[29-32] The adenoviruses are common pathogens of humans and cause a wide range of illnesses and symptoms such as common cold, enteritis, acute upper respiratory tract infections, or conjunctivitis.[14]

Table 1. Body weights and food intake in the adenovirus-36 (Ad-36) infected rats vs. the control group[*†]

Variables	Infected	Control	P
Body weight (g)			
Baseline	192.8 ± 16.3	195.30 ± 9.00	0.48
At the time of infection	229.0 ± 25.9	232.30 ± 16.60	0.60
12 weeks post-infection	304.0 ± 39.0	301.00 ± 36.50	0.82
Change[‡]	75.8 ± 27.8	70.80 ± 24.50	0.57
Food intake/d (g)			
Baseline	22.8 ± 1.0	22.60 ± 0.56	0.52
At the time of infection	23.1 ± 1.1	22.80 ± 0.58	0.36
12 weeks post-infection	25.9 ± 1.1	25.20 ± 1.60	0.26

[*] Values are means ± standard deviation; [†] Data at 12 weeks are for 11 rats from the control group and 46 rats from the infection group; [‡] Weight changes from the time of infection to 12 week post-infection

The presence of serum antibodies to adenoviruses is common in the general population, which makes them promising candidates for studying their potential role in human obesity with a particular emphasis on the Ad-36.[13,29] Clearly, Ad-36 is the first human virus associated with increased adiposity and significant weight gain in animals.[33] In addition, results from epidemiological studies have shown that Ad-36 infection might be associated with obesity in both children and adults.[29,34-40] However, more animal and human researches are needed to establish the contribution of Ad-36 in human adiposity. For ethical reasons, humans cannot be infected experimentally with Ad-36 to study the virus induced adiposity; therefore, animal models are used to determine the relevance of Ad-36 induced human obesity.[7,10] Rats are good research models for investigating human obesity and the metabolic and biochemical modulations induced by Ad-36. Rat's body provides a higher weight and adequate quantities of adipose tissue to be used in various mechanistic pathways representing the obesity process. Rat has become a standardized physiological and toxicological model because it behaves more similar to human.[20,41] Therefore, investigating Ad-36-induced obesity in rat model may lead to finding a therapeutic agent for preventing or treating Ad-36-induced obesity.

Conclusion

In the present study, Ad-36 had no statistically significant effect on weight gain in infected rats. Further studies with longer duration of follow-up are suggested. As a model of medical research, rat has many advantages over other animal models for further investigations about Ad-36-induced obesity. In addition, it is strongly recommended not to rely merely on weight measurement; body composition and or histopathological assessments would be accompany weight measurement.

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Conflict of Interests

Authors have no conflict of interests.

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Association between ABO blood group and severity of coronary artery disease in unstable angina

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Original Article

Abstract

BACKGROUND: ABO blood groups are genetically transmitted through chromosome 9 at locus 9q34. It is supposed that there is a locus on 9p21, which has a role in developing coronary artery disease.

METHODS: Our study population consisted of 309 patients with unstable angina admitted to the Ziaeian Hospital, Tehran, Iran, who underwent coronary angiography. The association between types of blood group (O and non-O) with the severity of coronary artery disease was investigated.

RESULTS: Compared to the non-O groups, the O group had more severe coronary artery involvement (P = 0.004).

CONCLUSION: Our study supports recent suggestions on the association between blood group and coronary artery disease. Further studies are needed to evaluate the effect of blood group on atherosclerosis.

Keywords: ABO Blood Group System, Blood Group, Coronary Artery Disease, Unstable Angina, Acute Coronary Syndrome, Myocardial Ischemia, Angina Pectoris, Atherosclerosis

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Introduction

Coronary artery disease (CAD) is the major cause of death all over the world and has multiple major risk factors such as aging, gender, dyslipidemia, hypertension and diabetes, smoking, and family history.[1] The high prevalence of a particular blood group in a community or geographical area may affect the incidence of the diseases.[2] The ABO system occurs because of polymorphism of complex carbohydrate with different antigenic structures of glycoproteins and glycol lipids at the surface of erythrocytes, as glycan units of mucin glycol proteins.[1,2] H-antigen precursor converts by A and B glycolsyl transferase into A or B determinants. These transferase enzymes have no function in group O and they express H-antigen.[3,4] ABO blood groups are genetically transmitted through chromosomes 9 at locus 9q34.[5,6] The ATP-binding cassette 2 [ABCA2] gene is also located on locus 9q34 which has some role in the cholesterol balance and lipid homeostasis.[7] A locus on 9p21 is supposed to have a role in developing CAD.[8,9] ABO (H) carbohydrate antigenic determinants expressed on the N-linked glycan chains of circulating plasma von Willebrand factor (VWF). High levels of VWF and factor VIII are associated with risk of thrombosis and an ABO relationship has been suggested.[6] The role of ABO blood group as a risk factor of venous thrombosis and ischemic heart disease had been recognized from 1960, and it has been postulated that it is due to the relation between the ABO group and level of pro-coagulant factor VIII and VWF. VWF levels are 25 percent higher in non-O groups, compared with group O.[9] Previous studies showed controversial results about the relation of ABO blood groups and ischemic heart disease in Italy, Iran and India.[10,11] We

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aimed to investigate whether ABO blood groups were associated with the severity of CAD and major cardiovascular risk factors in Iranian patients with moderate to high risk unstable angina.

Materials and Methods

The study was conducted on patients admitted to the coronary care unit of the Ziaeian Hospital affiliated to Tehran University of Medical Sciences, Tehran, Iran, from June 2014 to April 2015. Total of 309 patients with moderate to high-risk unstable angina according to thrombolysis in myocardial infarction (TIMI) risk score were consecutively included into this cross sectional study. We used convenient sampling method regardless of patient blood groups. Exclusion criteria were clinically significant heart failure, significant valvular heart disease and severe renal failure. Informed consent was obtained from all participants and the study protocol was approved by the ethic committee. Baseline clinical and demographic data including age, hypertension, diabetes mellitus, dyslipidemia, smoking habits and family history were recorded. Hypertension was defined as systolic blood pressure equal or more than 140 mm Hg, diastolic blood pressure equal or more than 90 mm Hg or history of anti-hypertensive medications. Diabetes mellitus was defined as previous history of diabetes and history of medical treatment. Dyslipidemia was defined as total cholesterol > 200 mg/dl or low-density lipoprotein cholesterol (LDL-C) >130 mg/dl or history of medical treatment. Smoking was defined as person who currently smokes. Family history was defined as history of myocardial infarction or coronary artery disease in the first degree relatives in men < 45 and in women < 55 years of age. Blood sampling for lipid profile study [triglyceride, cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C)] was done after 12 hours fasting and checked using proper kit. Coronary angiography was performed in the standard manner with angiography system Artis Zee Multipurpose (SIEMENS) and the results were assessed by two cardiologists separately. Severity of coronary artery stenosis according to the severity categorization of Jones et al.[12] was defined as mild (< 50%), moderate (50%-70%) and severe (> 70%) stenosis of coronary lumen. According to the previous studies, blood groups were divided in two groups, O and non-O.[13]

Results are presented as mean ± standard deviation (SD) for quantitative variables. Categorical variables are shown as frequencies and percent. Chi-square test was used for categorical variables. To evaluate the adjusted effect of blood group on severity of CAD, the unconditional logistic regression method was used and presented as odds ratio (OR) and 95% confidence interval (CI). Statistical significance was determined as a P-value of ≤ 0.05. All statistical analysis were performed using SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA).

Results

Totally, 309 patients [mean age 59.6 ± 11.7 years, 60% (185) men and 40% (124) women] with coronary artery involvement were assessed. The distribution of blood groups were as follows: O 53.1% (164), A 30.1% (93), B 11% (34), AB 5.8% (18). Table 1 presents the characteristics of study population and also compares the patients by blood group O versus non-O.

Table 1. Clinical and laboratory characteristics of the patients with O and non-O blood groups

Variables	Total [n = 309 (100%)]	Blood group O [n = 164 (53.1%)]	Blood group non-O [n = 145 (46.9%)]	P
Age (mean ± SD)	59.6 ± 11.7	60.3 ± 11.8	58.8 ± 11.5	0.274
BMI (mean ± SD)	27.5 ± 4.1	27.8 ± 4.2	27.3 ± 3.9	0.292
Triglyceride (mean ± SD)	162.9 ± 80.3	162.3 ± 88.6	163.7 ± 69.9	0.879
Cholesterol (mean ± SD)	204.2 ± 50.5	204.8 ± 47.1	203.6 ± 54.3	0.836
HDL-C (mean ± SD)	44.6 ± 12.9	43.1 ± 13.8	46.1 ± 11.8	0.035
LDL-C (mean ± SD)	130.6 ± 40.1	130.3 ± 38.7	130.1 ± 41.8	0.836
Smoking [n (%)]	146 (47.2)	77 (52.7)	69 (47.3)	0.911
Diabetes [n (%)]	154 (49.8)	89 (57.8)	65 (42.2)	0.098
Hypertension [n (%)]	175 (56.6)	95 (54.3)	80 (45.7)	0.626
History of MI or admission in CCU [n (%)]	92 (29.8)	46 (50.0)	46 (50.0)	0.481
History of HPL [n (%)]	73 (23.6)	36 (49.3)	37 (50.7)	0.461
Family history of CAD [n (%)]	135 (43.7)	67 (49.6)	68 (50.4)	0.285
Severity of CAD				0.004
Mild and Moderate	187 (60.5)	87 (46.5)	100 (53.5)	
Severe	122 (39.5)	77 (63.1)	45 (36.9)	

SD: Standard deviation; BMI: Body mass index; MI: Myocardial infarction; CCU: Coronary care unit; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; HPL: Hyperlipidemia; CAD: Coronary artery disease

Table 2. Adjusted and unadjusted effect of blood group on the severity of coronary artery disease

Effect	OR (95% CI)	P
Severity of coronary artery disease (unadjusted)	1.967 (1.23-3.13)	0.005
Severity of coronary artery disease (adjusted)*	1.968 (1.22-3.15)	0.005

*Adjusted for high-density lipoprotein cholesterol and diabetes
Hosmer and Lemeshow goodness of fit test, P = 0.675
OR: Odds ratio; CI: Confidence interval

Regarding cardiovascular risk factors, 49.8% had diabetes mellitus, 56.6% had hypertension and 43.7% had family history of premature coronary artery disease. In order to assess the severity of coronary artery involvement in different blood groups, they were classified into two categories, O and non-O.

There was no statistically significant difference between blood group O (n = 164, 53.1%) and non-O blood groups (n = 145, 46.9%) in terms of diabetes mellitus, history of myocardial infarction or smoking. HDL-C showed statistically significant difference between O and non-O blood groups (P = 0.035, effect size = 0.23). There was a statistically significant difference between mild, moderate and severe coronary artery involvement with O and non-O blood groups (P = 0.004). Variables with P-value < 0.200 in O and non-O blood groups in table 1 were included in adjusted model to eliminate the potential confounder effect. Table 2 represents the adjusted and unadjusted effect of blood group on severity of CAD.

Discussion

CAD is the leading cause of death all over the world, so evaluation of all aspects that may predispose to CAD is important.

Our study agree with the recent suggestions on the association of blood groups with CAD. In our study, patients with blood group O had more severe form of coronary involvement. Carpeggiani et al. reported that mortality of ischemic heart disease was more common in non-O blood group which is opposed to our result.[10] In the study of Amirzadegan et al., there was no significant difference between frequency of ABO blood group in patients with CAD in an Iranian population.[11] As shown by Biswas et al. in a Bengali Asian-Indian population, AB blood group decreased the risk of CAD and the O blood group was more frequent in CAD which is the same as our result.[14] In the study of Dodiya et al. on 256 patients with CAD, a significant association was observed between ABO blood group and CAD. Early onset of CAD was more common in blood group O and A compared with B and AB.[15] In the study by Sujirachato et al., patients with coronary atherosclerosis and blood group O had increased sudden cardiac deaths that were more common in women.[16] As shown by Whincup et al.[17] in British men and McKeigue et al.[18] in Asians in northwest London, blood group A was more frequent in patients with CAD, which was opposed to our study. Biswas et al.[14] showed that blood group O was associated with low HDL-C level, which was the same as our result. Although HDL-C showed statistically significant difference between the O and non-O groups, but the difference was not clinically significant.

Controversies between the association of blood group and CAD can be due to several confounding factors such as diabetes mellitus, hypertension and smoking. Other important factor is the rule of race and genetic which may have different impact on relationship between blood groups and coronary artery involvement among Asian and European. In addition, socioeconomic condition, environmental and Life style may have some effect on correlation of ABO and CAD.

The limitation of this study was study design, which was cross sectional. the causality could not be confirmed in a cohort study. In this study, the majority of patients were men and we recommend a gender specific population study.

Conclusion

ABO blood group seems to have an impact on the risk of coronary artery involvement and the type of blood group effects on severity of CAD. There may be different responses to usual medications used in the CAD. Identifying the specific target of ABO blood group, which plays a role in thrombosis and atherosclerosis, can lead to development of targeted therapeutic medications.

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Conflict of Interests

Authors have no conflict of interests.

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The effect of educational intervention on weight loss in adolescents with overweight and obesity: Application of the theory of planned behavior

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Original Article

Abstract

BACKGROUND: The increased prevalence of overweight and obesity in children and adolescents is associated with type 2 diabetes, hypertension, dyslipidemia, and cardiovascular diseases. The theory of planned behavior (TPB) efficiently explains the ability of perceived behavioral control and possibly attitude to enhance the motivations of the obese people to lose weight. Our aim was to investigate the effect of TPB-based education on weight loss in obese and overweight adolescents.

METHODS: In an interventional study, simple random sampling was used to select 86 overweight and obese adolescents aged 13-18 years in the pediatric clinic at the Isfahan Cardiovascular Research Institute. Anthropometric measures and TPB constructs were collected using a researcher-made questionnaire. The questionnaires were filled out before and six weeks after the intervention. Participants received 5 sessions of training based on the constructs of the TPB.

RESULTS: A significant increase was observed in the mean score for knowledge and TPB constructs (attitudes, subjective norms, perceived behavioral control, intention, and behavior) six weeks after the educational intervention ($P < 0.001$). Moreover, significant decrease in body mass index ($P < 0.001$), weight ($P = 0.001$), and waist circumference ($P < 0.001$) of adolescents were found after the educational intervention.

CONCLUSION: The TPB-based interventions seem to be effective in losing weight in obese and overweight adolescents. This theory serves as a helpful theoretical framework for health-related behaviors and can be an appropriate pattern to plan for educational interventions.

Keywords: Adolescents, Education, Obesity, Behavior

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Introduction

Currently, the prevalence of overweight and obesity are increasing worldwide. Over 1.12 billion people worldwide are projected to be overweight and obese up to 2030.[1] Overweight and obesity prevalence is increasing not only among adults but also especially in children and adolescents. These two conditions are known as the most common eating disorders among children and adolescents in the USA.[2]

Overweight and obesity prevalence has markedly increased in the recent decades in Iran due to changes in lifestyle and inappropriate eating behaviors. On average, 5.0%-13.5% of children and 3.2%-11.9% of adolescents under 18 years in Iran have been reported to be overweight and obese.[3] Increase in prevalence of overweight and obesity among adolescents is associated with early maturation, type 2 diabetes, hypertension, dyslipidemia, cardiovascular diseases, and some types of cancer.[4] Besides that, overweight and obesity are associated with certain cognitive and social problems that can affect children's and adolescents' psychosocial health including discrimination, low self-esteem, depression, dissatisfaction with body image, exposure to negative labels, and social exclusion in addition to adverse effects on physical health.[5]

In addition to inappropriate eating habits,

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physical inactivity or decline in physical activity are considered as predisposing factors for overweight and obesity in children and adolescents.[6]

Epidemiological and clinical studies have confirmed the role of low-calorie diet, intensified physical activity, and cognitive strategies to change behaviors.[7] These strategies include self-monitoring, problem-solving, planning, stress management, and gaining other children's social supports in managing adolescents obesity and associated cardio-metabolic risks. Findings, however, are not always consistent.[8]

Human behaviors are physical actions and observable emotions. Health education is required to recognize behavior and, if necessary, replace it with new behavior to develop effective programs.[9] This determines the role of models and theories in behavioral sciences in health education.[10]

The theory of planned behavior (TPB) is one of the known patterns of behavior change. According to the TPB, intention is the most important determinant of behavior. Intention itself is influenced by three independent constructs, i.e. attitude, subjective norms, and perceived behavioral control (PBC).[11] Attitude represents a positive or negative evaluation of behavior, subjective norms refer to perceived social pressure to do or not to do a particular behavior and PBC is the perceived ease or difficulty of a particular behavior that directly or indirectly affects the behavior. This theory states that people decide to exhibit a behavior when they evaluate it to be positive and believe that there are influencing and important people who think that they should perform that behavior, and perceive that they have control over doing that behavior.[12]

To develop a behavior based on theoretical principles, it is necessary to identify the most effective constructs on development of that behavior, and their direct and indirect effects so that more effective educational interventions can be developed and planned.[13]

Although the TPB has been frequently used in studies to predict exercise and healthy eating habits, a few number of such studies have considered weight-reduction behavior. In addition, most studies that have focused on populations other than obese women would supplement the applicability of the TPB to weight reduction.[14] The TPB has been demonstrated to be a helpful theoretical framework for many health-related behaviors. The determinants offer strong correlations to predict desirable behaviors.[14] Because the obesity that remains since childhood and adolescents can increase the risk of developing metabolic syndrome and cardiovascular diseases in youth and adulthood, it is essential to promote effective educational programs for weight loss in adolescents. Thus, our aim was to investigate the effect of TPB-based education on weight loss in obese and overweight adolescents.

Materials and Methods

This interventional study was conducted at the Isfahan Cardiovascular Research Institute, Isfahan, Iran, from July to September 2016. The probability of making type I error and the power of the hypothesis test was considered 0.05 and 0.9%, respectively. The standard deviation difference was 6 according to Muzaffar et al. study[15] and total sample size was estimated to be 86. After obtaining ethical clearance (ethical code number IR.SSU.SPH.REC.1394.74), total of 100 adolescents and their parents were invited to a meeting in Cardiovascular Research Centre through convenience sampling based on their records in the Pediatric Clinic. After receiving explanations about the study, they provided written informed consent to participate. The inclusion criteria were being 13-18 years old, volunteering to participate in the study and having body mass index (BMI) ≥ 25 according to age and gender.

The exclusion criteria were suffering from diseases such as hypothyroidism and Cushing's syndrome, having the history of taking drugs, BMI ≥ 25 for athletes, and refusing to participate in the study.

After selecting 86 adolescents, the pretest questionnaires were distributed. All of the cases consented to cooperate with the study. Figure 1 presents the flowchart of study participants.

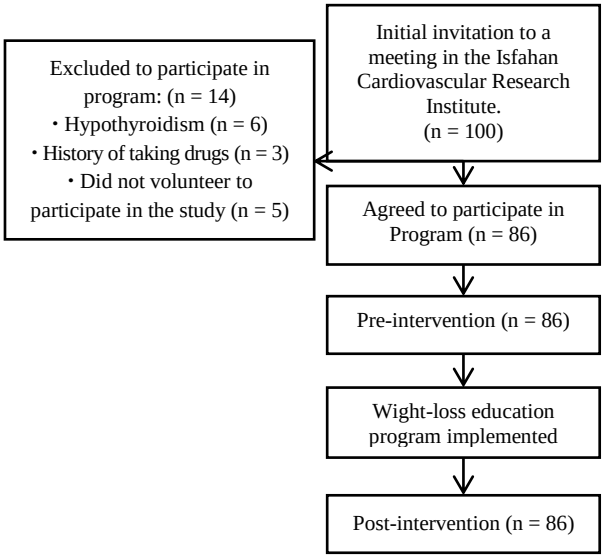

Figure 1. The study flowchart

After the data were collected, the TPB-based intervention for the adolescents and their parents included five 60-min educational sessions of speech, group discussion, role playing, questions and answers and pamphlets, booklet, photographs, movie screening, and PowerPoint displays. the man topics covered in the training sessions are as follows:

- Increasing knowledge of adolescent's about obesity and its signs, risk factors, symptoms, complication
- Development of positive attitudes and correction of false beliefs
- Talking about the role of nutrition in preventing overweight and obesity, and the importance of exercise in weight loss
- Strengthening social supports through holding the sessions with the presence of at least one of the parents and explaining the role of family members, friends, and teachers in weight loss
- Strengthening self-efficacy

Self-efficacy is influenced by four main information sources including experience, vicarious experience, verbal persuasion, and physiological and affective states that can be integrated either alone or in combination into a rehabilitation program.[16,17] In the session where vicarious experience was discussed to improve self-efficacy, a number of successful adolescents in weight loss were invited who participated in previous Pediatric Clinic programs. Furthermore, for strengthening self-efficacy in other sessions, three sources of self-efficacy including experience, verbal persuasion and physiological and affective states were used. We asked adolescents at the end of each session to watch the photographs and a short movie in the classroom. The adolescents were also recommended to enlist their goals in losing weight after watching the photographs and short movie. The photographs were about patients that suffered from overweight and obesity and the short movie showed that people could overcome any difficulty. Verbal persuasion was conducted in two ways: verbal encouragement by the researcher during education intervention, and telephone follow-ups throughout the study. This part of the intervention continued on a weekly basis for 6 weeks after education intervention. Topics from the previous sessions were reviewed and the subjects were offered educational booklet. Summative evaluation was conducted six weeks after completing the educational intervention using the same self-report questionnaires administered in the pretest.

Anthropometric parameters including weight, height, and waist circumference were measured twice using standard tools, before and six weeks after the educational intervention. Height and weight were measured with minimal clothing and without shoes. Height was recorded to the nearest 0.5 cm. Weight was also measured to the nearest 0.1 kg using a balance (Seca) scale. The waist circumference was measured at the midpoint between the bottom of the rib cage and the top of the iliac crest at the completion of exhalation. BMI was calculated as weight (kg) divided by height in squared meter. Based on the International Obesity Task Force (IOTF) definition, BMI was classified into three categories of normal (BMI $\leq$ 25 kg/m^2), overweight (BMI $\geq$ 25 kg/m^2) and obese (BMI $\geq$ 30 kg/m).[18]

We designed questionnaire according to a study guideline,[19] and scientific resources and then elicited the expert comments including the professors of health education, nutrition and exercise physiologist to develop the items.

The questionnaire was developed based on the TPB (Table 1).

Table 1. Some illustrative items of the Questionnaire

TPB constructs	Questions	Scale
Knowledge	Which of the following diseases is created with overweight and obesity?	True /False
Attitudes	Weight loss decreases the risk of cardiovascular disease, for me. Prevention of cardiovascular disease …	Absolutely agree to Absolutely disagree It is very important to It is not very important
Subjective norms	My parents regularly encourage me for weight loss. What is the opinion of parent's importance for my weight loss?	Absolutely agree to Absolutely disagree Much frequently to Never
PBC	The use of low-caloric diet for weight loss is difficult for me.	Absolutely agree to Absolutely disagree
Intention	I intend to get regular physical activity in free time for weight loss.	Absolutely agree to Absolutely disagree
Behavior	Do you have fruits and vegetables 2 or 3 time each day?	Never, Rarely, Sometimes, Frequently, Always

TPB: The theory of planned behavior; PBC: Perceived behavioral control

The questionnaire consisted of seven sections: demographic items, questions for knowledge measurement (10 items, α = 0.88, true/false scale), and items on five TPB constructs i.e. attitude (20 items, α = 0.68, 5-point Likert scale), subjective norms (12 items, α = 0.79, 5-point Likert scale), PBC (10 items, α = 0.78, 5-point Likert scale), intention (7 items, α = 0.74, 5-point Likert scale), behavior [10 items, α = 0.67, 5-point Likert scale).

To determine the content validity, 10 specialists and professionals outside the team were consulted in the field of health education and health promotion (n = 8) and nutrition (n = 2). To investigate the reliability, a list of the items was completed by 35 adolescents aged 13-18 years with similar characteristics to target population in two successive 14-day periods. The total reliability of the instrument was 0.75 based on the Cronbach's alpha. Because the alpha values for the questionnaire used in this study were over 0.7, the instrument was considered reliable.[20]

Data were analyzed by SPSS software (version 20.0, IBM Corporation, Armonk, NY, USA). Quantitative data were expressed as mean standard deviation and qualitative as frequencies and percentages. The impact of the intervention on anthropometric indexes and constructs of TPB including knowledge, attitude, subjective norms, PBC, intention and behavior was assessed by paired t-test procedures. To investigate the normal distribution of the data, Kolmogorov-Smirnov test was used. $P < 0.050$ was considered significant.

Results

The mean age of the participants was 15.37 ± 1.54 years. At baseline, the mean BMI of the adolescents was 29.89 ± 4.38 kg/m^2, mean weight 82.46 ± 16.22 kg, and mean waist circumference 97.39 ± 10.87 cm. Table 2 shows the demographic data.

In adolescents, six weeks after the educational intervention, the mean scores for knowledge and the TPB constructs increased significantly ($P < 0.001$, Table 3). Besides that, after the educational intervention, the mean value of weight ($P = 0.001$), BMI and waist circumference ($P < 0.001$) of the adolescents decreased significantly (Table 4).

Discussion

The present study was conducted to investigate the effect of a TPB-based educational intervention on weight loss in overweight and obese adolescents in the Isfahan Cardiovascular Research Institute. Although the TPB has been heavily applied in studies that predict exercise and healthy eating habits, few of these studies have addressed the weight reduction behavior. Furthermore, few of the studies investigated change in body weight as a real behavioral outcome.[14]

Table 2. Socio-demographic characteristics of the participants

Variable	n (%)
Age years	
13	17 (19.8)
14	7 (8.1)
15	18 (20.9)
16	20 (23.3)
17	19 (21.1)
18	5 (5.8)
Sex/gender	
Girl	51 (59.3)
Boy	35 (40.7)
Educational level	
Grade 6-9 high school	25 (29.1)
Grade 9-12 high school	61 (70.9)
Maternal education	
Illiterate	0
Primary	7 (8.1)
Secondary	13 (15.1)
High school	46 (53.5)
College	20 (24.1)
Father education	
Illiterate	0
Primary	10 (11.6)
Secondary	25 (29.6)
High school	30 (34.8)
College	21 (24.0)
Mother's occupation	
Employed	13 (15.1)
Housewife	73 (84.9)
Father's occupation	
Self-employed	54 (62.8)
Worker	6 (7.0)
Employed	13 (15.1)
Retired	13 (15.1)
Number of family members	
Three	12 (14.0)
Four	52 (60.5)
Five	22 (23.3)
Monthly Family Income	
Less than RLs6,000,000 ($194)	0
RLs6,000,000- RLs10,000,000 ($194 to $323)	42 (48.8)
RLs10,000,000- RLs20,000,000 ($323 to $645)	31 (36.0)
More than RLs20,000,000 ($645)	13 (15.1)

Table 3. Comparison between the mean scores of adolescents' knowledge and the theory of planned behavior components

Variable	Before the intervention (n = 86)	6 weeks after the intervention (n = 86)	Mean difference (n = 86)	P*
	Mean ± SD	Mean ± SD	Mean ± SD	
Knowledge	21.43 ± 7.09	34.50 ± 2.12	13.06 ± 7.17	< 0.001
Attitude	84.18 ± 5.96	90.47 ± 4.34	6.29 ± 6.71	< 0.001
Subjective norms	47.48 ± 5.55	51.27 ± 4.87	3.79 ± 5.74	< 0.001
Perceived behavioral control	31.44 ± 7.35	42.16 ± 4.45	10.72 ± 6.99	< 0.001
Intention	28.66 ± 3.89	30.75 ± 3.83	2.93 ± 5.08	< 0.001
Behavior	20.88 ± 6.73	29.54 ± 4.98	8.66 ± 6.73	< 0.001

*Comparison between before and 6 weeks after the intervention, paired t-test
SD: Standard deviation

This study revealed the knowledge, attitude, social norms and PBC might affect intentions of overweight and obese adolescents to control their weight through educational intervention. We observed an increase in the adolescents' mean score for knowledge six weeks after the completion of the intervention.

This is consistent with the results of Hazavehei et al.,[21] Moradi et al.,[22] and Alizadeh et al.[23] The increase in knowledge and other constructs may represent the participants' access to information as well as their participation in the course held by the researcher about obesity and health issues for the adolescents and their parents.

There was a significant difference between attitudes of adolescents 6 weeks after educational intervention. More clearly, after the intervention, most adolescents believed they were at risk of obesity. Schifter and Ajzen reported a poor correlation between attitude and final body-weight changes,[24] whereas Palmeira et al. found that attitude was associated with body-weight reduction.[25] The high variance among individuals could explain these inconsistent findings. Development of a desirable attitude to promote the practicing of target behavior is one of the strategies that have been much frequently emphasized in the education about obesity prevention and control.[26] Therefore, educational interventions are expected to create an environment in which people can logically evaluate the consequences of practicing the current behavior and the positive outcomes of accomplishing the recommended behavior.

Subjective norms refer to an individual's perception about a particular behavior which is influenced by the judgment of others, including parents, sibling, friends, and teachers.[27] The mean score for subjective norms showed so increase after the intervention. This is in agreement with the results of McConnon et al.,[28] and Kothe et al.[29] However, Schifter and Ajzen,[24] and Ahmadi Tabatabaei et al.,[30] studies indicated no significant difference in the mean difference of subjective norms scores before and after the training. In the present research, the high score of subjective norms in the participant before the intervention implied that parents, relatives, doctors, health workers, friends and teachers had a high expectation of the population under study for weight reduction, with parents having the greatest share. Other measures taken in this study were holding training sessions attended by the parents, especially mothers, and preparing the pamphlets and booklets to strengthen the subjective norms of people influencing the participants, including the fathers and other family members.

Table 4. Mean and standard deviation of anthropometric indices in the adolescents' at the baseline and 6 weeks after the intervention

Variable	Before the intervention (n = 86)	6 weeks after the intervention (n = 86)	Mean difference (n = 86)	P*
	Mean ± SD	Mean ± SD	Mean ± SD	
Body mass index (kg/m^2)	29.89 ± 4.38	29.42 ± 4.23	-0.46 ± 0.78	< 0.001
Weight (kg)	82.46 ± 16.22	81.73 ± 15.84	-0.73 ± 1.96	0.001
Waist circumference (cm)	97.39 ± 10.87	95.43 ± 10.52	-1.96 ± 2.92	< 0.001

*Comparison before and 6 weeks after the intervention, paired t-test
SD: Standard deviation

The mean score on PBC in the present study showed that before the intervention, adolescents had low ability to control overweight and obesity. After the intervention, the mean score on PBC increased significantly. This is consistent with the results of Caron et al.,[31] Karimy et al.[32] and Pakpour Hajiagha et al.[12] In contrast, in the study by Ahmadi Tabatabaei et al., no significant difference was observed in the mean difference of scores of perceived behavioral control before and after the intervention.[30] The PBC of TPB addresses volitional control. However, there are controversies with the distinction between self-efficacy and PBC.[14] Self-efficacy is considered as an important predictor of behavior. People with high levels of self-efficacy remove obstacles ahead through improving self-management skills and perseverance, stand in the face of difficulties, and have more control over issues.[13,16]

Self-efficacy and perceived barriers are common variables in several theoretical frameworks concerned with health behaviors.[14] Therefore, certain opportunities to promote self-efficacy should be taken into account in designing the educational programs.

The mediator of behavior is intention, which is the perceived likelihood that an action is performed to achieve a targeted behavior; more clearly, no behavior occurs without intention.[11] High levels of intention can have optimal effects in increasing the behavior.[8] In the current study, the mean score on behavioral intention increased significantly. As a general rule, the more optimal attitude, subjective norms, and PBC are, the stronger an individual's intentions for adopting a behavior will be.[12] The results of the studies performed by Luszczynska et al.,[8] Giles et al.,[33] and Kothe et al.[29] are in agreement with the findings of the present study.

We found a significant difference between the mean score of weight loss behavior before and after the intervention. This shows the positive effects of the education on adolescent's behavior. Gardner et al.,[34] and Chung and Fong[14] demonstrated that behaviors for losing weight increased among women with obesity after the interventions. This is in agreement with the results of our study.

TPB-based educational programs, throughout six-week follow-up, were effective in reducing BMI, weight, and waist circumference in adolescents with overweight and obesity in our study, which is in agreement with the results of the studies by Pasdar et al.,[35] Duangchan et al.,[36] and Kazemi and Mazloom.[37]

The authors believe that although the mean BMI and weight of the adolescents decreased in the present study, it should be mentioned that BMI and weight changed slightly due to the adolescents' negligible height growth within the short duration of the educational intervention to the post-test. Because BMI normally increases with aging, maintaining BMI and even, in most cases, slight changes in this variable can be considered an achievement.[38]

It has been shown that theories that include self-efficacy such as transtheoretical model[17] and social cognitive theory[25] are the most predictive models for behavior change.

These findings also raise the question of whether the TPB can be used in designing and developing intervention methods for weight reduction programs. This study showed that the TPB can be effectively used in weight reduction programs targeting obese adolescents.

Strengths and limitations: There were a number of strengths to this study. First, this study has an explicit theoretical basis: the TPB was used in the current study as a theoretical framework to design, implement, and evaluate an intervention. Second, this study has been the first to utilize the TPB to develop and evaluate an intervention specifically designed for weight loss in adolescents with overweight and obesity in the Isfahan Cardiovascular Research Institute.

There were also a number of limitations to the present study. The outcomes were evaluated only 6 weeks after the educational intervention. Thus, future studies with longer follow-up periods are recommended for better evaluation. In addition, the final evaluation in this study was based on the adolescents' self-reports, which could be a source of bias. Hence, future studies can use a combination of self-reports, direct observation of the behavior, and parents report.

Conclusion

The TPB-based interventions were effective in losing weight in adolescents with overweight and obesity. Additionally, all the TPB constructs played a key role in weight loss in adolescents. The TPB efficiently explains the ability of PBC and possibly the attitude to increase the intention of obese adolescents to obtain superior weight loss results.

Implications: This theory can serve as a helpful theoretical framework for health-related behaviors and also it can be an appropriate pattern to plan for educational interventions. Health knowledge, attitudes, and behaviors are learned during

childhood and adolescence. Receiving education based on models whose efficiency has already been confirmed, such as TPB, in the early years of life and repeating it in adolescents contribute greatly to the prevention and treatment of overweight and obesity, metabolic syndrome, and cardiovascular diseases as well as reduction in risk factors for health. Due to the appropriate educational field and cost-effective educational intervention at school, the generalization of such training programs in other areas seems critical.

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Conflict of Interests

Authors have no conflict of interests.

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The effect of exercise training on upregulation of molecular markers of bile acid metabolism in the liver of ovariectomized rats fed a cholesterol-rich diet

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Original Article

Abstract

BACKGROUND: Small heterodimer partner (SHP) is an important transcriptional factor involved in the regulation of glucose, lipid, and bile acid metabolism in the liver. SHP has been reported to be down-regulated in ovariectomized (Ovx) mice and up-regulated by estrogens suggesting a link between estrogens and SHP. The aim of the present study was to determine the effects of exercise training on SHP and key molecular markers of cholesterol and bile acid homeostasis in Ovx rats under cholesterol feeding.

METHODS: Our main experimental group was composed of Ovx rats fed a high-cholesterol diet (Ovx-Chol) that was compared to a group of Ovx rats fed a standard diet (Ovx-SD) and a group of sham operated rats fed the cholesterol diet (Sham-Chol). These three groups of Ovx and sham rats were subdivided into either voluntary wheel running (Tr) or sedentary (Sed) groups for 5 weeks. The mRNA expression of all genes was measured by quantitative real-time polymerase chain reaction.

RESULTS: Liver total cholesterol levels were not affected by exercise training in any of the experimental conditions. Cholesterol feeding in both sham and Ovx rats resulted in significantly higher hepatic cholesterol accumulation than in Ovx-SD ($P < 0.001$). Hepatic low density lipoprotein receptor (LDL-R) involved in cholesterol uptake from circulation was not influenced by training. A main effect of training was, however, found for transcripts of SHP and cholesterol 7 alpha-hydroxylase (CYP7A1, $P < 0.050$). CYP7A1 is the main gene involved in bile acid biosynthesis from cholesterol.

CONCLUSION: These results suggest that voluntary wheel running modulates cholesterol metabolism in Ovx animals through up-regulation of SHP and bile acid formation.

Keywords: Exercise, Cholesterol 7 Alpha-Hydroxylase, Rat, Cholesterol, Low Density Lipoprotein Receptor

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Introduction

Accumulated evidence from human and animal studies shows that estrogen deficient state leads to disturbances in fat and cholesterol metabolism.[1,2] While most studies were limited to assessment of plasma cholesterol levels, recent studies indicated that hepatic cholesterol metabolism is also affected by estrogen withdrawal.[3,4] Considering that the liver is a master regulator of cholesterol metabolism, there is a need for a better understanding of the liver response under estrogen withdrawal. Nutritional approaches have frequently been used to investigate the response of the liver to estrogen deficient conditions.[5,6] For instance, a large hepatic cholesterol accumulation was observed in ovariectomized (Ovx) animals when fed a high-fat and/or high-fat high-cholesterol diet.[4,7] In addition to hepatic cholesterol accumulation, it has been shown that the combined effect of cholesterol diet and ovariectomy resulted in suppression of transcripts of hepatic bile salt export pump (BSEP) and Na^+-taurocholate cotransporting polypeptide (NTCP), two transporters of bile acids in liver.[8] From this last study, it appears that a better

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knowledge of the contribution of bile acid transport/metabolism, which is the main way of elimination of excess cholesterol transiting through the liver, can shed some light on how liver regulates cholesterol metabolism in Ovx animal.

In addition to nutritional approaches, there is some evidence that exercise training may also affect hepatic cholesterol metabolism. For instance, it has been reported that voluntary wheel running increased cholesterol conversion into bile acids based on the observation of increased fecal bile acid excretion and consequently decreased atherosclerotic burden in low density lipoprotein receptor (LDL-R) deficient mice.[9] However the underlying molecular mechanisms for these observations have not been fully explored.

One of the key molecules involved in bile acid metabolism is small heterodimer partner (SHP). SHP interacts with several nuclear receptor family members. Through these interactions, SHP is involved in diverse metabolic pathways, including cholesterol, bile acid, triglyceride, and glucose homeostasis.[10,11] The interaction of SHP and farnesoid X receptor (FXR) is a well-known relationship that results in bile acid homeostasis. FXR is known as a bile acid receptor and bile acid-activated FXR induces SHP gene expression that results in inhibition of cholesterol 7 alpha-hydroxylase (CYP7A1) gene transcription.[12-14] CYP7A1 catalyzes the rate-limiting step in cholesterol conversion into bile acids in liver.[15] Moreover, it was also reported that SHP is down regulated in Ovx mice and alternatively, up-regulation of SHP by estrogens suggests that there is a link between estrogens and SHP.[16] Therefore, the main aim of the study was to determine the effect of training on key molecular markers of hepatic FXR/SHP/CYP7A1 pathway involved in bile acid metabolism in Ovx rats fed a cholesterol diet. We also targeted gene expression of the molecules involved in hepatic cholesterol metabolism including LDL-R and low-density lipoprotein receptor-related protein 1 (LRP1).

Materials and Methods

Female Sprague-Dawley rats (n = 49) weighing 180–200 g were obtained from Charles River (St-Constant, PQ, Canada) and housed individually to monitor food intake. The animals had ad libitum access to food and tap water. Their environment was controlled in terms of light (12 h light–dark cycle starting at 06:00 AM), humidity and room temperature (20–23 °C). Body weight and food intake were monitored bi-weekly from the start of experiment. All experimental procedures were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of the University of Montreal in agreement with the Canadian Council on Animal Care's rules (CCAC-CCPA).

At 1 week after their arrival, rats underwent either a bilateral ovariectomy (Ovx, n = 34) or a bilateral sham-operation (Sham, n = 15) according to the technique described by Robertson et al. under isoflurane anaesthesia.[17] After surgery, all animals were injected with antibiotics (Tribrissen 48%; 0.125 cc/kg, subcutaneously) and analgesics (carprofen; 4.4 mg/kg, subcutaneously) for 3 days. Then sham and Ovx rats were given either a standard diet (SD) or a high-cholesterol diet (Chol). The Chol diet consisted of a standard diet (SD) supplemented with 0.25% cholesterol (SD + Chol). Our main experimental group was composed of Ovx rats fed a high-cholesterol diet (Ovx-Chol, n = 17) that was compared, on one hand, to a group of Ovx rats fed a standard diet (Ovx-SD, n = 17) to observe the effects of the diet and, on the other hand, compared to a group of sham rats fed the cholesterol diet (Sham-Chol, n = 15) to observe the effect of estrogen withdrawal. These three groups of Ovx and sham rats were also subdivided into either voluntary wheel running (Tr) or sedentary groups (Sed). Tr rats were placed in freely rotating wheel cages while Sed rats were placed in blocked running wheel cages. Each wheel cage was equipped with a sensor connected to a computerized data acquisition system enabling the continuous sampling of running data from individual rats. The rats were on diet and training for 5 weeks.

Rats were euthanized between 09:00 and 12:00 AM. Food was removed from the cage overnight before sacrifice. Rats refrained from exercising ~24 h before sacrifice. Immediately after complete anaesthesia with isoflurane, the abdominal cavity was opened following the median line of the abdomen. Approximately 4 ml of blood was collected from the abdominal vena cava (< 45 s) into syringes treated with ethylenediaminetetraacetic acid (15%; EDTA). Blood was centrifuged (3000 rpm; 4 °C; 10 min; Beckman GPR Centrifuge; Beckman Coulter) and the plasma was kept for further analyses. Immediately after blood collection, the liver median lobe was removed and freeze-clamped. This sample was used for cholesterol and mRNA determinations. Several organs and tissues were removed and weighed (Mettler AE 100) in the

following order: uterus, urogenital, retroperitoneal and mesenteric fat deposits. The urogenital fat pad included adipose tissue surrounding the kidneys, uterus and bladder as well as ovaries, oviducts and uterus. The retroperitoneal fat pad was taken as that distinct deposit behind each kidney along the lumbar muscles. The mesenteric fat pad consisted of adipose tissue surrounding the gastrointestinal tract from the gastroesophageal sphincter to the end of the rectum, with special care taken in distinguishing and removing pancreatic cells. All tissue samples were frozen in liquid nitrogen immediately after being weighed (Mettler AE-100). All tissue samples were stored along with plasma samples at −80 °C until analyses were performed.

Liver total cholesterol (TC) levels were determined with some adaptations of the procedure described by Folch et al.[18] Briefly, 0.1 g of liver was homogenized in a chloroform–methanol mixture (2:1, v/v). The chloroform layer was collected and evaporated overnight. After adding 10% Triton X-100 in isopropanol, the sample was assayed for total cholesterol using commercial kits according to the manufacturer's instructions (Wako Diagnostics and Chemicals USA, Richmond, VA, USA).

RNA isolation and quantitative real-time (RT) polymerase chain reaction (PCR): Total RNA was extracted from frozen liver using RNA extraction Mini kit (Invitrogen) according to the manufacturer's protocol. Then RNA was treated with DNase (Invitrogen) in order to avoid genomic contamination. Total RNA (2 µg) was reverse-transcribed into complementary DNA using high capacity complementary DNA reverse transcription kits (Applied Biosystems). RT samples were stored at -20 °C. Gene expression for β-actin was determined using a pre-validated Taqman Gene Expression Assay (Applied Biosystems, Rn01462661, Foster City, CA). Gene expression level for target genes was determined using assays designed with the Universal Probe Library from Roche. The primer sets used to generate amplicons are presented in (Table 1). To validate the efficiency of the qPCR assays, we used a mix of the samples tested in the study. The ABI PRISM® 7900HT (Applied Biosystems) was used to detect the amplification level and was programmed with an initial step of 3 min at 95 °C, followed by 40 cycles for 5 s at 95 °C and 30 s at 60 °C. All reactions were run in triplicate and the average values of threshold cycle (C_T) were used for quantification. β-actin was used as endogenous control. The relative quantification of target genes was determined using the $\Delta\Delta C_T$ method. Briefly, the C_T values of target genes were normalized to an endogenous control gene (β-actin) ($\Delta C_T = C_{T\ target} - C_{T\ \beta\text{-actin}}$) and compared with a calibrator: ($\Delta\Delta C_T = \Delta C_{T\ Sample} - \Delta C_{T\ Calibrator}$). Relative quantification (RQ) was calculated using the Sequence Detection System (SDS) 2.2.2 software (Applied Biosystems) through the following formula: $RQ = 2^{-\Delta\Delta C_T}$.

All data are presented as mean ± standard error. Statistical significance ($P < 0.050$) was determined using a 2-way analysis of variance (ANOVA) for non-repeated measures with exercise and surgery-diet as main factors. Interpretation of the comparisons was made only between the Ovx-Chol and the Ovx-SD groups on one hand and between the Ovx-Chol and the Sham-Chol groups on the other hand. Fisher's least significant difference (LSD) post hoc test was used in the event of a significant interaction effect. For a significant surgery-diet effect without interaction, Fisher's LSD from a one-way ANOVA was used.

Table 1. Oligonucleotide primers used for quantitative real-time polymerase chain reaction

Gene	Forward primers	Reverse primers
CYP7A1	Ggagcttatttcaaatgatcagg	cactctgtaaagctccactcactt
FGFR4	Ttgaggcctctgaggaaatg	tcttgctgctccgagattg
FXR	Ccacgaccaagctatgcag	tctctgtttgctgtatgagtcca
HMG-CoAr	Caaccttctacctcagcaagc	acagtgccacacacaattcg
LDL-R	Tgctactggccaaggacat	ctgggtggtcggtacagtg
LRP1	Aatcgagggcaagatgacac	ccagtctgtccagtacatccac
NTCP	Aaaatcaagcctccaaaggac	ttgtgggtacctttttccaga
SHP	Cctcggtttgcatacagtgtt	aggttttgggagccatcaa
SREBP2	Gtgcagacagtcgctacacc	aatctgaggctgaaccagga
ActB	Cccgcgagtacaaccttct	cgtcatccatggcgaact
Cyclophilin B	Acgtggttttcggcaaagt	cttggtgttctccaccttcc

CYP7A1: Cholesterol 7 alpha-hydroxylase; FGFR4: Fibroblast growth factor receptor 4; FXR: Farnesoid X receptor; HMG-CoAr: 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDL-R: Low-density lipoprotein-receptor; LRP1: Low density lipoprotein receptor-related protein 1; NTCP: Na+-taurocholate cotransporting polypeptide; SHP: Small heterodimer partner; SREBP2: Sterol regulatory element binding protein 2; ActB: Actin Beta

Table 2. Anthropometric parameters and food intake

Variables	Ovx-SD		Ovx-Chol		Sham-Chol	
	Sed	Tr	Sed	Tr	Sed	Tr
Final body weight (g)	438.70 ± 9.30	452.50 ± 14.10	433.20 ± 10.60	450.60 ± 11.60	372.30 ± 13.20$^{\dagger\dagger\dagger,\delta\delta\delta}$	346.60 ± 10.60$^{\dagger\dagger\dagger,\delta\delta\delta}$
Intra-abdominal fat pad weights (g)	37.80 ± 2.80	39.60 ± 3.90*	39.30 ± 3.80	34.30 ± 4.30*	30.20 ± 4.50$^{\dagger\dagger\dagger,\delta\delta\delta}$	14.30 ± 2.80$^{*,\dagger\dagger\dagger,\delta\delta\delta}$
Food intake (kcal/day)	100.10 ± 2.90	114.70 ± 3.70***	101.80 ± 2.20	113.00 ± 3.10***	92.60 ± 5.60$^{\dagger,\delta}$	101.80 ± 2.50$^{***,\dagger,\delta}$
Uterus (g)	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.54 ± 0.08$^{\dagger\dagger\dagger,\delta\delta\delta}$	0.55 ± 0.07$^{\dagger\dagger\dagger,\delta\delta\delta}$

Ovx: Ovariectomized; Sham: Sham operated; SD: Standard diet; Chol: Standard diet + 0.25% cholesterol; Sed: Sedentary group; Tr: Trained group

Values are mean ± standard error

* Significantly different from respective Sed group ($P < 0.050$); *** ($P < 0.001$); † Significantly different from respective Ovx-SD group ($P < 0.05$); ††† ($P < 0.001$); $^{\delta}$ Significantly different from respective Ovx-Chol group ($P < 0.05$); $^{\delta\delta\delta}$ ($P < 0.001$)

Results

Anthropometric parameters, food intake and total distance run: Running did not significantly impact on final body weight in any of the experimental groups; However, running increased ($P < 0.001$) food intake in all trained groups (Table 2). On the other hand, final body weight ($P < 0.001$) as well as food intake ($P < 0.050$) were lower in Sham-Chol group compared to both Ovx groups. Intra-abdominal fat pad weight, which was composed of urogenital, retroperitoneal and mesenteric fat deposits, was decreased ($P < 0.050$) by training in both sham and Ovx groups fed the cholesterol diet, whereas Ovx-SD group showed slightly higher intra-abdominal fat weight under training. Similar to body weight, intra-abdominal fat pad weight was significantly ($P < 0.001$) higher in the two Ovx groups compared to Sham-Chol group. The Chol diet as compared to the SD diet in Ovx animals had no effect on the final body weight, food intake and intra-abdominal fat pad weight. Uterus weight was higher in sham rats compared to Ovx groups confirming total ovariectomy in Ovx rats (Table 2). Total running distance was 6.09 ± 0.39 km/d in Sham-Chol rats. Ovx-SD and Ovx-Chol rats ran 2.79 ± 0.30 and 2.82 ± 0.33 km/d, respectively.

Molecular markers of bile acid metabolism: The most significant effects of training in the present study were found for hepatic gene expression of SHP and CYP7A1 with higher values measured in Tr compared to Sed rats in all experimental conditions ($P < 0.05$, Figure 1 a). The SHP and CYP7A1 responses were not significantly affected by the surgery and the diet. However, their transcription factor, FXR mRNA was decreased in cholesterol-fed rats. There was no impact of exercise training on FXR transcript (Figure 1 a). Hepatic gene expression of NTCP, involved in bile acids uptake at the basolateral membrane of hepatocytes, and fibroblast growth factor receptor 4 (FGFR4) were decreased ($P < 0.001$) following the cholesterol diet but their gene expressions were not affected by training (Figure 1 b). Hepatic FGFR4 mediates the effect of intestinal fibroblast growth factor 15 (FGF15) on suppression of CYP7A1 in liver.

Molecular markers of hepatic cholesterol synthesis and uptake: Gene expression levels of sterol regulatory element binding protein 2 (SREBP2), a key regulator of hepatic cholesterol content, as well as its target genes including 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoAr) and LDL-R were not changed by training (Figure 2). Cholesterol feeding in both sham and Ovx rats led to lower ($P < 0.010$) gene expression of SREBP2 and all of its aforementioned target genes compared to Ovx rats fed the SD (Figure 2). HMG-CoAr is involved in cholesterol biosynthesis in liver. These results imply that the hepatic transcript of SREBP2 and all its target genes involved in hepatic cholesterol biosynthesis and cholesterol uptake from circulation were down regulated by the cholesterol diet.

In addition to LDL-R, LRP1 is also involved in hepatic cholesterol uptake form circulation. Running had no impact on the gene expression level of LRP1 similar to LDL-R. On the other hand, the expression level of LRP1 was lower ($P < 0.010$) in Ovx-Chol than in both Ovx-SD and Sham-Chol groups (Figure 2). These findings indicate that LRP1 gene expression was significantly reduced by the combined effect of cholesterol feeding and ovariectomy.

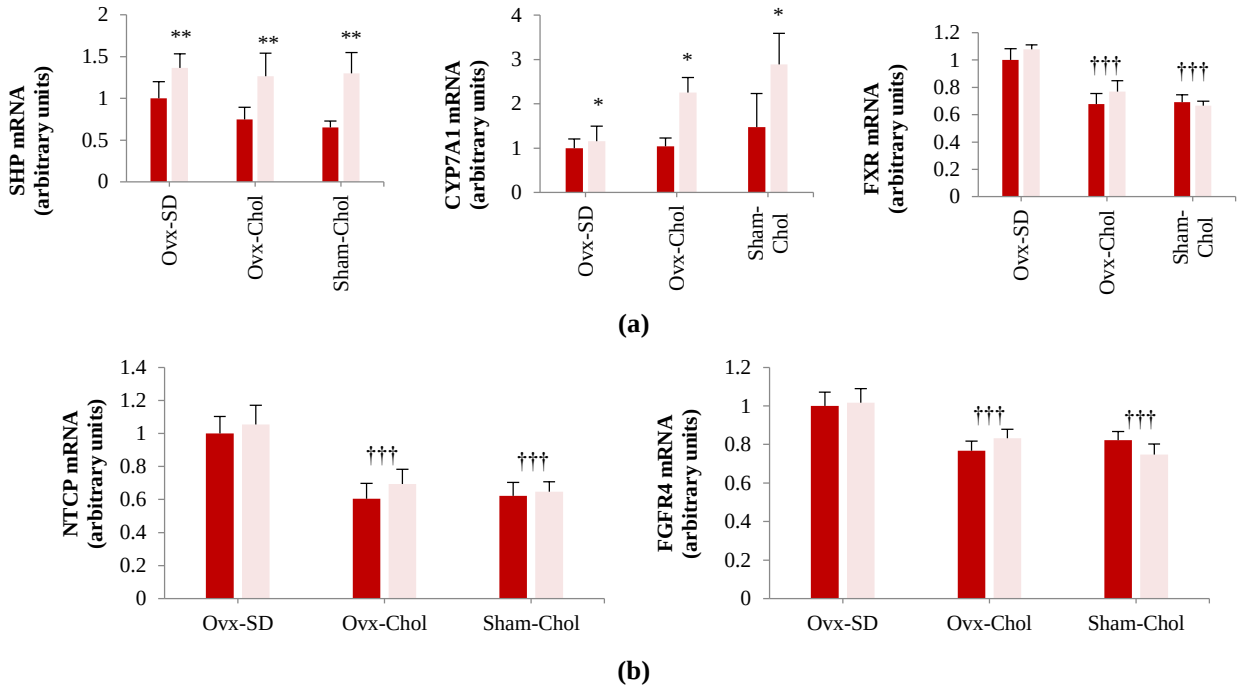

Figure 1. Hepatic mRNA expression of genes related to bile acid metabolism in ovariectomized (Ovx) rats fed a standard diet (SD) (Ovx-SD), Ovx rats fed a standard diet + 0.25% cholesterol (Chol) (Ovx-Chol) and sham operated (Sham) rats fed the Chol diet (Sham-Chol) in sedentary (■) or trained (□) state

Values are mean ± standard error

(a) * Significantly different from respective sedentary group ($P < 0.050$), ** ($P < 0.010$); ††† Significantly different from respective Ovx-SD group ($P < 0.001$)

SHP: Small heterodimer partner; CYP7A1: Cholesterol 7 alpha-hydroxylase; FXR: Farnesoid X receptor

(b) ††† Significantly different from respective Ovx-SD group ($P < 0.001$)

NTCP: Na^{+}-taurocholate cotransporting polypeptide; FGFR4: Fibroblast growth factor receptor 4

Liver TC content: Liver TC content was not affected by training in any of the nutritional conditions (Figure 3). Nevertheless, cholesterol feeding in both Sham and Ovx rats led to significantly ($P < 0.001$) higher hepatic cholesterol accumulation than in Ovx rats fed the SD. Moreover, liver TC was significantly higher in sham than in Ovx animals fed the cholesterol diet ($P < 0.050$, Figure 3).

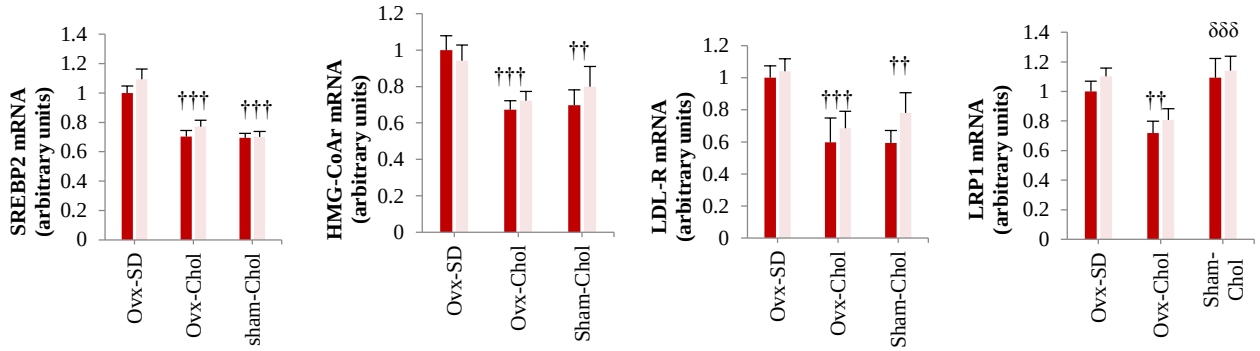

Figure 2. Hepatic mRNA expression of genes involved in hepatic cholesterol biosynthesis and cholesterol uptake from the circulation in ovariectomized (Ovx) rats fed a standard diet (SD) (Ovx-SD), Ovx rats fed a standard diet + 0.25% cholesterol (Chol) (Ovx-Chol) and sham operated (Sham) rats fed the Chol diet (Sham-Chol) in sedentary (■) or trained (□) state

Values are mean ± standard error

† Significantly different from respective Ovx-SD group ($P < 0.050$), †† ($P < 0.010$), ††† ($P < 0.001$) δδδ Significantly different from respective Ovx-Chol group ($P < 0.001$)

SREBP2: Sterol regulatory element-binding protein-2; HMG-CoAr: 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDL-R: Low-density lipoprotein-receptor; LRP1: Low-density lipoprotein-receptor-related protein-1

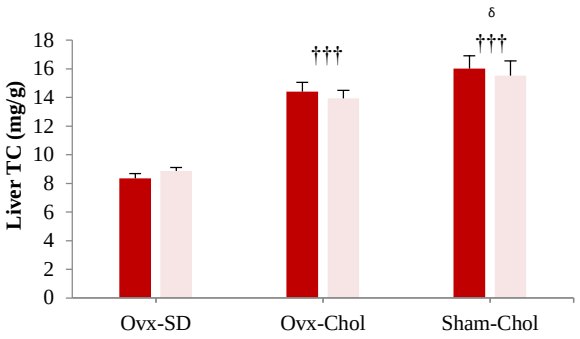

Figure 3. Liver total cholesterol (TC) content in ovariectomized (Ovx) rats fed a standard diet (SD) (Ovx-SD), Ovx rats fed a standard diet + 0.25% cholesterol (Chol) (Ovx-Chol) and sham operated (Sham) rats fed the Chol diet (Sham-Chol) in sedentary (■) or trained (□) state

Values are mean ± standard error

††† Significantly different from respective Ovx-SD group ($P < 0.001$); δ Significantly different from respective Ovx-Chol group ($P < 0.050$)

Discussion

The main finding of our study is an increased gene expression of two key markers of bile acid metabolism (SHP and CYP7A1) found in the liver of exercise-trained rats. This finding was observed in animals fed a standard diet but more importantly when rats were fed a high-cholesterol diet and that independently of estrogen levels. This observation suggests that exercise training may help overcome a cholesterol load in liver by stimulating bile acid metabolism. On the other hand, exercise training was associated with no change in SREBP2, HMG-CoAr, LDL-R, and LRP1 transcripts indicating an absence of molecular effects on key markers of cholesterol synthesis and hepatic cholesterol uptake from the circulation.

FXR/SHP/CYP7A1 pathway: Exercise training has been for a long time associated with changes in plasma cholesterol levels favouring a decrease in LDL-cholesterol and an increase in high-density lipoprotein (HDL) levels, the latter being in line with an increase in the so-called reverse cholesterol transport.[19,20] However, the basic pathways responsible for these exercise-induced beneficial effects are poorly understood. In recent years, Meissner et al.[9] and Meissner et al.[21] reported an increased fecal bile acid excretion in healthy and especially in hypercholesterolemic mice suggesting that bile acid metabolism may be involved in the action of exercise training on cholesterol metabolism. An important novel finding of our study is the increase in mRNA expression of SHP and CYP7A1with exercise training in all experimental conditions. The importance of the present SHP up-regulation by exercise training is enlighten by findings of previous studies indicating that missense mutations and polymorphisms in the promoter and coding regions of SHP in human were associated with severe early-onset obesity and diabetes.[22,23] SHP is, therefore, an excellent candidate that may link cholesterol/bile acid regulation to glucose and lipid adaptations known to occur with exercise training. In contrast to SHP and CYP7A1, their nuclear receptor FXR was not affected by exercise training. Hepatic FXR transcript was decreased by the cholesterol diet in both Sham and Ovx rats suggesting that there is no bile acid accumulation in liver and as a result there is not inhibition on CYP7A1 gene expression. Bile acids function as natural ligands for the transcription factor FXR.[24] Bile acids induce FXR activation which leads to stimulation of SHP gene expression and subsequently results in inhibition of CYP7A1 gene transcription.[12] CYP7A1 is the main enzyme involved in bile acid biosynthesis from cholesterol in liver.[15] In line with the FXR transcript reduction in cholesterol fed rats, increased hepatic TC accumulation suggests that there is a need to convert the extra cholesterol to bile acids as a way to eliminate excess cholesterol in the form of bile acids from the liver. The observed increase in the hepatic CYP7A1 transcript is in line with the concept that exercise training may regulate excess cholesterol through bile acid metabolism. In concert with our finding, an increase in hepatic gene expression of CYP27A1, a second important hepatic enzyme of bile acid formation from cholesterol, has been previously reported in trained mice fed a lithogenic diet for 12 weeks, thus favouring the catabolism of cholesterol to bile acids and reducing gallstone formation.[25] On the other hand, Meissner et al.[9] and Meissner et al.[21] did not find any changes in gene expression of CYP7A1 in mice assigned to voluntary wheel running in spite of an increase in fecal bile acid excretion. However, Pinto et al.[26] recently reported an increase in hepatic CYP7A1 gene expression in the liver of mice trained for 6 weeks while there were no differences in the [3H] cholesterol excretion into feces between the sedentary and exercise groups. In the present study, CYP7A1 transcripts were increased with exercise training in Ovx as well as in sham rats fed the cholesterol diet indicating that this exercise effect takes place independently of estrogen levels. Taken together, the present data support the

concept that increased bile acid biosynthesis following exercise training may contribute to elimination of cholesterol accumulation in liver.

Relationship between SHP and CYP7A1: As mentioned earlier, SHP is known to suppress the gene expression of CYP7A1, the rate-limiting enzyme involved in conversion of cholesterol into bile acids.[12,14,15] It has been recently reported that mRNA levels of hepatic SHP were reduced in Ovx mice while estrogen administration up-regulated SHP expression through binding to its proximal promoter. SHP promoter has an estrogen receptor responsive element (ERE) site.[16,27,28] Moreover, it was shown that this estrogen receptor α binding site on the SHP overlaps with the known FXR binding site on the SHP promoter. The combination of ethinyl estradiol plus FXR agonists did not produce an additive induction of SHP expression in Ovx mice, suggesting that simultaneous occupancy of this site by both estrogen receptor and FXR could not happen. Surprisingly, it has been also reported that induction of SHP by ethinyl estradiol did not inhibit expression of the well-known SHP target genes, CYP7A1 or CYP8B1.[27] It is expected that activation of SHP inhibits expression of CYP7A1,[12] which was not observed under estrogen treatment. Furthermore, it seems that SHP may also act independently of FXR. Lack of inverse relationship between SHP and CYP7A1 in the present study is in line with preceding mentioned finding that suggests that stimulation of SHP by estrogens may not result in suppression of CYP7A1 transcripts.[27] It seems that exercise training in our study imitated the effect of estrogen therapy on SHP transcripts. In fact, the present finding that exercise training also up-regulated SHP expression in Ovx animals extends previous findings showing that exercise training provokes estrogen-like effects on the expression of several genes involved in the regulation of lipid metabolism in liver.[29]

In contrast to SHP and CYP7A1, gene expressions of other markers of bile acid metabolism in liver, including NTCP and FGFR4 were all decreased by the cholesterol diet in sham and Ovx rats but not influenced by the training state. Down-regulated gene expression of NTCP suggests that there is less bile acid influx to the liver from the enterohepatic circulation. Decreased transcript levels of FGFR4 by cholesterol feeding may indicate that the inhibitory effect of intestinal FGF15 on CYP7A1 which acts through hepatic FGFR4 might be reduced. This lack of inhibitory effect on CYP7A1 would reinforce the interpretation that increased CYP7A1 gene expression following exercise training might be the mechanism to eliminate extra cholesterol from the liver and ultimately from the body.

Hepatic HMG-CoAr, LDL-R, and LRP1: Cholesterol feeding in our study resulted in lower hepatic transcripts of SREBP2 and its target gene, HMG-CoAr in both sham and Ovx animals. Moreover, hepatic expression of LDL-R and LRP1, two other target genes of SREBP2 which are involved in hepatic cholesterol uptake from circulation, were suppressed by the cholesterol diet. It seems that hepatic cholesterol accumulation might be a reason for the suppression of hepatic cholesterol biosynthesis and cholesterol uptake from plasma. It might be a protective response to prevent more cholesterol accumulation in the liver. These findings are in concert with the previous study that showed cholesterol feeding led to a reduction in SREBP2, LDL-R and LRP1 expression in the liver.[4] Meissner et al.[21] reported that fecal excretion of bile acids and neutral sterols in running mice was a reflection of elevated endogenous hepatic cholesterol synthesis in running group compared to sedentary mice. This is hardly the case in the present study since cholesterol feeding resulted in lower expression of HMG-CoAr in both sham and Ovx rats regardless of exercise intervention. Therefore, higher CYP7A1 mRNA expression under training is likely a consequence of hepatic dietary cholesterol accumulation. It thus seems that exercise does not modulate a cholesterol load by reducing cholesterol synthesis, but rather by stimulating bile acid metabolism. Moreover, a training effect was not observed in mRNA expression of SREBP2, LDL-R, and LRP1 in liver. Previously it was reported that exercise training increased mRNA expression of SREBP2 in Ovx rats but had no effect on its target genes in liver.[4] These data, therefore, do not provide any evidence that exercise training affects hepatic cholesterol uptake from the circulation through the LDL-R pathway. On the other hand, Wen et al. reported that a high fat diet plus exercise for 8 weeks led to an increase in SREBP2 protein with elevated levels of hepatic LDL-R mRNA in mice due to a reduction in cholesterol accumulation in liver.[30]

Liver TC: Higher CYP7A1 expression in response to training suggests higher bile acid synthesis and more excretion of the cholesterol from the liver in the form of bile acid. Therefore, less cholesterol accumulation might be expected within the liver. However, liver TC content was not

changed by training under all the experimental conditions. On the other hand, exercise running in other studies resulted in decreased hepatic cholesterol content in male mice.[21,30] It is possible that the reported effects of training in Ovx rats under the present duration of observation are only at the molecular levels. Considering the rats were fed a diet rich in cholesterol, hepatic TC content response to a training stimulus may need a longer time course of study.

Conclusion

In conclusion, the results of the present study indicate that exercise training modulates hepatic cholesterol metabolism through the up-regulation of SHP and bile acid metabolism. It seems that increased mRNA expression of SHP and subsequently higher gene expression levels of CYP7A1 is a positive response triggered by exercise to alleviate hepatic cholesterol accumulation and help to drive cholesterol out of the liver. Elevated cholesterol turnover induced by exercise training may contribute to improve hepatosteatosis and decrease the risk of atherosclerosis.

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

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Mobile mass in the aortic arch: A case report

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Case Report

Abstract

BACKGROUND: The finding of a floating mass in the aortic arch is rare and the management remains controversial.

CASE REPORT: We describe a 42-year-old woman with an embolic infarction in whom transesophageal echocardiography revealed a mobile mass in the aortic arch that was characterized as atherothrombi with an evidence of embolic infarction in the territory of the middle cerebral artery. Treatment with antiplatelet and anticoagulants failed to resolve the mass and is surgically resected.

CONCLUSION: In conclusion, the presence of mobile aortic mass seems to carry a high embolic risk. The optimal treatment for mobile aortic arch atherothrombi remains to be elucidated.

Keywords: Embolism, Echocardiography, Transthoracic Echocardiography, Transesophageal Echocardiography

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Introduction

The finding of a floating mass in the aortic arch is rare and the management remains controversial. Complex aortic arch atheromatous plaque (plaque thickness ≥ 0.44 mm or plaque with mobile elements) is a potential source of emboli that has become increasingly common these days due to the advent and extensive use of echocardiography.[1] We report the case of a 42-year-old woman with embolic infarction in whom the transesophageal echocardiography (TEE) study revealed a mobile mass in the aortic arch.

Case Report

A 42-year-old woman presented to the neurology emergency department with headaches, lack of mobility in right hand and foot and lost speech ability. On physical examination, there was complete Broca's aphasia and right hemiplegia. Brain computed tomography (CT) scan showed an evidence of embolic infarction in the left middle cerebral artery (MCA) zone and the treatment for cerebrovascular accident was started. Then, the heart and carotids were evaluated.

Carotid Doppler ultrasound and electrocardiogram (ECG) were normal. The heart rhythm in ECG was normal showing a sinus rhythm. Transthoracic echocardiogram (TTE) was normal in chambers and heart valves, A suspicious mobile mass was seen in the aortic arch in assessment of suprasternal notch. To investigate further, the CT angiography of the thoracic aorta was performed and a filling defect was observed in the aortic arch. TEE was performed emergently. Hypermobile mass was seen in the aortic arch with the dimensions of 4.0 × 0.5 × 0.5. On the histopathology, the resected intra-aortic mass showed a blood clot. Regarding the nature of mass, hypermobility and lack of intracerebral hemorrhage in brain CT scan, the decision was made to urgently resect the tumor. The patient was immediately transferred to the operating room.

Midsternotomy was performed right after receiving heparin and arterial cannulation of the right atrium was performed with a grade 7 cannula. Anonymous artery was cannulated with a cannula number 14 and the patient was cooled to 18 degrees and then was given health care assistant (HCA). The base of the anonymous artery was clamped and arterial flow was maintained through the anonymous artery cannula to 800 cc/kg. First arc opening and cutting was undertaken during septum.

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A red mobile mass of 4.0 × 0.5 × 0.5 cm was observed at the junction of the ascending aorta and arch, with a soft consistency which was attached with a pike of 0.5 × 0.5 cm in aortic tissues. The free end of the mass reached the left carotid artery at the time of mobility which was consistent with the cerebrovascular accident (CVA) signs in the patient. Mass was removed with a margin of 2 mm from the wall of the aorta and aortic wall was repaired and the patient was warmed. After taking the appropriate course of action, the patient was referred to the heart department on the second day. The patient's clinical condition improved on the second day and she was discharged with aspirin and warfarin after 8 days. A follow-up echocardiogram one month later showed no change in the aortic arch and she had an uneventful surgical resection of the mass.

Discussion

In conclusion, "the presence of mobile aortic mass seems to carry a high embolic risk".[1] Echocardiographic evaluation of the aortic arch is mandatory in patients with embolism and mass and no obvious source of emboli.[1-3] Initial assessment can be performed by the suprasternal TTE view, followed by TEE, which is considered as the most reliable method for the detection of aortic arch atheroma.[1] The optimal treatment of mobile aortic arch atherothrombosis remains to be elucidated.[1,4,5]

TEE is an accurate, reproducible and widely applicable method for the diagnosis of aortic arch atheromatosis. However, even the application of TTE suprasternal observation can be diagnostic, especially in cases who are suffering from large atherothrombotic plaques in the aortic arch.[4-6] TEE may be combined with the Doppler ultrasound and color Doppler to evaluate blood flow across the heart's valves. In this case, the TTE suprasternal observation confirmed the presence of one mobile mass in the aortic arch, but for conducting an exploration through the rest of the thoracic aorta, the application of TEE was necessary.[1] In addition, the application of the real-time 3D echocardiogram provided the morphological picture of the mass as well as the aortic wall.[2,7] Future studies are needed to show whether the better visualization of aortic atheromas (in terms of size, protrusion, mobility) and aortic wall by real-time 3D echocardiography will provide additional information regarding the potential for embolism and the appropriate treatment.[1]

The management of aortic arch atherothrombi is still not crystal clear considering different opinions. Today, the aggressive management of hypertension and hypercholesterolemia as the risk factors and also the use of antiplatelets in patients suffering from symptomatic aortic atheroma is considered as an intelligent and widely-accepted therapeutic strategy.[2,7] Anticoagulants should be reserved for mobile atheromas.[8] Surgical thrombectomy or atherectomy should be applied in patients with a history of embolism and persistent mobile atheromas despite anticoagulation.[2,4] In our patient, the administration of anticoagulants and antiplatelets did not resolve the mass; therefore, the mass was removed surgically.[1]

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Conflict of Interests

Authors have no conflict of interests.

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Transcriptional activity of tumor necrosis factor-alpha gene in peripheral blood mononuclear cells in patients with coronary slow flow

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Short Communication

Abstract

BACKGROUND: Coronary slow flow (CSF), an angiographic phenomenon that is characterized by a delayed coronary blood flow in the absence of obstructive coronary artery stenosis, is known as a disorder of the coronary microcirculation. Inflammation has an important role in the vascular hemostasis and endothelial dysfunction especially regarding monocyte adhesion and infiltration. Pro-inflammatory cytokines released by inflammatory cells result in endothelial cell dysfunction and cardiovascular diseases. It has been demonstrated that tumor necrosis factor-alpha (TNF-α) mainly influences the vascular homeostasis and endothelial dysfunction. In the present enquiry the transcriptional activity of TNF-α gene in peripheral blood mononuclear cells (PBMCs) of patients with CSF was compared with healthy controls in order to further survey the role of TNF-α in pathophysiology of CSF.

METHODS: The study was carried out on 30 patients with CSF and 30 matched healthy controls. To analysis gene expression of TNF-α, total mRNA was isolated from PBMCs. The quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was used to compare the transcriptional activity of TNF-α gene between patients with CSF and controls.

RESULTS: The mean ± standard error of mean of fold in CSF patients and controls were 0.20 ± 0.04 and 1.38 ± 0.27, respectively. The mRNA mean expressions of TNF-α (fold) were different in tested groups, which indicated a significant decrease in TNF-α in patients with CSF group (P = 0.0001).

CONCLUSION: Expression of TNF-α was decreased in patients with CSF. Changes in TNF-α expression suggest a potential role for altered immune function in the pathophysiology of CSF.

Keywords: Inflammation, Tumor Necrosis Factor-alpha, Cytokines, Slow Flow Phenomenon, Coronary Angiography

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Introduction

Coronary slow flow (CSF) is defined as an angiographic phenomenon that is identified by the delayed opacification of the distal vessel in the absence of coronary artery stenosis. Etiology of this phenomenon is controversial.[1] The overall prevalence of CSF is 1%-7% among patients undergoing diagnostic angiography as the consequence of clinical distrust of cardiovascular disorders.[2] CSF is frequent among current smokers and has several clinical findings such as unstable angina, metabolic syndrome, high resting microvascular endothelial tone, and high-minded aortic stiffness.[3] CSF has been correlated to obesity as well as male gender.[4] Predictors of CSF are gender, body mass index (BMI), hypertension, a low level of high-density lipoprotein cholesterol, and high hemoglobin.[5] Pathogenic mechanism of CSF is

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complex and related to coronary microcirculation,[6] endothelial dysfunction,[7,8] atherosclerosis,[8,9] inflammatory parameters[10], and anatomic properties of coronary arteries.[11] The results of a recent study showed that endothelial function was impaired in CSF.[12] Also, plasminogen activator inhibitor-1 (PAI-1), angiotensin-converting enzyme (ACE) and endothelial nitric oxide synthase (eNOS) genes polymorphisms have not been associated with the risk of CSF. Several RNA based biomarkers have been studied in the case of human disease such as coronary heart disease,[13,14] and CSF.[15] The objectives of different studies were to investigate the pathophysiology of CSF.[12-17] The coronary microcirculation is under control of anatomical factors of pre-arterioles, arterioles, capillaries, and venules as well as several systemic factors.[18] Inflammatory cells and inflammation has an important role in the vascular homeostasis and endothelial dysfunction especially regarding monocyte adhesion and infiltration.[18] Outcome of troubled balance because of inflammation in the endothelial cells changes from an anti-inflammatory state to a pro-inflammatory condition. Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 (IL-1) are important mediators released by inflammatory cells and result in endothelial cell dysfunction and cardiovascular diseases.[19] Several inflammatory factors have been increasingly recognized regarding vascular dysfunction and vascular disease including cytokines and cell adhesion molecules and C-reactive protein and other markers.[20-23] TNF-α as a pro-inflammatory cytokine has several roles such as induction of expression of cell adhesion molecules including receptor for advanced glycation end products,[24] intercellular adhesion molecule-1 (ICAM-1) and E-selectin,[25] oxidized low-density lipoprotein (ox-LDL) receptor-1 by nuclear factor kappa B (NF-kappa B) activation[26] and eNOS activation.[27] It has been demonstrated that TNF-α mainly influences the vascular homeostasis and endothelial dysfunction with definite pathologies.[28-31] The genetics of human cardiovascular disease is complex and include several genetic risk factors.[32] Gene expression profiling in human cardiovascular disease shows an important role for IL-1β in coronary artery disease.[33] TNF-α is known as an ultimate mediator of the acute phase response and is involved in production of other inflammatory mediators including chemokines with important role in recruitment of leucocytes to the site of inflammation.[34,35] Elevated plasma and myocardial levels of TNF-α have been recognized in patients with heart failure.[36,37] The human TNF-α gene maps on chromosome 6, at 6p21.33 between the class I HLA-B and the class II HLA-DR genes.[38] TNF-α gene has 1 transcript and 4 exons.[38] In the present study, the transcriptional activity of TNF-α gene in peripheral blood lymphocytes (PBMCs) of patients with CSF was compared with healthy controls to assess the role of TNF-α in pathophysiology of CSF.

Materials and Methods

This case-control investigation was approved by Medical Ethics Committee of Urmia University of Medical Sciences, Urmia, Iran (IR.umsu.rec.1393.26). The case group contained of 30 patients with CSF. The study subjects included individuals who had a history of chest pain and angina and thrombolysis in myocardial infarction (TIMI) frame count (quantitative way of assessing coronary artery flow) greater than 23 frames. One proficient cardiologist checked all individuals in case group via angiography and confirmed the diagnosis of CSF. The control group contained of 30 healthy subjects who were matched with the case group in terms of gender, age, and BMI. Exclusion criteria were positive medical history regarding coronary ectasia, coronary intervention associated with slow flow, myocardial inflammation, surgery and infectious diseases within past six months, familial hypercholesterolemia, and congenital heart defects.[35] Study subjects completed the written informed consent. Ten ml whole blood was obtained from study subjects and collected in ethylenediamine tetraacetic acid (EDTA)-containing tubes. RNA was isolated from PBMCs using RNX™- Plus solution per manufacturer's instructions (CinaGen Co., Tehran, Iran). RNA samples were stored at -80 °C upon the next stage. The quality of extracted RNA was tested by BioPhotometer (Eppendorf AG, Hamburg, Germany). The RNA with poor quality (OD 260/280 ratio < 1.6, normal ratio > 1.8) was discarded. First strand cDNA synthesis was carried out using RevertAid First Standard synthesis kit (Fermentas, Lithuania) according to the manufacturer's instruction under settings of 65 °C for 5 min, 25 °C for 5 min, 42 °C for 60 min and 70 °C for 5 min. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was done via Maxima™ SYBR Green qPCR Master Mix (Thermo Scientific), primer pairs (Table 1) (GenFanavaran Co., Tehran, Iran)[35] on a real-time

Table 1. Oligonucleotide primers used for quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

Gene	Sequences	Amplicon size	Reference
HPRT	5'-cctggcgtcgtgattagtgat-3' (forward)	131 bp	35
HPRT	5'-agacgttcagtcctgtccataa-3' (reverse)		
TNF-α	5'-cccaggcagtcagatcatcttc-3´ (forward)	85 bp	
TNF-α	5'-agctgcccctcagcttga-3´ (reverse)		

HPRT: Hypoxanthine-guanine phosphoribosyl transferase; TNF-α: Tumor necrosis factor-alpha

PCR machine (Bio-Rad iQ™5 Multicolor Real-Time PCR Detection System). The PCR settings were as follows: initial denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 seconds and 58°C for 60 seconds. Real time PCR reactions were performed in duplicate. The cycle threshold (Ct) values were normalized against to endogenous reference gene of hypoxanthine-guanine phosphoribosyltransferase (HPRT).[35] Livak method was used for $2^{-\Delta\Delta Ct}$ analysis.[39]

All parameters were expressed as means ± standard deviation (mean ± SD) or mean ± standard error of mean (SEM). Relative amounts of mRNA expressions were compared between two groups using the independent sample t-test. Statistical analyses were done by SPSS for Windows (version 16, SPSS Inc., Chicago, IL, USA). In order to check the normality of the distribution, Kolmogorov-Smirnov test was performed. Differences were considered to be significant at P-value < 0.0500.

Results

The demographic data of the tested groups is summarized in table 2. In tested groups, the mean differences in systolic and diastolic blood pressures were statistically significant, but for other parameters, the difference was not significant. About 60.7% of cases were regular smokers and 26.7% of cases had family history of coronary heart disease.

The mean ± SEM of fold in patients with CSF and controls were 0.20 ± 0.04 and 1.38 ± 0.27, respectively. The mRNA mean expressions of TNF-α (fold) were different in tested groups, which indicated a significant decrease in TNF-α in patients group (P = 0.0001). Figure 1 shows gene expression of TNF-α in CSF patients and controls.

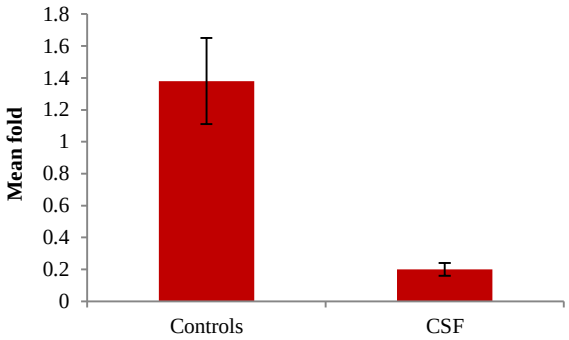

Figure 1. Tumor necrosis factor-alpha (TNF-α) mean fold changes in patients with coronary slow flow and controls
CSF: Coronary slow flow

Discussion

In the present investigation, analysis showed a significant decrease in the expression of the TNF-α gene in PBMCs of patients with CSF compared to controls. To the best of our knowledge, this is the first study which assessed the quantitative expression of TNF-α gene in PBMCs of patients with CSF. Our findings showed that TNF-α might be donated in CSF in a distinct way. TNF-α as a pleiotropic cytokine is associated with the acute response of inflammation and critically occupied in the pathogenesis of atherosclerosis and heart complications.[40] The role of TNF-α is dose dependent and also partially associated with the signal transduction regarding certain receptor.

Table 2. Demographic characteristics of participants

Parameter	Controls (n = 30)	CSF (n = 30)	P
Age (year)	51.37 ± 11.89	53 ± 11.83	0.5960
Sex (female:male)	8:22	8:22	1.0000
Body mass index (Kg/m^2)	27.44 ± 3.60	26.93 ± 4.46	0.6260
Heart rate (n)	78.12 ± 10.03	74.16 ± 7.69	0.1040
Systolic blood pressure (mmHg)	137.48 ± 13.79	128.03 ± 15.85	0.0050
Diastolic blood pressure (mmHg)	88.72 ± 10.31	81.86 ± 22.08	0.0070

CSF: Coronary slow flow

TNF-α and its related signal transducers regarding two receptors participate in cardiovascular disorders.[40] TNF-α receptor type 1 (TNFR1) with a molecular mass of 60 kDa is expressed in all cell types; but, TNF-α receptor type 2 (TNFR2) with a molecular mass of 80 kDa is expressed on cells of the immune system and on the endothelial cells.[4,40] In vascular dysfunction, TNF-α changes smooth muscle cell function and cell interactions and results in heart dysfunction.[4,41] TNF-α inhibits eNOS in a dose dependent manner and leads to vascular inflammation.[4,41] Patients with CSF were more likely to present with acute onset angina and abnormal ECG prompting emergency admission and rapid angiographic assessment.[42,43] Several investigations studied the pathophysiological mechanisms of CSF with conflicting findings such as inflammation and endothelial dysfunction,[44] and oxidative stress.[45] Endothelial dysfunction and inflammation have also been understood as mechanisms related to the CSF.[5] Several markers have also been shown to be associated with the CSF pathogenesis.[45] CSF is distinguished from cardiac syndrome X and microvascular angina and is understood as a separate clinical finding.[43-46] Inflammation and oxidative stress mechanisms play an important role in acute CSF but not in the case of chronic CSF.[6,18,46] As TNF-α levels are elevated in chronic inflammatory conditions, this suggests a potential role for altered immune function in the pathophysiology of CSF.

Future studies with big number of samples are essential to confirm the role of TNF-α in pathogenesis of CSF regarding TNF-α receptors and also more details including cytokines, as well as policies focusing on mechanisms involved in CSF.

Conclusion

Expression of TNF-α was decreased in patients with CSF. This phenomenon may affect the function of microvasculature and increases the risk.

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Conflict of Interests

Authors have no conflict of interests.

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