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Effect of Walnut on Lipid Profile in Obese Female in Different Ethnic Groups of Quetta, Pakistan

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Abstract: Four week controlled study was designed to observe the effect of 40 g of walnut in daily breakfast on Body Mass Index (BMI), total Cholesterol (CHO), Triglyceride (TG) High Density Lipoprotein (HDL) cholesterol and Low Density Lipoprotein (LDL) cholesterol in obese female subjects of various ethnics, i.e. Baloch (B), Pathan (P), Hazara (H) and Punjabi (PU) residing in Quetta region of Balochistan. A batch of 32 obese female subjects, 8 from each ethnic group as a control and another batch of 32 obese females 8 from each ethnic group as treated were selected. Twelve hour fasting blood samples a day after stoppage of walnut were taken from obese control and obese treated subjects. Daily walnut consumption demonstrated considerable drop in body weight in all ethnic groups residing in Quetta locality. Marked and statically significant reduction in total cholesterol was noticed in all ethnic groups i.e. 3.6% ($p<0.05$), 5.4% ($p<0.001$), 5.8% ($p<0.01$) and 7% ($p<0.001$) in B, P, H and PU groups respectively. Baloch subjects showed significant reduction of 7.8% in TG ($p<0.001$), similarly significant lowered TG was also observed in PU group. A significant increase ($p<0.001$), ($p<0.05$) in HDL cholesterol had been observed in B and P group respectively in walnut consuming subjects. In walnut consuming female subjects significant reduction ($p<0.05$), ($p<0.01$), ($p<0.01$) in LDL cholesterol levels was noted in B, P and PU subjects respectively. The positive influence of walnut on lipid profile suggests that walnut rich diet may have beneficial effects beyond changes in plasma lipid level.

Key words: Walnut, cholesterol, triglyceride, high density lipoprotein, low density lipoprotein

INTRODUCTION

Obesity is now considered a major public health problem of epidemic proportion that threatens millions of lives in the United States (Department of Health and Human Services, 2008) and worldwide (James, 2008). Obesity is linked to a number of chronic diseases (US Surgeon General, 2008) and lipoprotein disorder is among the most common metabolic disease occurring in normal human subjects as well as diabetic subjects. Plasma cholesterol levels are moderately decreased when low cholesterol diets are used (Toth, 2004). The effect of dietary cholesterol on plasma cholesterol levels may be influenced by the types of fatty acid consumed which may be saturated or unsaturated (Mcpherson and Spiller, 1996).

Different types of dietary lipids have been shown to affect lipid metabolism and serum lipid profile differently. Nuts have favorable fatty acid and nutrient profile, which are low in saturated fatty acids and high in monounsaturated and polyunsaturated fatty acids. The study of McManus *et al.* (2001) demonstrated that following a Mediterranean-style moderate fat weight loss diet, such as peanuts and walnut, were able to improve weight loss and keep weight loss for longer period than people following the traditionally recommended low fat diet. The study of Sabate and Ang (2009) also emphasize that long term nut consumption is linked with lower body weight and lower risk of obesity. Findings of Bes-Rastrollo *et al.* (2007) further support and recommended that nut consumption is an important component of a cardio protective diet and those participants who fulfill risk factors for obesity and they ate nuts two or more times per day had a significantly lower risk of also allay fears of possible weight gain.

Walnuts are distinguished from other nuts by virtue of their higher polyunsaturated fat content (and importantly their α-linolenic acid [ALA] content) combined with antioxidants in the form of γ-tocopherol (Storlien *et al.*, 1996; Storlien *et al.*, 2000). In subjects with dyslipidemia (total cholesterol >5.17; LDL >3.36; triglycerides >2.26 mmol/l), a low-fat diet supplemented with walnuts was found to reduce total cholesterol compared with a habitual or low-fat diet and LDL compared with a low-fat diet alone (Almario *et al.*, 2001). Feldman (2002) found that there was evidence of decreased total cholesterol and LDL cholesterol in diets of at-risk subjects supplemented with two to three servings of walnuts per day, with no net gain in body weight. Tapsell *et al.* (2004) demonstrated that lipid profile of patients with type 2 diabetes improved after including 30 g walnuts/day. The studies of Zhao *et al.* (2004) and Ros *et al.* (2004) observed that compared to the average American diet, both the LA and the ALA diets including walnuts lowered total cholesterol 11%, LDLs 11-12% and triglycerides

18%. Walnut polyphenolics are effective inhibitors of *in vitro* plasma and LDL oxidation (Anderson *et al*., 2001). The findings of Lavedrine *et al*. (1999) showed that the positive effect of walnut consumption on blood HDL cholesterol and apo A1 is of special interest since these lipid parameters have been shown to be negatively correlated with cardiovascular morbidity.

In Pakistan the province of Balochistan in North West has its capital city of Quetta. The region of Quetta is inhabited by various ethnic groups since centuries. It is also characterized of higher altitude at an average of 1600 meter. The present study is carried out to assess the effect of walnut consumption in the female population of different ethnic groups in this specific environment.

MATERIALS AND METHODS

The study was performed in different ethnic groups [Pathan (P), Baloch (B), Hazara (H) and Punjabi (PU)] in Quetta, Balochistan. Participant volunteers were recruited from the local community, primarily through newspaper advertisements and through pasting posters in all departments of University of Balochistan, Bolan Medical College, hospitals, telephonic messages, emails and by counseling in different communities and also in various medical camps.

Volunteers were screened and those were excluded from the trial that consumed nuts frequently, had known nut allergies, smoked cigarettes and had history of hypertension or atherosclerotic or metabolic disease. Meetings with the selected volunteers were held to explain the protocol of the study. Thirty two female obese subjects, 8 from each ethnic group, participated in the study. Selection of the subjects were according to the WHO, 1998 criteria where BMI = 30-34.9 is considered as obese I (at a moderate risk of co-morbidities), BMI = 35-39.9 is obese II (at a severe risk of co-morbidities), and BMI > = 40 is obese III (at a very severe risk of co-morbidities).

All the subjects volunteered to take daily 40 g walnut in their breakfast along with normal eating habits for four weeks. At the end 4 week they were sampled for blood after a 12 h fast and general data was collected. BMI in the general observations and blood samples before and after the walnut consumption were subjected for estimation of total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol with commercial kits.

Statistical analysis was undertaken with statistical program of Sigma Stat 3.5. Student t test was used for comparison between normal and obese subject groups and $p<0.05$ was considered as statistically significant.

RESULTS

Body mass index: Daily walnut consumption demonstrated considerable drop in body weight in all ethnic groups residing in Quetta locality. Walnut showed its most beneficial effects on Hazara group and statistically significant ($p<0.01$) reduction of 5.8% was observed. However, other groups also exhibited noticeable decline in BMI with 2.9, 4 and 3.8% in B, P and PU groups, respectively.

Total cholesterol: Consumption of 40 g walnut daily in breakfast in obese females caused statistically significant reduction in cholesterol level in all ethnic groups. The decrease was 3.6% ($p<0.05$), 5.4% ($p<0.001$), 5.8% ($p<0.01$) and 7.1% ($p<0.001$) in B, P, H, PU, respectively (Fig. 1). The cholesterol level, however, ranged between 216.1±1.9 to 245.1±1.3 mg/dl and 204.5±1.5 to 236.0±3.2 mg/dl in control and walnut treated obese female, respectively.

Triglyceride: In obese females, Baloch group exhibited statistically significant ($p<0.001$) 7.8% reduction after walnut consumption. A marked decrement ($p<0.05$) was also observed in H and PU subjects. The TG level in control obese female ranged between 217.5±2.3 to 235.9±2.8 mg/dl and after walnut consumption it ranged between 207.5±1.8 to 225±2.6 mg/dl. Walnut seems to be effective in lowering TG in obese subjects (Fig. 2).

HDL cholesterol: Walnut consumption in obese females showed remarkable statistical significant increase ($p<0.001$) in HDL cholesterol level. A significant increase ($p<0.05$) was also observed in P group to a value of 6.5%. The level of the fraction in obese control females varied between 39±0.8 to 41.5±0.8 mg/dl and ranged between 41.1±0.9 to 43.8±0.6 mg/dl in walnut consumed group (Fig. 3).

LDL cholesterol: Low density lipoproteins were effectively decreased following walnut consumption for four weeks in breakfast in female obese subjects. Baloch ($p<0.05$), P ($p<0.01$) and PU ($p<0.01$) groups exhibited appreciable and statistically significant decrease in LDL cholesterol level after walnut. In the control obese females, the LDL cholesterol level ranged

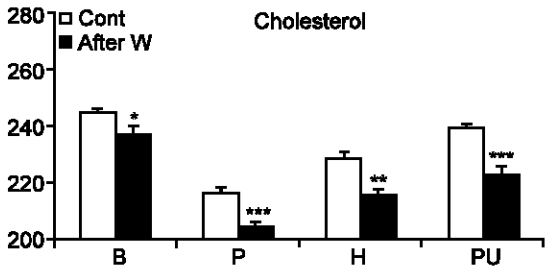

Fig. 1: Serum cholesterol mg/dl in obese female subjects of control (Cont) and Walnut (W) taking categories in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. *$p<0.05$, **$p<0.01$, ***$p<0.001$

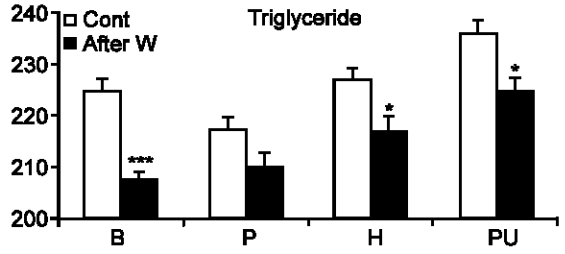

Fig. 2: Serum triglycerides mg/dl in obese female subjects of control (Cont) and Walnut (W) taking categories in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. *p<0.05, **p<0.01, ***p<0.001

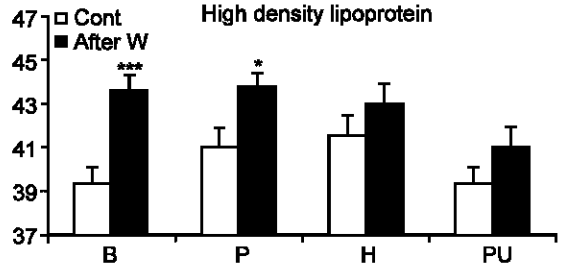

Fig. 3: Serum LDL cholesterol mg/dl in obese female subjects of control (Cont) and Walnut (W) taking categories in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. *p<0.05, **p<0.01, ***p<0.001

between 151.5±0.9 to 163.6±1.7 mg/dl and in walnut treated group it ranged between 144.7±1.8 to 156.6±1.9 mg/dl (Fig. 4).

DISCUSSION

Obesity increased the prevalence of almost every abnormal lipid profile. Being overweight, in turn, is recognized as a significant risk factor for chronic diseases such as arteriosclerosis, ischemic heart disease and diabetes, all of which are major causes of morbidity and mortality (Kannel *et al*., 1991 and Kissebah and Krakower, 1994). Rapidly increasing obesity prevalence rates necessitate weight management to be a priority for the prevention and treatment of chronic diseases.

The results of present study clearly have demonstrated that daily walnut consumption in obese subjects reduced weight in all ethnic groups. However, the reduction was statistically significant (p<0.05) in Hazara group with 7.6%. These findings agree with the results of Bes-Rastrollo *et al.* (2009) and associated with a slightly lower risk of obesity. Our findings agree with the results from 2 prospective cohort studies. In a 28-mo prospective study (the SUN Study) conducted in Spain in free-living university graduates (n = 8865), a significant inverse association between nut consumption and weight gain was reported (Bes-Rastrollo *et al*., 2007). In

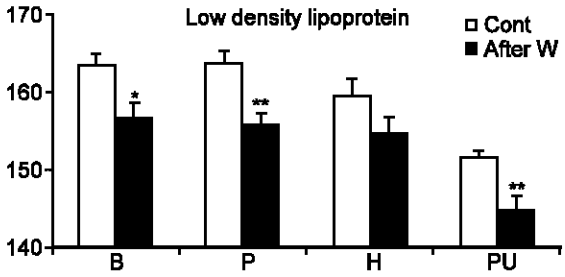

Fig. 4: Serum HDL cholesterol mg/dl in obese female subjects of control (Cont) and Walnut (W) taking categories in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. *p<0.05, **p<0.01, ***p<0.001

the Nurses' Health Study, a slightly lower risk of obesity was found among those who consumed nuts regularly during 16 y of follow-up (Jiang *et al*., 2002). Two reviews summarize the findings of 15 human intervention trials that evaluated the effects of nut consumption on body weight changes (Garcia-Lorda *et al*., 2003; Rajaram and Sabate, 2006) and concluded that self-selected diets that included nuts in free-living populations did not have a tendency to increase body weight. In a randomized crossover trial that ate an average 35 g walnuts/d for 6 mo, found minimal weight gain, which was less than predicted given the reported dietary intake of walnuts (Sabate *et al*. 2005). Modest consumption of almonds (i.e., 2 oz/d) for 6 mo did not cause a significant or biologically meaningful changes in body weight reported in another randomized crossover trial (Fraser *et al*., 2002). Alper and Mattes, 2002 assessed peanut consumption in 15 healthy normal-weight adults and reported a significantly lower body weight (1 kg) than the average weight predicted (3.6 kg) after 19 wk of consuming 505±118 calories/d of peanuts. More recently, Hollis and Mattes (2007) showed in a randomized crossover trial that daily consumption of 1440 kJ almonds (~344 calories/d) for 10 wk did not promote weight gain. From a public health point of view, it is important to highlight a tendency toward lower risk of obesity, which suggests that nuts as a factor in the context of a healthy diet that can help to prevent weight gain or at least to regulate weight gain and to avoid the risk of developing obesity among those participants with a higher frequency of nuts consumption.

Given the known effects of diet fats, the composition of the fatty acids found in walnuts should lower serum cholesterol levels (Ahrens *et al*., 1957; Keys *et al*., 1957; Keys *et al*., 1965a; 1965b; 1965c; 1965d). About 81% of the calories of the walnut are derived from the fat, which accounts for 58% of their weight. Walnut fat is qualitatively similar to that of some commonly used oils extracted from the grains and seeds. The ratio of polyunsaturated to saturated fat in walnuts is 7.1, one of

the highest naturally occurring foods. Walnut contains relatively large amounts of the n-3 linolenic acid, about 7 g/100 g of edible portion, or 12% of their total fat content (Hepburn *et al*., 1986). Thus, they can be considered as alternative food source of n-3 fatty acids that does not add cholesterol to their diet (Weiner, 1986). The results of present study demonstrated that in obese subjects, walnut exhibits significant reduction in cholesterol level in all studied ethnic groups of Quetta. The reduction was 3.6, 5.4, 5.8 and 7.1 in B, P, H and PU groups. Triglycerides were also decreased in obese female subjects in all ethnic groups except P. Appreciable reduction was observed in B ($p<0.001$), H ($p<0.05$) and PU ($p<0.05$) groups. In obese females B ($p<0.001$) and P ($p<0.05$) groups' only explicit significant elevation after daily walnut consumption in HDL level. All studied ethnic groups except H group showed statistically significant decreased LDL in obese female following walnut consumption and the decrease ranged between 4.3-4.9%. Although it is known that, for every 1% decrease in plasma LDL cholesterol, there is 2% reduction in Cardiovascular Disease (CVD) risk (Levine *et al*., 1995). There are suggestions that walnut-rich diets may have beneficial effects beyond changes in plasma lipid levels.

Our findings from the walnut diet are in accord with previous feeding studies in which a decrease of LDL cholesterol in the range of 8-16% was observed at intakes of 56-84 g (2-3 oz/d) of walnuts in normal and hypercholesterolemic patients (Sabate *et al*., 1993; Griel and Kris-Etherton, 2006). Walnuts are rich in linoleic acid and ALA, which are known to lower cholesterol when they replace SFA or MUFA in the diet, possibly by increasing the receptor-mediated uptake of LDL cholesterol (Mori *et al*., 2000). The cholesterol-lowering effect of walnuts is, however, not entirely due to the fatty acid makeup of this food. Consistent with what we and others have found (Sabate *et al*., 1993; Griel and Kris-Etherton, 2006), the predicted decrease in LDL cholesterol in this study was smaller than the observed decrease, suggesting that other non lipid components of walnuts may have mediated some of the cholesterol-lowering effects.

Studies of Ros *et al*. (2004) demonstrated that walnut diet significantly reduced total cholesterol and LDL cholesterol. Serum total cholesterol and LDL cholesterol concentrations in adults who followed the walnut diet (4.87±0.18 and 2.77±0.15 mmol/L, respectively) were lower than in those who followed the control diet (5.14±0.18 and 3.06±0.15 mmol/L, respectively). Incorporating 1.5 oz (42.5 g) of walnuts in a diet based on the current dietary guidelines markedly decreased total cholesterol and LDL cholesterol by 5.4% and 9.3%, respectively. Rajaram *et al*. (2009) and Sabate (1999) reported that a regular consumption of nuts lowers the risk of myocardial infraction and death from ischemic heart disease. Consumption of walnut has favorable effects on human serum lipid profiles, with a decrease in total and LDL cholesterol as well as triglycerides (Abbey *et al*., 1994; Zambon *et al*., 2000; Sabate *et al*., 1993; Chisholm *et al*., 1998)) and an increase in HDL cholesterol and apolipoprotein A1 (Lavedrine *et al*., 1999).

The findings obtained in the present study further support to the existing recommendations that walnut rich diet may have beneficial effects beyond changes in plasma lipid level.

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