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COMPARISON OF LIPID PROFILE AND ANTIOXIDANT ENZYMES AMONG SOUTH INDIAN MEN CONSUMING COCONUT OIL AND SUNFLOWER OIL

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ABSTRACT

In this study, we compared the lipid profile and antioxidant enzymes of normal and diabetic subjects consuming two different types of oil as cooking medium. 70 normal, healthy subjects were taken as controls and 70 subjects with Type 2 diabetes were recruited in patient group. Each group was further subdivided into two subgroups of 35 subjects each, consuming coconut oil and sunflower oil respectively as cooking medium. Samples of blood were collected and analyzed for serum total cholesterol, triacylglycerols, and cholesterol in lipoprotein fractions. Total glutathione and glutathione peroxidase were measured in erythrocytes and superoxide dismutase in serum. Triacylglycerols, LDL and VLDL cholesterol levels were high in the diabetic subjects compared to the controls. Total glutathione and glutathione peroxidase values showed significant decrease in diabetic subjects as compared to the controls, while superoxide dismutase values showed significant difference between coconut oil consuming groups. Though lipid profile parameters and oxidative stress were high in Type 2 diabetic subjects compared to controls, no pronounced changes for these parameters were observed between the subgroups (coconut oil vs. sunflower oil).

KEY WORDS

Type 2 diabetes, Lipid profile, Glutathione, Glutathione peroxidase, Superoxide dismutase.

INTRODUCTION

Coconut has been an important component of the diet of Kerala population for decades. Coconut is consumed mainly as fresh kernel, coconut milk or as coconut oil derived from dried kernel. Coconut oil contains approximately 90% saturated fats. Saturated fats are known to contribute to coronary artery disease (CAD) by causing hypercholesterolemia, an established risk factor for CAD. However, most of the saturated fats of coconut oil are medium chain fatty acids having 10 to 12 carbon atoms, which are preferentially transported through the portal venous system to the liver. On the hepatocellular level, these medium chain fatty acids do not require carnitine acyltransferase for their transport across the inner mitochondrial membrane (1). Thus, medium chain fatty acids being more available for oxidation provide body with a rapid source of energy (2) and are considered to be less implicated in the accumulation of body fat (3).

A general belief that coconut oil is a major contributor to the rise in the incidence of CAD among the Kerala population have made the people shift to alternate cooking oils like sunflower oil, which is rich in linoleic acid, an essential, ω-6 fatty acid. Long chain fatty acids like linoleic acid are incorporated into chylomicrons and follow the lymphatic system. In the hepatocytes, they form triacylglycerols and phospholipids, and esterify cholesterol to give cholesterol esters (4).

Though there are studies that indicate a positive correlation between consumption of coconut oil and development of CAD (5,6), most of the recent investigations conducted in animals as well as human beings contradict claims that coconut oil increases the risk of atherosclerosis and heart disease (7,8). Coconut oil and its association with newly identified risk factors for CAD have also been studied, though not extensively (9). Though the effect of coconut oil on lipid profile has been dealt in detail by many investigators, studies on its effect on oxidative

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stress in animals and humans are scanty (10) and have not been understood properly.

In this study, we compare the lipid profile and anti oxidant enzymes (total glutathione, glutathione peroxidase and superoxide dismutase) of subjects who were consuming coconut oil (saturated, medium chain fatty acid) and those subjects consuming sunflower oil (polyunsaturated, long chain fatty acid).

MATERIALS AND METHODS

The study was conducted in the departments of Comprehensive Health Care, Endocrinology, Cardiology and Biochemistry at Amrita Institute of Medical Sciences, Kochi. The appropriate institutional review board approved the protocol used in this study, and all subjects gave informed consent for the study. Male subjects aged 35 to 65 years from middle class background, who presented to the hospital for routine health check/diabetic evaluations, were recruited for the study. The subjects furnished details regarding diet in a dietary questionnaire provided on their recruitment. The following groups of subjects were studied. Group 1 consisted of 35 control subjects consuming coconut oil (mean age: 44.9 ± 7 years), and Group 2 consisted of 35 control subjects consuming sunflower oil (mean age: 45.2 ± 8.6 years). Group 3 consisted of 35 subjects with Type 2 diabetes consuming coconut oil (mean age: 55.1 ± 8.6 years), and Group 4 consisted of 35 subjects with Type 2 diabetes consuming sunflower oil (mean age: 53.6 ± 7.4 years). All the subjects recruited were consuming the respective oil as the predominant cooking medium for over a period of six years. The subjects derived approximately 13 to 20% of their total calories from the oil considered. Subjects without any known morbidities, who were free from history of CAD as confirmed by a normal resting 12-lead ECG and absence of inducible ischemia on TMT were recruited as controls. Type 2 diabetes was diagnosed based on WHO diagnostic criteria for diabetes (fasting blood glucose levels >126mg/dL) (11). Type 2 diabetic subjects were on insulin or oral hypoglycemic agents, but none of them had previously diagnosed CAD nor were on lipid lowering therapy. The mean HbA_{1C} value for the diabetic subjects was 8.24 ± 2.02 %. 2 ml of fasting blood in EDTA and 2 ml with clot activators were collected from each subject.

The samples were centrifuged immediately and serum / plasma were separated. Serum was analyzed for lipid profile. Erythrocytes were washed thrice with ice-cold physiological saline, lysed with deionized water and used for determination of total glutathione (GSH) and glutathione peroxidase (GPx). Superoxide dismutase (SOD) was estimated in the serum.

Total cholesterol, HDL cholesterol, LDL cholesterol and triaclyglycerol (TAG) concentrations in serum were measured using kits from Randox in Hitachi 911/912 auto analyzers. VLDL cholesterol concentrations were calculated from TAG values. GSH was estimated in erythrocytes using the method of Beutler et al (12). The GSH concentration was expressed as nmol /g of hemoglobin. GPx was assayed in erythrocytes biochemically according to Paglia and Valentine (13) and as modified by Lawrence and Burk (14). Results were expressed as specific activities, where an enzyme unit represented 1 nmol NADPH oxidized per minute/g of hemoglobin. Serum SOD was assayed based on the method of Marklund and Marklund (15) and as modified by Nandi and Chatterjee (16). One unit of SOD activity is defined as the amount of enzyme required to inhibit the autooxidation of pyrogallol by 50% under specified conditions. The results have been expressed as U/ml serum. The hemoglobin concentration was determined by the cyanomethemoglobin method (17). Results were expressed as mg/dl.

Table 1: Mean values and standard deviations of serum lipid levels of subjects

	Group 1 Control Coconut oil	Group 2 Control Sunflower oil	Group 3 Diabetic Coconut oil	Group 4 Diabetic Sunflower oil
Mean age	44.9±7	45.2±8.6	55.1±8.6	53.6±7.4
Plasma lipids				
Total cholesterol (mg/dL)	161.3±30.7	157.1±28	172.4±35.9	179.1±32.3*
Triacylglycerols (mg/dL)	136.5±44.7	125.2±38.3	162.1±47.9*	151.2±37.4*
HDL cholesterol (mg/dL)	47.8±10	44.3±8.5	43.8±10.3	39.9±9.8*
LDL cholesterol (mg/dL)	78.3±24.2	82.6±26.9	108.2±35.4*	121.7±34.9*
VLDL cholesterol (mg/dL)	27.3±8.9	25.00±7.7	32.4±9.6*	30.2±7.5*

Values are mean ± SD, * $p < 0.05$ compared to controls. No significant changes were observed between subgroups (Groups 1& 2 and groups 3& 4).

Table 2: Mean values and standard deviations of antioxidant enzyme levels of subjects

	Group 1 Control Coconut oil	Group 2 Control Sunflower oil	Group 3 Diabetic Coconut oil	Group 4 Diabetic Sunflower oil
GSH (nmoles/g Hb)	7.14±0.7	6.88±0.73	5.5±0.87*	5.26±0.95*
GPx (nmol of NADPH oxidized/minute/g Hb)	18.3±1.8	18.7±2.1	16.8±2.2*	17±1.6*
SOD (U/ml serum)	5.59±1.14	5.22±1.22	4.67±0.98*	5±1.1

Values are mean ± SD, * $p < 0.05$ compared to controls. No significant changes were observed between subgroups (Groups 1& 2 and groups 3& 4).

Statistical analysis was carried out in SPSS, version 11.0. Independent 't' test was used to compare normal analytes and "Mann Whitney U test" was applied to compare non-normal analytes. The differences were considered significant if the p value was <0.05. Comparisons were drawn between coconut oil and sunflower oil consumers of control group and diabetic group, as well as between controls and diabetics consuming the same oil (between groups 1 & 2, 3 & 4, 1 & 3 and 2 & 4).

RESULTS

Table 1 shows the mean ± SD of Total cholesterol, Triacylglycerols, HDL cholesterol, LDL cholesterol and VLDL cholesterol of subjects. Total cholesterol showed significant difference only between control subjects and Type 2 diabetics consuming sunflower oil (between groups 2 and 4). Triacylglycerols, LDL cholesterol and VLDL cholesterol levels were high for the diabetic subjects of both groups (groups 3 and 4) compared to their respective controls (groups 1 and 2). Though the HDL cholesterol levels of the diabetic subjects were lower than the controls, the values attained statistical significance only for subjects consuming sunflower oil (between groups 2 and 4).

Mean ± SD of GSH, GPx in erythrocytes and serum SOD from the subjects are given in Table 2. GSH and GPx values were showing significant decrease for diabetic subjects (groups 3 and 4) compared to their controls (groups 1 and 2), while SOD values showed significant variation between coconut oil consuming groups only (groups 1 and 3). Lipid profile or oxidative stress parameters did not show significant changes between coconut oil and sunflower oil subgroups.

DISCUSSION

Atherogenic dyslipidemia is common among Indians and a major risk factor for CAD. This condition is characterized by borderline high LDL cholesterol (130-160 mg/dL), low HDL cholesterol (<35 mg/dL), high triacylglycerols (>150 mg/dL) and increased small dense LDL particles (18). Lipid profile parameters of the diabetic subjects of the present study showed considerable variation from the controls. 11.4% of our diabetic subjects had atherogenic lipid profile compared to 2.8% of the control subjects. LDL cholesterol level was significantly high for diabetics compared to the controls. Increased concentrations of LDL cholesterol may be more pathogenic in diabetic patients compared to non-diabetic subjects (19). Oxidized LDL induces vessel wall dysfunctions that are associated with the development of atherosclerosis (20). Also, a statistically insignificant increase was observed for TAG concentrations of both coconut oil groups compared to the respective subgroups.

There has been numerous animal as well as human studies in which lipid profile parameters on consuming coconut oil and other dietary fats were compared. The results obtained are not uniform and are highly conflicting. There are studies that indicated that coconut oil consumption might result in undesirable lipid profile changes compared to safflower oil (21) and soya bean fat (22). Yet, there are other studies that have failed to find any association of coconut oil with adverse lipid profile changes (23) and some that showed that coconut oil consumption has beneficial effects compared to other dietary fats (24). The effectiveness of polyunsaturated fatty acids in reducing serum cholesterol and LDL cholesterol has been elucidated (25), though several studies reported that polyunsaturated fats lower HDL cholesterol when substituted for saturated fats (26,27). HDL cholesterol concentrations were low for sunflower oil groups compared to coconut oil groups in this study also, though the results were not statistically significant. Results from Table 1 fail to provide any indications that coconut oil consumers have undesirable lipid profile pattern and increased risk for CAD compared to sunflower oil consumers. This finding is in harmony with an earlier study conducted in Kerala population, which indicates that habitual consumption of coconut and coconut oil along with normal diet has no specific role in the causation of coronary heart disease in Kerala population (28).

Coronary artery disease is now considered as an inflammatory disease and accumulating evidence is now available to suggest that oxidative stress may contribute or aggravate the process of atherosclerosis (29,30). Oxidative stress may be defined as an imbalance between the production and degradation of reactive oxygen species such as superoxide anion, hydrogen peroxide, lipid peroxides, and peroxynitrite. Enzymatic inactivation of reactive oxygen species is achieved mainly by glutathione peroxidase, superoxide dismutase, and catalase (31). In mammalian cells, glutathione and the glutathione peroxidases constitute the principal antioxidant defense system (32,33). Results of recent studies indicate that oxidative stress occurs in patients with CAD despite being clinically stable and under medical treatment (34).

Diabetes is a major risk factor for atherosclerosis and diabetes is exacerbated by oxidative stress (35). Hyperglycemia induces oxidative stress to the cells (36), which may cause injury to the vascular endothelium. Abou-Seif et al have shown that Type 2 diabetic subjects have reduced plasma concentrations of SOD and GSH when compared to controls (37). Zitouni et al had concluded that GPx activity was reduced for diabetics compared to controls (38). In the present study, there is a significant reduction in the GSH and GPx levels among the diabetic subjects compared to the controls (Table 2), which clearly indicate that, their anti oxidant defenses are weak compared to the healthy population. A reduction in serum SOD activity was noticed for diabetic subjects in this study (Table 2) and earlier studies have reported similar results (37, 39). The mean HbA_{1C} value of the diabetic subjects in this study was above the desired level indicating that poorly controlled hyperglycemia may have contributed to elevated oxidative stress in these subjects. It has been reported earlier that subjects with poor glycemic control have depressed antioxidant capacity (40). Also, diabetic subjects in this study belong to a higher age group when compared to the controls, and that oxidative stress increases with advancing age have been established earlier (41).

The role played by various dietary fats on oxidative stress in humans has not been elucidated so far. The effect of various dietary fats on antioxidant enzymes and indicators for oxidative stress has been investigated in animals. The formation of the promutagenic, exocyclic DNA adducts in the liver of rats, which are markers for DNA damage by lipid peroxidation, was found to be highest in sunflower oil fed rats when compared to coconut oil, olive oil or rapeseed oil (42). It was found that rats fed with coconut oil have low susceptibility to lipid peroxidation compared to olive or sunflower oil diets (43). Since there was no noticeable variation for the anti oxidant enzymes of the subjects consuming either of the oils, it may be concluded that the type of dietary fat consumed may not be a major contributory factor to oxidative stress in this population. The preponderance of risk factors for CAD such as hypertension, diabetes mellitus and dyslipidemia among Kerala population is very high and these factors may be contributing to the increased incidence of CAD in the state, rather than the oil consumed.

Subjects considered in this study were deriving approximately 13 to 20% of their total calories from the cooking oil. There were no significant differences for the parameters considered between the subjects consuming coconut oil and sunflower oil with similar clinical conditions. Hence, it may be concluded that the consumption of coconut oil in moderation, as a part of routine diet, may not contribute to the risk for CAD, directly by affecting the lipid profile or indirectly by aggravating oxidative stress. It may not be the type of cooking oil, rather its quantity that may be contributing to the risk of CAD. Further studies are needed to divulge how dietary fats interact with the antioxidant defense mechanisms of the body and alter them.

The increased oxidative stress in diabetics compared to the controls may be due to the age difference between the subjects of the two groups. The absence of significant results between subgroups may be due to the small study population. A prospective study with larger sample size is planned to establish the observations made in the present study.

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