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Effects of Apple Supplement and Unsupervised Home-based Brisk Walking on Low Density Lipoprotein Cholesterol

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Abstract

Elevated levels of cholesterol present in Low-Density Lipoprotein (LDL) are directly associated with atherosclerosis. Therefore, the present study was an attempt to analyze the effect of apple supplement, restricted dietary fat and unsupervised home-based brisk walking program on the level of LDL-Cholesterol (LDL-C). Fourteen participants (11 males and 3 females) recruited voluntarily from Punjabi University, Patiala, constituted the study group. They were further subdivided into 2 groups. Whereas Group I consisted of participants with body mass index > 25 kg/m^2 and LDL-C≥ 110 mg/dl, Group II consisted of members with body mass index between 20-25 kg/m^2 and LDL-C<110 mg/dl. Members in Group I (n = 7) were given apple fruit (Pyrus Malus) twice a day free of charge and were advised unsupervised home-based walking activity for three weeks. Members in Group II (n = 7) were given apple fruit alone. During the period of the study all participants were advised dietary fat restriction and their fasting serum lipid profiles were estimated at 0 and 3 weeks. After 3 weeks of the study period, Group II lowered LDL-C by 18.57 mg/dl (95% confidence interval = 9.05-28.09, p=0.03), so did group I by 38.33 mg/dl (95% confidence interval = 25.62-51.04, p=0.01). Apple supplement combined with dietary advice to reduce fat intake and unsupervised home-based brisk walking program may result in significant reduction in LDL-C level in sedentary individuals.

Keywords: apple, brisk walking, dietary restriction, home-based, LDL-C, low-fat, Physical activity, sedentary, unsupervised

1. Introduction

Research from experimental animals, laboratory investigations, epidemiology, and genetic forms of hypercholesterolemia indicate that elevated serum level of Low-Density Lipoprotein Cholesterol (LDL-C) is an important independent risk factor for Coronary Heart Diseases (CHD) (Stefanick *et al*., 1998; Davidson *et al*., 1998; NCEP, 2001; Grundy *et al*., 2004; Zoeller, 2007). Fruit intake, reducing intake of low-fat dairy products, and engaging in a regular Physical Activity (PA) are considered as a protective factor for many chronic diseases **(**U.S. Department of Health and Human Services, 1996; Krauss *et al*., 2000; Hyson, 2002; WHO/FAO, 2003; Tully *et al*., 2005; Varady & Jones, 2005) and dietary guidelines have consistently recommended increased intake of fruits and vegetables, low-fat diet and PA (Krauss *et al.*, 2000). However, the effect of combined use of fruits and PA on lowering LDL-C is not determined. The two mechanisms have a potential to lower serum cholesterol and LDL-C. Most studies on the role of apples on health have focused on their lipid-lowering effects and more recently on their anti-oxidative properties. The fiber in apples is thought to play an important role in the reduction of lipids (Aprikan *et al*., 2003).

Extensive studies on experimental animals indicate that the addition of different types of dietary fibers have advantageous physiological effects such as reducing cholesterol levels in serum (Sembries *et al*., 2004). Apples are good sources of dietary fiber and have been shown to lower serum cholesterol concentrations in hypercholesterolemia (Brown *et al*., 1999; Bazzano *et al*., 2003; Boyer & Liu, 2004; Sembries *et al*., 2004; Edijala *et al*., 2005). Consuming apples lowered cholesterol and LDL-C on obese Zucker rats (Boyer & Liu, 2004). On the other hand, it is generally accepted that dietary advice to lower fat intake (King & Gibney, 1999), low-fat diets, in particular reducing saturated fat intake, significantly lowers total and plasma LDL-C concentrations. However, such diets also decrease High-Density Lipoprotein concentrations (HDL-C) and may increase plasma triacylglycerol concentrations (Hannah *et al*., 1997; Kasim-Karakas *et al*., 2000; Tully *et al*., 2005).

Many studies support moderate-intensity (such as brisk walking) to high-intensity exercise 3 to 5 times per week for at least 30 min per session to manage or improve cholesterol profiles (Tully *et al*., 2005; Zoeller, 2007). Regular exercise such as 30 min of brisk walking daily and increased levels of PA can lower levels of total cholesterol and LDL-C (U.S. Department of Health and Human Services, 1996; NCEP, 2001; Bernstein *et al*., 2002). Walking is a very acceptable form of exercise to a wide proportion of the population. It does not require any formal training or special equipment and can be performed in an individual's own locality and time (Tully *et al*., 2005).

Therefore, we carried out an experimental trial among normal and overweight 11 males and 3 females to determine primarily the effect of apple supplement and dietary advice to reduce fat intake and unsupervised home-based brisk walking program on the level of LDL-C.

2. Materials and Methods

Participants

This study is a one-time experimental trial involving a normal and overweight sedentary individual, which is being carried out in Panjabi University, Patiala (PUP), India. The study was not randomized and controlled. Rather fourteen (3 females) voluntary participants, who were regular students pursuing their undergraduate and graduate program, were recruited. Subjects were selected based on the following criteria: they were (1) between the ages 18-26 years, (2) in good health, with no history of cardiovascular or metabolic disease, (3) a body mass index between 20-40 kg/m^2, (4) non-smokers or abusing alcohol, (5) not taking medications known to interfere with lipid profile, (6) sedentary, and (7) no musculoskeletal contraindications to exercise were included in the study. Brief discussion about the experiment was organized to the participants to ensure better understanding of the protocols and procedures, benefits and associated risks of the study prior to the intervention; hence consent was acquired from the participants. In addition, seven participants (2 females) with body mass index > 25 kg/m^2 and level of LDL-C ≥110 mg/dl) were grouped into Group I (an exercise plus apple group); and the rest seven participants (a female) with body mass index between 20-25 kg/m^2 and level of LDL-C<110 mg/dl were grouped into Group II (apple group).

Procedures

Prior to the experiment, participants were instructed to fast between 8 to 11 hours for blood sampling. On the pre- and post-blood sampling days (i.e., 22 February 2007 and 16 March 2007, respectively), all participants were asked to arrive to the laboratory at 9:00 AM. They were asked to undergo one time 5ml blood sample and body weight measures before and after the experiment. Written guidelines were given to each of the participants indicating the importance of fasting from food and avoid PA in the preceding blood sampling day and this was verbally reiterated to each participant a day prior to the pre- and post-blood testing periods. All participants were instructed by a dietitian to reduce their fat (mainly saturated, trans-fatty acid and cholesterol food stuffs) prior to the experiment. Adherence to dietary fat intake was assessed on the basis of advice and guidelines. Guiding principles associated with this had been given to all participants. Furthermore, either of the group's intakes of dietary fat was not supervised or standardized; rather it was on the basis of dietary advice or guidelines to reduce the fat intake. No advice was given to restrict the participants' normal habitual eating practices. Participants in both groups were instructed to eat two apples: one in the morning (before breakfast) and one in the evening (before dinner) (without removing the skin) per day for 3 wk.

Intervention

Participants who were assigned in Group I received free of charge red royal apple fruit (Pyrus Malus) that weighs 250 gram on average twice a day and engaged in an unsupervised home-based moderate-intensity (brisk walking) activity for the period of 3 wk. Brisk walking was defined as a pace faster than normal and leaves the individual slightly breathless but still able to converse. Participants received detailed demonstration regarding how to do the brisk walking. Adherence to advice was assessed using a self-reported diary and stopwatch. In other words, all participants were instructed to record on their diary the time only they covered during brisk walking activity for 3 consecutive wks. The diary was prepared in such a manner that the total minutes of brisk walking were partitioned into the following categories based on CDC/ACSM guidelines (Pate *et al*., 1995) recommending a minimum of 30 min of activity daily: 0 min, 1-29 min, 30-59 min, 60-89 min, 90-119 min, 120-149 min, and 150 plus min. Bearing the small number and sedentary nature of participants in mind, we amended the categories as follows: 0 min, 1-29 min, 30-59 min and 60 min or more. Following the collection of the diary report, the total time that participants spent in brisk walking activity was estimated. On the other hand, Group II participants received a free of charge red royal apple fruit that weighs 250 gram on average twice a day. They were also instructed to refrain themselves from any kind of PA.

Blood sampling Procedures

Following 8 to 11 hr overnight fast, 5 ml of venous blood was taken under aseptic conditions by venipuncture with the participant in a seated position. Blood samples were obtained at 0 and 3 week. Blood samples were collected in serum tubes, allowed clotting for 1 hr by keeping it undisturbed, and then the serum was separated in a clean, dry test tube centrifuged on Microsil laboratory at 3000 rpm for 5 min.

Analytical procedures

All the pre- and post- blood samples were analyzed on fully automatic analyzer (Metrolab 2300 Random Access, Clinical analyzer made in Argentina) at the Government Medical College, Patiala, and Department of Biochemistry. The whole blood was analyzed to perform a detail lipid profile estimation including total lipids, serum cholesterol, triglycerides, HDL-C and LDL-C. Also, the participants' lipid profile results were obtained nearly four hours following blood collection.

Statistical analysis

A paired samples t-test was employed to examine differences in the level of LDL-C from pre- to post-intervention for the two groups using the SPSS software program, version 15.0 (Statistical Package for Social Science, Chicago, IL). In all cases, $p < 0.05$ was taken as the level of significance in two-tailed tests.

3. Results

The post level of LDL-C of one participant from Group I could not be calculated due to elevated triglycerides and total lipids. So, data for Group I was treated for 6 participants. On the other hand, similar level of LDL-C was observed in the pre- and post-test of one participant in Group II. The participant reported that she started taking medications (namely Desogestrol and Ethinylestradiol) in the mid of the intervention. Thus, the medications might have interfered with the lipid profiles. Table 1 and 2 show the lipid and lipoprotein variables of the participants at pre- and post- intervention. After 3 weeks of intervention, Group II lowered LDL-C by 18.57 mg/dl (95% confidence interval = 9.05 – 28.09, P = 0.03); Group I lowered by 38.33 mg/dl (95% confidence interval = 25.62 – 51.04, P = 0.01). There was no significant change in Cholesterol; 19.29 mg/dl (95% confidence interval = -8.39 – 46.96, P = 0.139) obtained in Group II while a change in cholesterol of 36.67 mg/dl (95% confidence interval = 17.69 – 55.63, P = 0.04) was observed in Group I. With respect to HDL-C and triglyceride levels, no significant changes were obtained in both groups from pre-to-post interventions.

Table 1. Changes in Total Lipids, Cholesterol, Triglycerides, HDL-C & LDL-C for Group II (BMI ≤ 25 kg/m^2, level of LDL-C < 110mg/dl)

				Paired differences		
	Pre	Post	Change	Mean	SEM	P
Total Lipids	476.43	470.71	-0.0119	5.71	39.79	0.891
Cholesterol	151.43	132.14	-0.127	19.28	11.30	0.139
TG	125.00	140.71	0.125	-15.71	30.64	0.626
HDL-C	37.14	35.00	-0.057	2.14	2.40	0.407
LDL-C	85.00	66.43	-0.22	18.57	3.89	0.03

4. Discussion

Such experimental design is the first to examine the usefulness of regular consumption of apple fruit combined with dietary advice to reduce fat intake and unsupervised home-based brisk walking in lowering LDL-C. To date CVD remains one of the leading causes of morbidity and mortality worldwide.

Table 2. Changes in Total Lipids, Cholesterol, Triglycerides, HDL-C & LDL-C for Group I (BMI > 25 kg/m^2, level of LDL-C$\geq$ 110mg/dl)

				Paired differences		
	Pre	Post	Change	Mean	SEM	**P**
Total Lipids	435.83	425.00	-0.025	10.83	26.06	0.695
Cholesterol	172.50	135.83	-0.21	36.67	7.37	0.04
TG	70.00	90.83	0.29	-20.83	23.64	0.419
HDL-C	39.17	34.17	-0.11	4.17	3.96	0.341
LDL-C	121.67	83.33	-0.32	38.33	4.94	0.01

Participants in Group I were reported that they were engaged in brisk walking activity. The diary report for brisk walking program is presented in the following table:

Table 3. Descriptive Statistics for unsupervised brisk walking activity (n=6)

Walking Minutes	Sum	Average walking minutes in 20 days	Percentage
0	13	0.65	3.25%
1-29	31	1.55	7.75%
30-59	58	2.9	14.5%
60+	18	0.9	4.5%

Available evidence indicates that persons who consume more fruits and vegetables often have lower prevalence of important risk factors for CVD, including hypertension, obesity, and type 2 diabetes mellitus. Some large, prospective studies showed a direct inverse association between fruit and vegetable intake and the development of CVD incidents such as CHD and stroke (Ignarro *et al*., 2007). Indeed, the health protective effects of fruit and vegetable intake observed in epidemiological studies may be due, in part, to the presence of antioxidants in these foods (Hyson *et al*., 2000).

Evidence suggests that a diet high in fruits and vegetables may decrease the risk of chronic diseases, such as CVD and cancer and phytochemicals including phenolics, flavonoids and carotenoids from fruits and vegetables may play a key role in reducing chronic disease risk. Apples are a widely consumed, rich source of phytochemicals, and epidemiological studies have linked the consumption of apples with reduced risk of some cancers, CVD, asthma, and diabetes. In the laboratory, apples have been found to have very strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation, and lower cholesterol. Apples, and especially apple peels, have been found to have a potent antioxidant activity and can greatly inhibit the growth of liver cancer and colon cancer cells (Boyer & Liu, 2004). Studies found that apples had a greater cholesterol lowering effect than the other two fruits such as peaches and pears (Leontowicz *et al*., 2002). A number of studies have indicated that LDL oxidation is reduced in vitro by a variety of fruits or fruit extracts (Hyson *et al*., 2000).

Long-term consumption of a low-fat diet might be associated with even greater weight loss and additional improvement of plasma lipid concentrations. Moreover, a diet low in saturated fat has become accepted (Franz *et al*., 2002; Gerhard *et al*., 2004).

On the other hand, a home-based walking program may be a safe and effective way for high-risk individuals to increase their PA. Walking is a moderate-intensity form of PA that carries less risk for adverse cardiovascular or musculoskeletal events than more vigorous forms of PA. However, little is known about the risk for adverse events among high-risk individuals in response to unsupervised lifestyle activity programs that emphasize moderate intensity PA like walking. A recent study showed that individuals at high risk for CVD could safely start an unsupervised home-based walking program with a low risk for adverse reactions to walking (Goodrich *et al*., 2007). Walking, either in the form of supervised group walks or unsupervised home-based walking, is one of the easiest, safest, and most inexpensive type of exercise to promote, and it is also one of the most popular forms of exercise among those with and without chronic illness. Programs that encourage PA during leisure time or unsupervised home-based activities have better long-term adherence rates (Richardson *et al*., 2005).

Results of recent studies indicated that 30 min, 12- week self-paced, unsupervised brisk walking, 5 days per week improves fitness and decreases cardiovascular risk (Tully *et al*., 2005). The study added to the evidence of the health benefits of moderate-intensity exercise to sedentary adults. Home-based programs of moderate intensity activity with ongoing professional contact have been suggested to be more likely to lead to longer term changes in activity levels (Hillsodon *et al*., 1995) than programs delivered in more formal settings.

In the present study, a significant decrease in total cholesterol and LDL-C levels was noted following post-intervention. In addition, participants in both groups showed elevated triglycerides and lowered HDL-C. However, the decrease in HDL-C was coupled with large reductions in both LDL-C and total-C. Moreover, in this study LDL-C decreased by 22% in Group II; whereas 32% of the reduction in LDL-C was found in Group I in the period of 3 wk. Apparently, one participant had shown a significant reduction (a change of -0.38889 mg/dl) of LDL-C in Group II. Conversely, the post- test of LDL-C concentration (a change of -0.15385 mg/dl) of one participant in Group I showed similar result as those of Group II participants. The reasons behind all those observed changes, however, are unclear. Specifically, whether the LDL-C reduction was due to apple supplement or voluntary restriction of dietary fat intake or unsupervised home-based brisk walking program or the combination of the three is uncertain.

5. Implications

The results of our analyses may have several implications for sedentary people who are at risk for developing CVD and for researchers and health care professionals.

First, the addition of fruits and vegetables in conjunction with healthy diet and physical activity has the capacity to reduce the risk of CVD/CHD and should be more emphasized either in the clinical settings or in a controlled and targeted studies though it is difficult to evaluate prospectively the causal relationship of diet and physical activity on major CVD events.

Second, the findings indicate that awareness of unsupervised home-based walking program is the safest, effective in the promotion and achieving the sedentary individual's PA goals despite the fact that previous studies have shown that self-reported measures tend to be overestimates of actual activity (Bassett *et al*., 2000). Also, long-term participation in walking program can produce more desirable result than it does in the short-term ones. Third, the addition of fruits in a normal habitual eating practice, following the voluntary restriction of dietary fat intake and engaging in regular PA in an unsupervised home-based manner has increased the participant's understanding toward changes in lipid profiles particularly LDL-C and health maintenance in general.

Lastly, the most encouraging thing in this study is that the participants were in normal range of LDL-C level and were not clinically disturbed. Therefore, if similar study is conducted on those individuals who are clinically disturbed LDL-C level, there may be a chance to get remarkable outcomes. Besides, if blood sampling was taken 3 wks after stopping eating apple, it would equally be important.

Some limitations of our study deserve comment. Firstly, as we explained in the methodology section, the study was not randomized and the participants were motivated to take part in the intervention; hence, we cannot extrapolate adherence to the general population. Nevertheless, the findings document that benefits are possible in motivated participants. Secondly, the sample size was inadequate to examine in greater detail. Thirdly, unsupervised diet to reduce fat intake may affect the finding and lead to unreliable inference. Lastly, the relationship between cholesterol and time of brisk walking was not determined.

6. Conclusion

In summary, we observed that sedentary individuals are at low risk from CVD if they could engage themselves safely in an unsupervised home-based walking program along with healthy diet and lifestyle. This study addressed the gap in the literature by targeting the population (sedentary individuals) that regular fruit consumption, unsupervised home-based walking program and advice to lower dietary fat intake may play an important role in lowering LDL- ("bad") C concentrations, which is the major cause for CVD/CHD.

In general, from this novel study an apple a day in conjunction with voluntary restriction of dietary fat intake especially saturated fatty acids, trans fatty acids and cholesterol food stuffs and engaging in a home-based regular PA (such as brisk walking) may keep LDL- ("bad") C away in sedentary individuals. However, further study in a large sample size sedentary individuals or high- risk individuals is needed.

In conclusion, integrated approaches (such as including fruits, vegetables, and fiber foods, limiting high fat intake particularly saturated fatty acids, trans-fatty acids, cholesterol and high sugar, promoting regular PA) are highly influential in health maintenance and tackling the risk factors. Apple fruit supplement, which is high in fiber content and high in antioxidant potent, combined with dietary advice to reduce fat intake and unsupervised home-based moderate-intensity PA (such as brisk walking) at least 30 minutes a day may result in significant reduction in serum lipids particularly LDL-C level in sedentary individuals. An intervention of this type may help reduce the risk of CVD/CHD in high-risk individuals.

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