Lipid Profiles in Mixed Diet and Plasma of Dhaka University Students

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Abstract

This study was conducted to determine the fatty acids composition and other lipids of diet and plasma of the resident students of Dhaka University. Dietary interview revealed that 88% of dietary fat was vegetable origin. The saturated, monounsaturated and polyunstaurated fatty acids in mixed diet-1 were found 37%, 33% and 25%, and in mixed diet-2 were 20%, 26% and 53%, respectively. The plasma levels for saturated, monounsaturated and polyunsaturated fatty acids in the students were found to be 27%, 19% and 40%, respectively. Total unsaturated fatty acids in plasma of the students comprised about 58% of the total plasma fatty acid. The polyunsaturated and saturated fatty acid ratio (P/S) of the mixed diet-1 and mixed diet-2 were 0.67% and 2.65% respectively and 1.15% in plasma of the subjects. Blood cholesterol and triglyceride (TG) values for almost all the students were within the normal range. Considering all these, it can be concluded that dietary fat intake and its impact on the blood cholesterol, TG and fatty acids composition in the resident students were found satisfactory.

Key Words : Stndents, Dietary Fat Intake, Plasma Lipids

Introduction

Currently there is a great deal of interest and emphasis on the potential health benefits of maintaining good nutritional practices. Dietary fat intake has been an important focus of interest because high-fat diets and diets high in cholesterol have been implicated in contributing to various diseases such as coronary heart disease, atherosclerosis and certain forms of cancer[1-2]. Cardiovascilar disease accounts for a major proportion of adults in many parts of the developed world. Although genetic factors and stress (which may generate adverse behaviors, such as cigarette smoking) have been implicated

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in the aetiology of heart disease. There the recommendations are to modify nutritional practices to possible decrease the development of chronic diseases.

The lipid hypothesis[3] which states that dietary fat consumption alters blood lipid levels and that blood lipids inaugurate or exacerbate atherogenesis, has received support from a various epidemiological studies[4-7]. These studies together with dietary studies in individuals led the World Health Organization [8], and other institutions, including the National Institutes of Health, the American Heart Association[9], and the French Research Council, in 1981[10], to develop the new concept that reduction in dietary cholesterol through food rich in long chain unsaturated fatty acids could reduce the incidence of cardiovascular disease. Although fatty acids of dietary origin are involved in the regulation of fat metabolism in the maintenance of normal human physiology, only few research works were undertaken to measure the relationship between dietary lipid profiles with that of blood lipid profiles, particularly in Bangladeshi population. Only Ahmed *et al.*[11] analyzed fatty acid composition of full meal (lunche only) from six dormitories of Dhaka University, Bangladesh. So, the present study was designed to assess the relation between the dietary intake of fats and fatty acids with their concentrations in plasma.

Materials and Methods

Subjects : The participants (n= 40) in the study were the students of Dhaka University living in the University dormitory. They were between 19 and 29 years (mean: 23.21) of age, weighed from 44.2 to 69.5 kg (mean: 56.79) and had body mass indexes (kg/m^2) from 16.1 to 24.6 (mean: 20.27). None had any history of atherosclerotic disease and they were all apparently healthy as indicated by their medical history and answers to questions. Patients of hypertension and taking medication were excluded from this study. Subjects were selected as voluntary participants from the entire population of the dormitory. The protocol and objects of this study were fully explained to the subjects. Written consent was taken.

Collection of Food Samples : The food items served to the resident students were almost same everyday. Available food items in the canteen of the dormitory and foods taken by a selected individual person were collected on three consecutive days.

Lipid and Cholesterol Analysis : The lipid fraction of the diet was extracted according to the modified method of Floch *et al.*[12] With slight modification cholesterol in the diet was assayed according to the method of Person *et al.*[13]

Fatty Acid Analysis : For the determination of individual fatty acid in the diet the lipid extract was dried by nitrogen and used. Heptadecanoic acid (C_{17}) was added as an internal standard. After saponification, the fatty acid fractions were methylated according to the method of Morrison and Smith[14]. This methytated falty acid ester was then injected into a Gas Liquid Chromatography (GLC), Pye Unicam. A GC 304, Glass column, 1500 mm × 4 mm was used. The column was 10% DEGS on 100-120 mesh Diatomite CWA. Nitrogen was used as carrier gas at a flow rate of 32 ml/min. The Injector and Detector temperature were fixed at 210°C and 160°C respectively. The initial column temperature was 160°C, which was maintained for 5 minutes, it was later increased at a rate of 1°C/minute up to 200°C. The standards of different fatty acids were run simultaneously.

Blood Sampling and Analysis of Plasma Lipid Profiles : Five ml of venous blood was drawn from each subject in a heparinized tube. Aliquots of 20 microliter of this blood was immediately placed in a tube containing reagents for blood haemoglobin estimation according to the method of Van Campan *et al.*[15] by using a commercial kit (Cat No. 124229. Boehringer Mannheim, Germany). The remaining blood in the heparinized tube was allowed to stand at room temperature for approximately 30 minutes and then centrifuged at 3,000 rpm. After centrifugation, plasma was collected in a separate small tube. A portion was immediately used for the estimation of HDL- cholesterol according to the method of Brustein *et al.* and Lopez Verrilla[16,17] by using a commercial kit. The remaining plasma was stored at-20°C and later used for the analysis of total cholesterol, plasma triglyceride and free fatty acids.

Plasma total cholesterol and triglyceride were estimated by using commercially available kits (Boehringer) reagent according to the methods of Sidel *et al.*[18] and Fossati and Prencipe[19]. LDL- cholesterol was calulated according to the following formula mentnoied in the HDL-choleskerol kit literature.

LDL- cholesterol = Total cholesterol - (TG/5 - HDL cholesterol).

From the stored plasma, lipids were extracted according to Hann *et al.*[20]. Saponification and methylation of plasma fatty acids were carried out as the methods of diet saponification and methylation. But in plasma lipid was saponified with 1ml of 5N NaOH and 9ml of methanol and refluxed at 75-80°C for 1 hour. GLC treatment was also same as done in diet.

Results and Discussion

Available food items in the Canteen are shown in table 1 as mixed diet-1 and mixed diet-2. Mixed diet-1 contains the food of an individual person taken in a whole day. Since each student selects their foods from available items in the Canteen, so the food items of an individual student (mixed diet-1) and available food in the dormitory Canteen (mixed diet-2) throughout the day were more or less similar. Mixed diet-1 and mixed diet-2 contained soybean as cooking oil.

Moisture content of the mixed diet-1 was 71.4gm per 100gm diet on edible portion basis. Total lipid and cholesterol content wene 76.3gm and 308mg per total diet on dry weight basis respectively. Table 2 showed clear diference of the fatty acid composition of mixed diet-1 and mixed diet-2. Among the long chain saturated fatty acids, the major difference was observed in the amount of palmitic acid ($C_{16:0}$), which was 30.91% for mixed diet-1 and 15.08% for mixed diet-2.

Total polyunsaturated fatty acids were almost double in mixed diet-2 than in mixed diet-1. Both the lenoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) were found higher in mixed diet-2 (22.19% and 2.26%). The amount of both eicosatrienoic acid ($C_{20:3}$) and arachidonic acid ($C_{20:4}$) were found trace in both the diets. In case of total monounsaturated fatty acids, no major difference was found between the two diets. The most remarkable difference was noticed in case of P/S ratio, which was 0.67 for mixed diet-1 and 2.65 for mixed diet-2. The difference in fatty acid composition and P/S ratio between the two diets was due to the difference in food items and amount. Each student selects only one type of fish for their meal but mixed diet-2 contains all type of available fish. Probably this is the main cause of differences in fatty acids between the two different mixed diets. The cause of higher polyunsaturated fatty acids

Table 1. List of food items present in mixed diet-1 and 2

Mixed diet-1	Mixed diet-2
A. Breakfast :	A. Breakfast :
1. Parata 2. Dal (Bengal Gram) 3. Potato and papaya fried 4. Egg (Duck) 5. Tea	1. Parata 2. Dal (Bengal Gram) 3. Potato and papaya fried 4. Egg (Hen & Duck) 5. Halua 7. Tea
B. Snack	B. Snack
1. Biscuit 2. Banana	1. None 2. None
C. Lunch:	C. Lunch
1. Rice 2. Fish 3. Chicken 4. Potato 5. Bringle 6. Blsam Apple	1. Rice 2. Fish (Ruhi) 3. Fish (Katla) 4. Fish (Silver-cup) 5. Chicken 6. Egg (Duck) 7. Potato+ Cabbage 8. Bean + Tomato 9. Pumpkin
D. Snack	D. Snack
1. Dalpuri 2. Firnee 3. Nimki 4. Tea	1. Dalpuri 2. Patato chop 3. Shingara 4. Mogly parata 5. Tikia kabab 6. Pudding 7. Bengal Gram (fry) 8. Halua (Bengal Gram) 9. Halua (Carrot)
E. Dinner:	E. Dinner:
1. Rice 2. Mutton 3. Potato 4. Lentil	1. Rice 2. Mutton 3. Beef 4. Potato 5. Dal (Mug)

Mixed diet-1 : One person's whole day diet with snacks;

Mixed diet-2 : One-day whole meal and snacks available in the hall canteen.

observed in mixed diet-2 was due to nature of snacks and the presence of mugh dhal in this diet. The snacks in mixed diet-2 were cooked or fried with soybean oil, which is a good source of polyunsaturated fatty acid. The higher P/S ratio in mixed diet-2 was due to lower amount of saturated and higher

Table 2. Percent fatty acid composition of mixed diet-1 and 2

Fatty acid	Mixed diet-1	Mixed diet-2
C_{12}	0.14	Trace
C_{14}	0.99	0.42
C_{16}	30.91	15.08
$C_{16:1}$	0.32	0.78
C_{18}	4.67	40.49
$C_{18:1}$	32.05	24.93
$C_{18.2}$	22.19	45.25
$C_{18:3}$	2.26	6.88
$C_{20:3}$ & $C_{20:4}$	Trace	Trace
$C_{20:5}$	Trace	0.76
UK_1	3.54	0.55
UK_2	1.15	0.52
UK_3	0.50	0.35
UK_4	1.27	Trace
TSFA	36.71	19.99
TUFA	56.82	78.50
TMUFA	32.37	25.61
TPUFA	24.45	52.89
P/S ratio	0.67	2.65

UK_1: Unknown 1; UK_2: Unknown 2; UK_3: Unknown 3; UK_4: Unknown 4; TSFA: Total saturated fatty acid; TUFA: Total unsaturated fatty acid ; TMUFA: Total monounsaturated fatty acid; TPUFA: Total polyunsaturated fatty acid: P/S ratio: Ratio of polyunsaturated fatty acid to saturated fatty acid.

amount of polyunsaturated fatty acids. From the above fact students may be advised to choose food items, which are either fried or cooked with soybean oil in order to increase the intake of polyunsaturated fatty acids. Fatty acid composition of some foodstuffs available in the hall canteen varies in fatty acid pattern (Table 3).

Table 3. Percent fatty acid composition of some foodstuffs available in the hall Canteen

Fatty acid	Rice	Egg (Duck)	Lentil	Mugh dal	B. Gram dal	Kheshari dal
C_8	0.00	0.00	1.11	0.00	0.00	0.50
C_{10}	0.00	0.00	0.55	0.00	0.00	0.00
C_{12}	0.00	0.00	Trace	0.00	0.00	0.00
C_{14}	0.19	0.00	1.82	0.38	0.38	1.00
C_{16}	18.08	26.80	41.79	29.88	11.44	35.88
$C_{16:1}$	Trace	2.70	Trace	Trace	0.41	0.00
C_{18}	21.79	6.50	5.54	6.60	9.72	22.04
$C_{18:1}$	31.44	46.54	37.69	6.48	26.04	19.58
$C_{18:2}$	26.90	10.60	3.51	37.34	44.29	4.12
$C_{18:3}$	1.21	2.36	0.00	17.61	2.73	0.00
TSFA	40.06	33.30	50.81	36.85	24.53	59.41
TMUFA	31.44	49.24	37.69	6.48	26.45	19.58
TPUFA	28.11	12.96	3.51	54.95	47.02	4.42
P/S ratio	0.70	0.39	0.07	1.49	1.78	0.07

TSFA: Total saturated fatty acid; TFUA: Total unsaturated fatty acid; TMUFA: Total monounsaturated fatty acid; TPUFA: Total polyunsaturated fatty acid; P/S ratio: Ratio of polyunsaturated fatty acid to saturated fatty acid

Even though these foodstuffs are not a good source for fatty acids, they contain some fatty acids, which are important from nutritional point of view. Rice, the staple food in students diet contains 31.44% monounsaturated fatty acid ($C_{18:1}$). The amount of polyunsaturated fatty acid in rice was 26.90% linoleic acid ($C_{18:2}$) and 1.21% linolenic acid ($C_{18:3}$). Most interesting results were obtained in case of legumes. Despite the higher consumption of lentil by resident students as well as by the Bangladeshi common people, the fatty acid composition of mugh dhal and bengal gram dhal are superior to that of lentil. Mugh dhal contain 37.34% linoleic acid ($C_{18:2}$) and 17.61% linolenic acid (C18:3), these values for Bengal gram were 44.29% and 2.73%. But in case of lentil, lower quantity of linoleic acid (3.51%) were observed. The other pulse, kheshari is completely lack of linolenic acid ($C_{18:3}$) and contains only 4.42% of

linoleic acid ($C_{18:2}$). The major fatty acid observed in egg was oleic acid ($C_{18:1}$), 46.54%. Egg also contains 10.60% linoleic acid ($C_{18:2}$) and 2.36% linolenic acid ($C_{18:3}$). Alomost similar P/S ratio was observed in mugh dhal and Bengal gram (1.49 and 1.78 respectively). Ghafoorunissa[21] have shown that low socio-economic group maintained their normal fatty acid from cereal-based diet. So, it may be inferred from the present studies that the participating students obtained a good amount of fatty acids from their cereal and legume based diet.

Table 4 shows the individual values of blood haemoglobin, triglyceride (TG), total cholesterol, HDL-cholesterol and LDL-cholesterol of the resident students. Higher levels of serum TG along with increased serum cholesterol are responsible for creating atheromatous lesion in the artery[22]. In this study, only one student had high serum TG concentration (424.66mg/dl). In case of total serum cholesterol, all participants were found normal except four students whom cholesterol level was found slightly higher than 200mg/dl.

Table 4. Mean values of blood haemoglobin, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol of the resident students

Variable	Mean (± SD) (n = 40)	Range (minimum -maximum)
Haemoglobin (gm/dl)	14.63 ± 1.54	8.97-20.56
Triglyceride (mg/dl)	90.79 ± 60.62	26.48-424.66
Total cholesterol (mg/dl)	159.27 ± 29.02	1107.26-225.60
HDL-cholesterol (mg/dl)	34.13 ± 8.01	17.54-54.36
LDL-cholesterol (mg/dl)	106.97 ± 5.60	68.50-164.71

HDL-cholesterol=High Density Lipoprotein Cholesterol; LDL-cholesterol=Low Density Lipoprotein Cholesterol

Mean values of the percentage fatty acid composition in the blood sample of the subjects are shown in table 5. Fatty acid composition of the diet did not show the presence of caprylic acid (C_8) and capric acid (C_{10}). But plasma of the students contained these fatty acids. The mean value of caprylic acid in plasma was 0.82% and that of capric acid was 0.50%. The mean value of caprylic acid in plasma was 0.82% and that of capric acid was 0.50%. The amount of myristic acid (C_{14}) and palmitic acid (C_{16}) were 0.99% and 30.19% in

mixed diet-1 and 0.42% and 15.08% in mixed diet-2 respectively. The mean of this values in plasma of participants were 1.37% of myristic acid and 19.71% for palmitic acid. Siguel and Schaefer[24] observed 0.71% and 19.71% for $C_{14:0}$ and $C_{16:0}$ fatty acid in plasma. Long chain saturated fatty acid, especially stearic acid was not found in excess amount in plasma (4.01%). In literature 6.83% of this fatty acid in human plasma has been reported[23].

Table 5. Mean values of percent fatty acid composition of the subjects

Variable	Mean (n = 40)	±SD	Range (minimum-maximum)
C_8	0.82	1.14	0.00-4.98
C_{10}	0.50	0.53	0.06-2.51
C_{12}	0.04	0.03	0.00-0.09
C_{14}	1.37	0.51	0.53-2.79
C_{16}	19.84	2.89	13.47-27.92
$C_{16:1}$	2.73	1.08	1.00-5.48
C_{18}	4.01	1.06	1.81-6.36
$C_{18:1}$	15.93	3.85	7.66-29.73
$C_{18:2}$	29.93	5.58	19.00-45.35
$C_{18:3}$	1.86	0.77	0.55-3.91
$C_{20:3}$ & $C_{20:4}$	5.98	1.49	3.02-12.57
$C_{20:5}$	0.47	0.46	T-2.14
$C_{22:6}$	1.39	0.90	T-4.08

SD = Standard Deviation

On the other hand, monounsaturated fatty acids which are now considerd to be better fatty acids[23] were observed in plasma as 2.73% in case of palmitoleic acid ($C_{16:1}$) and 15.93% for oleic acid ($C_{18:1}$) Siguel and Schaefer[23] also reported these fatty acids as 1.72% and 18.21% in human plasma respectively. The polyunsaturated fatty acids are important for their essential fatty acid activity as well as their role in cholesterol lowering process[21]. Mixed diet-1 provided 22.19% linoleic acid, 2.26% linolenic acid with docosahexaenoic acid ($C_{22:6}$). Plasma of the students also reflected these fatty acids level. The mean values observed for these acids were 29.93% for linoleic acid, 1.86% for linolenic acid and 1.39% for docosahexaenoic acid. In human plasma 36.0%, 0.84% and 2.5% of $C_{18:2}$, $C_{18:3}$ and $C_{22:6}$ were observed by others[23]. There were differences

in fatty acid composition in between mixed diet-1, mixed diet-2 and blood. Mixed diet-1 was one-day diet for a particular subject; where as mixed diet-2 contained all foods available in the dormitory canteen. Each student selects food items according to their own choice. Since preference varies person-to-person and day-to-day, differences in plasma fatty acid composition among different students were also different.

However, blood cholesterol and TG values were within normal range in almost all students. The total levels of saturated, monounsaturated and polyunsaturated fatty acids in plasma, P/S ratio were found in between the level found in mixed diet-1 and mixed diet-2. It is generally accepted that a balanced lipid diet should comprise 33% saturated, 33% monounsaturated and 33% polyunsaturated fatty acids. In Europe certain Authors propose a different distribution: 1/4 saturated, 1/2 monosaturated and 1/4 polyunsaturated fatty acids. In the present study the results observed were as follows: in mixed diet-1 37%, 33%, 25% and in mixed diet-2 20%, 26%, 53% saturated, monounsaturated and polyunsaturated fatty acids, respectively. Dietary interview revealed that 88% of dietary fat was vegetable origin. Total unsaturated fatty acid in plasma of the students comprises about 58% of the total plasma fatty acid. Considering all these, it can be concluded that fat intake and its impact on the blood cholesterol, TG and fatty acid composition in the resident students were found satisfactory. However, these data should be used to execute further research. Therefore, diets should be modified to include long chain unsaturated fatty acids (or essential fatty acids such as linoleic acid). The French Research Council also published this suggestion in 1981[11].

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