EFFECTS OF DIETARY FLAVONOIDS INTAKE IN SAUDI PATIENTS WITH CORONARY HEART DISEASE

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Objectives: The aim of the study was to assess the dietary intake of flavonoids and their effect on serum lipid levels in Saudi patients with coronary heart disease (CHD).

Methodology: A cross-sectional study with a sample of 50 CHD patients and 50 controls. A 24-hour recall method was used to collect data on the dietary intake of macronutrients, flavonoids, and antioxidant vitamins. A food frequency questionnaire (FFQ) was used to collect data on habitual consumption during the year preceding the interview. Baseline data collection included medical history, anthropometric measurements, physical activity, and smoking status.

Results: CHD patients showed significantly less intake of fruits and vegetables compared to the controls. Serum lipids including total cholesterol (TC), triglycerides (TG), and low density lipoproteins (LDL) were found to be significantly higher in CHD patients than in the controls. The main sources of flavonoids in a typical Saudi diet are tea, fruits (apples), vegetables (onions), and chocolate. The intake of flavonoids and antioxidant vitamins was significantly lower in CHD patients compared to the controls. A negative correlation between the dietary intake of different flavonoids and serum LDL was observed in CHD patients. Significant correlation was found between the intake of flavonol and waist to hip ratio.

Conclusions: The findings of the study support a potential protective effect of dietary flavonoids in relation to CHD. The study showed that consuming more Flavonoids may have positive effect on lowering blood lipids.

Key Words: Flavonoids, antioxidants, coronary heart disease, lipid profile, Saudi Arabia.
INTRODUCTION
Flavonoids are polyphenolic compounds comprising one of the largest and ubiquitous groups of plant metabolites. They are considered an important part of the human diet. These compounds are known for their hormonal activity, but they are also potent antioxidants and tyrosine kinase inhibitors. Since oxygen free radicals and lipid peroxidation are thought to be involved in several conditions such as atherosclerosis, cancer, and chronic inflammation, the antioxidant activity of the flavonoids and phenolic compounds has gained primary interest. It has been reported that the intake of flavonoids was inversely related to mortality from coronary heart disease and also showed an inverse relationship to the incidence of myocardial infarction. This anti-atherosclerotic effect of flavonoids may be derived from their antioxidant properties, but that relationship remains unclear.

LDL-cholesterol and TG have long been recognized as the targets to treat for the primary and secondary prevention of cardiovascular disease (CVD). The presence of small dense LDL particles as well as higher C-reactive protein concentrations have been associated with a higher CVD risk. High circulating oxidized LDL concentrations have also been linked to an increased CVD risk. Oxidative modification of LDL has been implicated in human atherosclerosis. Another mechanism suggests that free radicals enhance thrombogenesis by increasing platelet aggregation. It has been postulated that flavonols and flavones can inhibit lipoxygenase and cyclooxygenase activity, leading to lower aggregation of platelets and a reduction in thrombotic tendency. The majority of strokes are caused by blood clots that lodge in narrowed arteries as an alteration that may have been caused in part by oxidative damage. Epidemiological studies showed that the intake of quercetin and other flavonoids and flavones had an inverse association with the CHD in Dutch population of elderly men. An inverse association between the intake of flavonols and CHD was also reported in a Finish study and in Japanese women.

In the past two decades, the Arabian Peninsula has witnessed a dramatic socioeconomic progress with social and dietary habits of a lifestyle that resembles those of western countries. Consequently, conditions commonly affecting affluent societies, such as CVD, diabetes mellitus, obesity, and cancer have started to emerge in this society. Several Saudi Arabian hospital-based studies have shown a rise in the occurrence of angina, myocardial infarction, and nephropathy. Physical inactivity, obesity, high dietary fat intake and hypercholesterolemia, and smoking are common variables in Saudi nationals. In a recent study of CHD risk factor, Abalkhail found that 18.8% of the total samples were smokers, and 50% of them were overweight. In view of the possible protective role of dietary flavonoids against CHD, it is evidently important to determine the intake of dietary flavonoids by the Saudi population and find its correlation with CHD.

MATERIALS AND METHODS
This study was conducted in Saudi Aramco Hospital in the Eastern province, Kingdom of Saudi Arabia. Fifty patients diagnosed as having CHD were selected from inpatient and outpatient clinics. Another 50 patients who had visited the hospital for regular medical check-ups were selected as controls. Both groups of patients were free from any other chronic diseases including cancer, diabetes, or renal disorders. Furthermore, both groups of subjects were not engaged any chemical or dietary therapies.

The subjects participated in the study after giving informed consent and patient anonymity was preserved. The study tool was designed to collect the data about the general characteristics (age, sex, education, Smoking status, marital status, activity level, and monthly income), anthropometric measurements, medical history, and dietary intake. This was done by using 24 hours-recall and food frequency questionnaires. Participants were asked to list and describe the food items they had eaten in the past 24 hours. Quantities as volumes and portion size were described using food models, various sized glasses, or spoons to help the patients recall the amount of food eaten. The composition of the prepared dishes was recorded and its micronutrient values were analyzed using food composition table. Food frequency questionnaire (FFQ) containing 75 commonly consumed food items was used to investigate the participants’ food habits.

Blood samples were collected after overnight fasting and the serum samples were stored at 20°C
till the estimation of lipid profile including TC, TG, HDL and LDL concentrations. These parameters were estimated by using the commercially available diagnostic kits (RANDOX; Randox Laboratories, CA, USA).

Statistical Analysis
The data is presented as Mean ± Standard Error (Mean ± S.E.), Student’s t-test was applied to find statistically significance at P<0.05. Pearson’s correlation was applied to find significant (P<0.05) disease causing factors.

RESULTS
Mean age of CHD patients was 56.60 ± 10.9 years and the mean age of the controls was 53.1 ± 10.0 years. The difference in age of the groups was not statistically significant. General characteristics including sex, education, activity level and income were similar in CHD patients and controls. However, a significant proportion (76% of the patients were smokers compared to only 14% of the controls (Figure 1).

The amount of food items consumed by CHD patients and controls are shown in the bar-graph (Figure 2). There was no significant difference of the mean consumption of meat, legumes, breads, and sweets within a typical week between cases and controls. The intake of vegetables (p<0.01), fruits (p<0.001), juices and beverages (p<0.05) was significantly low in CHD patients as compared to controls. However, more dairy products were consumed (p<0.01) by the CHD patients than controls.

Total energy intake in 24 hours estimated by using 24-hour recall methods showed no significant difference between the groups.

![Figure 1: CHD patients matched with controls in general characteristics as percent](image)

Similarly, there was also no significant difference in the consumption of carbohydrate and total fat. However, the intake of protein and saturated fat was found to be higher (p<0.05) in patients than controls. The total dietary intake of flavonoids including flavonols, kaempferol, quercetin, and catechin was significantly (p<0.01) lower in patients with CHD as compared to controls. The intake of β-carotene and vitamin C was significantly (P<0.001) lower in CHD patients than controls. CHD patients consumed significantly more vitamin E (p<0.05) and selenium (p<0.01) than controls. Serum concentrations of total cholesterol (p<0.01), triglycerides (p<0.05), and LDL (p<0.01) were found to be statistically higher in CHD patients than in the controls. The Mean serum HDL concentrations were similar in both groups (Figure 3).

A significantly negative correlation between serum LDL level with total flavonoids (p<0.01), flavonols (p<0.01), kaempferol (p<0.01), quercetin (p<0.05), and catechin (p<0.01) was observed respectively (Table 2). Correlation between dietary vitamin C intake and serum LDL level was significantly (p<0.01) negative. However, no significant correlation between vitamin E, β-carotene and dietary selenium, and serum lipid profile was observed (Table 2).

Table 1: Total daily intake of macronutrients, flavonoids and antioxidants calculated by 24 hr recall method

|                              | Control (n=50) | CHD patients (n=50) |
|------------------------------|---------------|---------------------|
|                              | (Mean ± SE)   | (Mean ± SE)         |
| **Macronutrients**           |               |                     |
| Total energy (Kcal)          | 2187 ± 62.05  | 2089 ± 67.29        |
| Carbohydrate (g)             | 315.85 ± 9.93 | 298.98 ± 11.96      |
| Protein (g)                  | 66.66 ± 2.06  | 84.70 ± 2.78        |
| Total fat (g)                | 54.23 ± 2.15  | 60.24 ± 3.11        |
| Saturated fat (g)            | 12.26 ± 0.36  | 14.26 ± 0.45        |
| **Flavonoids**               |               |                     |
| Total flavonoids (mg)        | 94.64 ± 6.01  | 49.38 ± 3.12        |
| Flavonols (mg)               | 23.25 ± 1.51  | 12.78 ± 0.80        |
| Kaempferol (mg)              | 5.33 ± 0.41   | 3.53 ± 0.27         |
| Quercetin (mg)               | 18.12 ± 1.27  | 9.42 ± 0.66         |
| Catechin (mg)                | 71.40 ± 5.00  | 36.60 ± 2.46        |
| **Antioxidants**             |               |                     |
| β-carotene (µg)              | 2084 ± 143.04 | 1012 ± 71.52        |
| Vitamin E (mg)               | 9.45 ± 0.55   | 11.14 ± 0.54        |
| Vitamin C (mg)               | 129.54 ± 7.68 | 69.00 ± 4.84        |
| Selenium (µg)                | 75.08 ± 3.45  | 95.66 ± 5.96        |

Statistical significance was calculated by using Student's t test

*p<0.05, **p<0.01, ***p<0.001
C (p<0.05). Similarly, significant (p<0.05) negative correlation was found between the flavonols and quercetin, and the intake of selenium. A significant negative correlation (p<0.05) between catechin and vitamin C was observed. No significant correlation was seen between flavonoids and the intake of saturated fat, smoking habit and BMI. However, there was a significant negative correlation (p<0.05) between the intake of flavonols and WHR (Table 3).

**DISCUSSION**

The antioxidant properties of dietary flavonoids has of late drawn considerable interest in the prevention of CHD. Currently, more than 5000 different flavonoids have been identified. In the present study, only two types of flavonoids (flavonols and catechin) which are known to be important because of their antinutagenic and antioxidant properties were considered.

Many epidemiological studies have shown an inverse association between dietary antioxidant vitamin like vitamin C, vitamin E and β-carotene, flavonoids, and a reduced risk for cardiovascular disease. The results of the present survey from FFQ showed that most of the CHD patients had a significantly low intake of fruit and vegetables. This suggests an association between low intake of fruit and vegetable and prevalence of CHD. In this study sample, the intake of all flavonols,
Table 2: Correlation between dietary flavonoids and antioxidants, and plasma lipids

|                      | Controls (CC) | CHD Patients (CC) |
|----------------------|---------------|-------------------|
| Total flavonoids intake |               |                   |
| Total cholesterol (mg/dl) | -0.001       | 0.191             |
| Triglycerides        | 0.060         | -0.025            |
| HDL (mg/dl)          | -0.153        | 0.116             |
| LDL (mg/dl)          | 0.117         | -0.561*           |
| Flavonol intake      |               |                   |
| Total cholesterol (mg/dl) | -0.043       | 0.055             |
| Triglycerides        | 0.050         | -0.138            |
| HDL (mg/dl)          | -0.250        | 0.131             |
| LDL (mg/dl)          | 0.034         | -0.369*           |
| Kaempferol intake    |               |                   |
| Total cholesterol (mg/dl) | -0.067       | 0.055             |
| Triglycerides        | 0.027         | -0.039            |
| HDL (mg/dl)          | -0.329*       | 0.098             |
| LDL (mg/dl)          | -0.067        | -0.441*           |
| Quercetin intake     |               |                   |
| Total cholesterol (mg/dl) | -0.032       | 0.044             |
| Triglycerides        | 0.052         | -0.153            |
| HDL (mg/dl)          | -0.205        | 0.124             |
| LDL (mg/dl)          | 0.059         | -0.292*           |
| Catechin intake      |               |                   |
| Total cholesterol (mg/dl) | 0.012        | 0.224             |
| Triglycerides        | 0.059         | 0.013             |
| HDL (mg/dl)          | -0.108        | 0.104             |
| LDL (mg/dl)          | 0.131         | -0.589*           |
| Dietary vitamin C intake |          |                   |
| Total cholesterol (mg/dl) | 0.086        | -0.263            |
| Triglycerides        | -0.077        | -0.067            |
| HDL (mg/dl)          | 0.014         | 0.096             |
| LDL (mg/dl)          | -0.060        | 0.373*            |
| Dietary vitamin E intake |          |                   |
| Total cholesterol (mg/dl) | -0.087       | 0.031             |
| Triglycerides        | 0.032         | -0.085            |
| HDL (mg/dl)          | 0.022         | -0.025            |
| LDL (mg/dl)          | -0.253        | -0.095            |
| Dietary β-carotene intake |          |                   |
| Total cholesterol (mg/dl) | -0.058       | 0.090             |
| Triglycerides        | 0.098         | -0.155            |
| HDL (mg/dl)          | -0.170        | 0.109             |
| LDL (mg/dl)          | 0.098         | 0.051             |
| Dietary selenium intake |           |                   |
| Total cholesterol (mg/dl) | -0.157       | 0.261             |
| Triglycerides        | -0.037        | 0.097             |
| HDL (mg/dl)          | -0.096        | -0.212            |
| LDL (mg/dl)          | -0.120        | 0.266             |

CC=Correlation coefficient *p<0.05

Table 3: Correlation between dietary flavonoids and dietary antioxidants, saturated fat, smoking, BMI and WHR

|                      | Controls (CC) | CHD Patients (CC) |
|----------------------|---------------|-------------------|
| Total flavonoids intake |               |                   |
| β-carotene (µg/d)    | 0.121         | -0.122            |
| Vitamin E (µg/d)     | -0.104        | 0.051             |
| Vitamin C (µg/d)     | -0.048        | -0.336*           |
| Selenium (µg/d)      | 0.074         | -0.207            |
| Dietary flavonol intake |           |                   |
| β-carotene (µg/d)    | 0.013         | -0.209            |
| Vitamin E (µg/d)     | -0.054        | 0.003             |
| Vitamin C (µg/d)     | -0.116        | -0.228            |
| Selenium (µg/d)      | -0.049        | -0.311*           |
| Dietary kaempferol intake |         |                   |
| β-carotene (µg/d)    | 0.150         | -0.110            |
| Vitamin E (µg/d)     | -0.153        | 0.071             |
| Vitamin C (µg/d)     | -0.052        | -0.258            |
| Selenium (µg/d)      | 0.079         | -0.229            |
| Dietary quercetin intake |          |                   |
| β-carotene (µg/d)    | -0.027        | -0.215            |
| Vitamin E (µg/d)     | -0.021        | -0.022            |
| Vitamin C (µg/d)     | -0.123        | -0.186            |
| Selenium (µg/d)      | -0.081        | -0.297*           |
| Dietary catechin intake |           |                   |
| β-carotene (µg/d)    | 0.142         | -0.086            |
| Vitamin E (µg/d)     | -0.108        | 0.019             |
| Vitamin C (µg/d)     | -0.023        | -0.350*           |
| Selenium (µg/d)      | 0.104         | -0.160            |
| Saturated fat intake |               |                   |
| Total flavonoids (mg/day) | 0.108       | 0.035             |
| Flavonols (mg/day)   | -0.086        | -0.069            |
| Kaempferol (mg/day)  | 0.026         | -0.097            |
| Quercetin (mg/day)   | -0.110        | -0.049            |
| Catechin (mg/day)    | 0.156         | 0.066             |
| Smoking habits       |               |                   |
| Total flavonoids (mg/day) | 0.000       | 0.057             |
| Flavonols (mg/day)   | 0.143         | 0.106             |
| Kaempferol (mg/day)  | 0.057         | 0.014             |
| Quercetin (mg/day)   | 0.154         | 0.134             |
| Catechin (mg/day)    | -0.043        | 0.038             |
| Body mass index (BMI) |             |                   |
| Total flavonoids (mg/day) | 0.085       | -0.085            |
| Flavonols (mg/day)   | 0.021         | -0.215            |
| Kaempferol (mg/day)  | -0.045        | -0.199            |
| Quercetin (mg/day)   | 0.038         | -0.191            |
| Catechin (mg/day)    | 0.095         | -0.037            |
| Waist to hip ratio (WHR) |           |                   |
| Total flavonoids (mg/day) | 0.028       | -0.104            |
| Flavonols (mg/day)   | -0.044        | -0.290*           |
| Kaempferol (mg/day)  | -0.064        | -0.230            |
| Quercetin (mg/day)   | -0.034        | -0.270            |
| Catechin (mg/day)    | 0.046         | -0.037            |

*p<0.05

Quercetin, kaempferol, and catechin were found to be significantly low in patients compared to controls. However, a comparison with other studies showed that the intake of flavonols and flavones was much higher in CHD patients and controls of the present study than had been reported in other populations.29,30 This could probably be due to inter-population variation in the bioavailability.31 A strong inverse correlation between the dietary intake of total flavonoids and flavonols with serum LDL levels was observed in CHD patients. A similar inverse relationship between intake of flavonols and flavones with the risk of coronary death was reported in a Dutch cohort study,13 in a French cohort study,29 in a Finnish cohort study,32 and in postmenopausal women.30
In the present study, catechin was found to be about 71 mg/day in controls and 36 mg/day in CHD patients, values that are much higher than those reported in other populations, and had shown an inverse relationship between catechin intake (mainly from black tea) with serum LDL in CHD patients. The possible explanation for the inverse association between catechin (from tea) and CHD is because it is thought that flavonoids from black tea may reduce myocardial infarction by reducing platelet aggregation, by inhibiting LDL oxidation, or by reducing inflammatory and thrombotic process involved in atherosclerosis. The ingestion of tea, especially green tea, had been reported to reduce lipid peroxidation, increase the resistance of LDL to oxidation, reduce total cholesterol level, and increases the HDL-cholesterol level in both normal and cholesterol-fed hamsters. However, in a recent study by Hakim et al of 3,430 Saudi adults aged 30-70 years, the consumption of black tea had been seen to have a potential protective effect in relation to CHD.

The intake of flavonoid was not associated with BMI and smoking habits. This agreed with the Finnish study but in conflict with the British study. However, the present study showed a significant correlation between the intake of flavonoids and WHR. Central obesity is considered a direct risk factor for CHD. This is supported by some studies in men which showed that the association between BMI and risk of CHD was weak while the association between WHR and risk of CHD was strong.

Vitamin E supplementation showed reduced CHD relative risk due to its role in inhibiting LDL peroxidation and platelet aggregation. However, the present study results showed that, vitamin E (α-tocopherol) had no relation with lipid profile. This was supported by the earlier in vitro studies from Esterbauer’s laboratory which reported that there was no correlation between the α-tocopherol content of LDL and its resistance to oxidation.

A significant inverse correlation between vitamin C and LDL level was found. This finding is supported by many studies that showed a significant negative correlation between ascorbic acid status and total serum cholesterol. Ascorbate supplementation has been shown to reduce lipoproteins oxidation in rats. In contrast, Ness et al found that plasma vitamin C was not correlated with serum total cholesterol or LDL. However, a high intake of vitamin C raises serum HDL cholesterol and lowers triglycerides. No correlation between the intake of selenium and lipoproteins were seen in the present study. Studies about the association between the intake of selenium and its role in CHD are highly conflicting. Some studies support our finding that revealed no association between cardiovascular risk and low intake of selenium. It has been shown that the intake of selenium is associated with lower plasma concentrations of total cholesterol and LDL-cholesterol plus very-LDL-cholesterol.

It can be concluded that in this study the intake of flavonoids was low in patients with CHD compared to controls. A negative correlation between the dietary intake of different flavonoids and serum LDL was observed in CHD patients. Furthermore, total flavonoids, flavonols, catechin, and vitamin C intake can affect the LDL levels but not other lipids such as total cholesterol, triglycerides, and HDL. Moreover, there is no relation between the intake of vitamin E, β-carotene, and selenium with CHD. Finally, smoking had no effect in the intake of flavonoids.

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