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Body Mass Index in Relation to Sialic Acid and Lipid Profile of Some Nigerian Students

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Abstract: The Body Mass Indices (BMI) and serum concentrations of High Density Lipoprotein (HDL), Total Cholesterol (TC), Total Sialic Acid (TSA) and Free Sialic Acid (FSA) were determined in thirty (30) apparently healthy students of Ahmadu Bello University, Zaria. Statistical analysis showed that the serum concentrations of TSA and FSA were significantly different ($p<0.05$) in males and females. Also, the Atherosclerotic Indices (AI) and serum concentrations of TC in students that take alcohol were significantly higher ($p<0.05$) than those that do not. These concentration values were however within normal range. A positive correlation was obtained between HDL and TC in all the students except those that consume alcohol. Between TC and TSA, a positive correlation was obtained in all the students, while between BMI and TSA, it was also positive except for those that do not take alcohol. A negative relationship existed between BMI and HDL in all the students, while between BMI and TC, all the correlations were positive except in males and smokers. It was also discovered that females and students that consume alcohol in Ahmadu Bello University, Zaria, have an increased risk to cardiovascular diseases.

Keywords: Lipoproteins, total sialic acids, free sialic acids

INTRODUCTION

Lipids, apart from being the principal form of stored energy in most organisms, play a variety of cellular roles. They serve as pigments (retina), co-factor (Vitamin A), detergents (bile salt), transporters (dolichol), hormones (sex hormones), extracellular and intracellular messenger (eicosanoids), derivatives of phosphatidal inositol, anchor for membrane protein (covalently attached fatty acids), prenine groups and phosphatidal inositol. Therefore, the ability to synthesize variety of lipids is essential to variety of organisms including man. One of such lipids is cholesterol – a steroid that modulates the fluidity of eucaryotic membranes and also the precursor of steroid hormones such as progesterone, testosterone, estradiol and cortisol. Cholesterol and other lipids present in diets are transported in blood by lipoproteins, such as, Very Low Density Lipoproteins (VLDL), Intermediate Density Lipoprotein (IDL), Low Density Lipoprotein (LDL), and High Density Lipoprotein (HDL) (Lehninger, 1993).

Sialic acids are major components of various receptor glycoproteins with a protective effect on serum glycoproteins and functions to regulate glycoprotein synthesis and metabolism in red blood cells (Schauer, 1982). Sialic acids play a major role in the viscosity of mucous secretions, which acts as lubricants and defensive agents in the body cavities or on body surfaces. Cell surface sialic acid may also be important in protecting against colonization and subsequent infection by bacteria on respiratory epithelial cells due to its ability to modulate cellular aggregation and attachment; hence membrane sialic acids prevent aggregation due to electrostatic repulsion in blood platelets (Schauer, 1982).

Earlier studies by Fontaine and Malmedier (1975) showed that the sialic acid content in VLDL is highest, whereas levels in IDL, LDL and HDL did not defer from each other. Other reports have shown that sialic acid contents decrease with increasing lipoprotein density from light VLDL to dense IDL, then being similar until dense LDL (Anber *et al.*, 1997; Millar *et al.*, 1999). Another study by Harada (1998) described decreasing sialic acid content from VLDL to LDL further to HDL. In these studies, the sialic acid content of protein and lipid were not separated.

An elevated level of cholesterol in the blood, known as hypercholesterolaemia, leads to its deposition within the arteries causing atherosclerosis, the major contributing factor of nearly all cardiovascular diseases. The ratio of Total Cholesterol to High Density Lipoprotein (TC: HDL) helps determine the Atherosclerotic Index (AI) of an individual (Martin *et al.*, 1986; Kukita *et al.*, 1982).

Watts *et al.* (1995) and Rastam *et al.* (1996), reported a positive correlation between high sialic acid levels and the incidence of cardiovascular diseases. Also, Wakabayashi *et al.* (1992) and Wu *et al.* (1999), showed that sialic acid could be linked to other cardiovascular disease risk factors, namely high serum cholesterol and triglyceride concentration and low HDL concentration.

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Also, sialic acid level has been shown to be elevated in diabetes mellitus (Crook *et al.*, 1993), and associated with cardiovascular risk in diabetic subjects (Pickup *et al.*, 1997).

Siervogel (1998) reported that age, diet and gender affect the serum level of cholesterol and high-density lipoprotein. In the present work we set the objective to study some of the lipid profiles and sialic acid concentration in the students of Ahmadu Bello University, Zaria, Nigeria. We show some correlations between the several indices that may be useful in the prediction of cardiovascular diseases.

MATERIALS AND METHODS

Experimental samples: In a study conducted in 2002, a total of thirty subjects, both males and females were randomly chosen from the students of Ahmadu Bello University, Zaria, Nigeria. These subjects were apparently healthy with ages between seventeen and thirty years old. They had to fast for about twelve hours before their blood samples were taken. The study was conducted after due clearance by the medical ethics committee of Ahmadu Bello University, Zaria, Nigeria. All reagents were of analytical grade and product of Sigma Chem. Co. Ltd., New York.

Sample collection: Blood was obtained from subjects by venipuncture, performed on the antecubital vein. The blood was then transferred immediately into labeled test tubes, allowed to coagulate and then centrifuged to obtain serum.

Preparation of reagents:

Thiobarbituric acid: This was prepared by dissolving 7.21 g thiobarbituric acid in a few mililitres of water and made up to 500 ml mark in a volumetric flask. The pH of the resultant solution was adjusted to pH 9.0 with 1.0 N NaOH (Aminoff, 1961).

Sodium arsenite: 2.0 g of Sodium arsenite was first dissolved in 20 ml of 0.5M HCl and made up to 100 ml mark in a volumetric flask using the same acid.

Periodic acid: 0.57 g of H_5IO_6 was dissolved in 20 ml of 0.125N sulphuric acid and made up to 100ml mark in a volumetric flask using the same acid.

Cholesterol reagent: Ferric perchlorate was mixed with 600 ml of ethyl acetate and 400 ml of concentrated sulphuric acid.

Butanol HCL: Butanol was mixed with HCl in a ratio of 95:5 respectively. This was mixed thoroughly.

Determination of absorption spectrum for sialic acid: Fifteen milligrams (15 mg) of N-Acetylneuraminic acid (Sialic acid) was weighed and dissolved in 1ml distilled water to make a solution of 15 mg/ml. Fifty microliters (50.0 µL) of solution was taken in a test tube and the color developed using the periodate-thiobarbituric acid assay (Aminoff, 1961). The color was extracted into the organic phase of n-butanol-HCl mixture and absorbance determined at different wavelengths (340 to 700 nm) in a quartz cuvette using a spectrophotometer. This is to determine the wavelength of maximum absorption (λmax) of sialic acid.

Preparation of standard curve for sialic acid: From a stock solution of 15 mg/ml of N-Acetylneuraminic acid (NANA), dilutions were made to the range of 1.0 mg/ml to 15mg/ml; in different test tubes. Fifty microlitres of the various concentrations were dispensed to different test tubes, and the colour developed using the periodate-thiobarbituric acid assay and their absorbance quantified at lmax, 549.

Periodate thiobarbituric acid assay for free sialic acid: To 50.0 µL of serum, 0.25 ml of 25 mM periodic acid was added, incubated at 37ºC for 10 min; after which 0.1 ml of sodium arsenite solution was added, mixed and allowed to stand for two minutes. One ml of 0.1 M thiobarbituric acid was added, mixed and incubated at 100ºC on a water bath for 10 min. A yellowish color occurred (a qualitative test for free sialic acid). The color was extracted into an organic phase by shaking the solution with 2.5 ml n-butanol-HCl (95:5) mixture. The absorbance of the colored solution was read at 549 nm in a spectrometer (Aminoff, 1961).

Periodate thiobarbituric acid assay for total sialic acid: A similar procedure like the preceding step was followed, except that 1 ml of 0.5M HCl was added initially to the serum and incubated at 80ºC for one hour, before 0.25 ml of 25 mM periodate was added and incubated at 37ºC for ten minutes. A cherry red/pink color resulted. The color was extracted into an organic phase by shaking the solution with 2.5 ml n-butanol-HCl mixture and absorbance read at 549 nm also.

Blood lipid analysis: The plasma samples were analyzed for total cholesterol (TC) and HDL-cholesterol according to the procedure described in the sigma cholesterol and HDL-cholesterol assay kits (Fortress diagnostics limited, Antrim).

Atherosclerotic index (AI):

$$\frac{\text{Value for concentration of total cholesterol}}{\text{Value for concentration of high density lipoprotein}}$$

Statistical analysis: Faculties within the University were divided into stratas, and students randomly picked. Data obtained were expressed as mean±standard error of mean (mean±SEM). The significance of the results was

Table 1: Value of HDL, TC, TSA, FSA, BMI, and AI in students of Ahmadu Bello University, Zaria, Kaduna State Nigeria (Mean±SEM, n=30)

Concentration analysis	Male	Female	Smoker	Non smokers	Alcohal	Non alcohal
HDL (mmol/L)	0.97±0.18	1.05±0.18	0.86±0.21	1.04±0.17	1.03±0.24	1.01±0.18
TC (mmol/L)	2.93±0.37	3.03±0.58	2.88±0.51	3.00±0.49	3.63±0.59*	2.88±0.38
TSA (mmol/L)	7.7±0.57*	13.8±0.15*	9.5±0.73	10.1±0.50	6.5±0.68	10.4±0.52
FSA (mmol/L)	2.6±0.17*	0.8±0.04*	2.00±0.21	1.70±0.17	3.00±0.25	1.5±0.13
BMI (mmol/L)	23.85±2.55*	22.9±2.24*	25.3±2.64	23.00±2.28	2404±1.79	23.19±2.47
(Kg/m^2) AI	3.12±0.61	2.98±0.81	3.1±0.33	2.97±0.66	3.85±0.83	2.93±0.59

*: Maen with the same superscript differ significantly

evaluated using t-test. Values of $p<0.05$ were regarded as statistically significant.

RESULTS

Effects of gender on serum concentrations of HDL, TC, TSA, and FSA as well as BMI (Table 1).

The serum HDL concentration in females (1.05±0.18 mmol/L) and males (0.97±0.18 mmol/L), TC concentration in females (3.03±0.58 mmol/L) and males (2.93±0.37 mmol/L), BMI in females (22.9±2.24) and males (23.85±2.55 kg/m^2) were not significantly different ($p<0.05$). The difference was however significant ($p <0.05$) with respect to TSA in females (13.8± 0.15 mg/ml) and males (7.7±0.57 mg/ml) as well as FSA in both genders (males (2.6±0.17 mg/ml) and females (0.8±0.04 mg/ml).

Effects of alcohol on serum concentration of HDL, TC, TSA, FSA and BMI: There was no significant difference ($p<0.05$) in the serum level of HDL in students that consume alcohol (1.03±0.24 mmol/L) and those do not (1.01±0.18 mmol/L). Total cholesterol level was however significant with respect to alcohol consumption. No significant difference was observed with respect to TSA, FSA and BMI in students that consume alcohol (6.5±0.68 mg/ml, 3.00±0.25mg/ml and 24.04±1.79 kg/m^2 respectively) and students not taking alcohol (10.4±0.52mg/ml,1.5±0.13 mg/ml and 23.19±2.47 kg/m^2).

Effects of smoking on serum concentration of HDL, TC, TSA, FSA and BMI: No significant difference was observed with respect to HDL and TC in smokers (0.86±0.21 mmol/L and 2.88±0.51 mmol/L) and non smokers (1.04±0.17 mmol/L and 3.00±0.49 mmol/L). There was also no significant difference with respect to TSA, FSA and BMI in smokers and non-smokers ($p<0.05$).

Relationship between HDL, TC, TSA, FSA and BMI: A positive relationship was observed between HDL and TC in all the students except those that take alcohol (Fig. 1). Also, serum TC concentration increased as TSA increased in all the students (Fig. 2). There was a positive correlation between BMI and TSA in all students except those that do not take alcohol (Fig. 3). In relation to HDL, a negative correlation was obtained with BMI (Fig. 4). Also, the correlation was positive between BMI and TC in all students, except in smokers and males (Fig. 5).

DISCUSSION

From the results obtained on body mass indices and serum concentrations of HDL, TC, TSA and FSA, all values of serum HDL, TC concentrations and AI were within normal range of 0.7-1.4, 2.5-6.0 and 2.4-6.0 mmol/L, respectively.

The absence of any significant difference with respect to TC and BMI in both males and females could be attributed to a possible involvement in exercises. This is corroborated by Xiao-Rong Xi *et al.* (1997) that daily physical exercise may be important in the prevention of CAD. However, the effect of exercise on HDL-cholesterol is controversial, because a study by Stubbe *et al.* (1983) reported a negative correlation between changes in plasma HDL levels and daily exercise intensity. Other factors could certainly contribute in part to changes in the levels of plasma lipids. The higher serum concentrations of TSA in females than males agrees with the report of Pickup *et al.* (1997), that TSA concentration is usually higher in women than men. Another report by Nayak and Bhakta (2005) showed that elevated TSA concentration is a risk factor for cardiovascular mortality in humans. Thus, these female students may be at risk of CAD.

The absence of any significant difference with respect to HDL in students that consume alcohol and those that do not, agrees with works by Ben *et al.* (1991), El-Sayed and Al-Bayatti (2001), who had previously reported that alcohol intake does not affect HDL concentration. This, however, contradicts the report of Hendricks *et al.* (2001), that moderate alcohol consumption increases HDL concentration and so reduces CAD risk. In the present work, the extent of alcohol consumption was ill-defined, since most consumers are rather regular drinkers. However, the significantly lower TC levels in students that do not take alcohol confirm the reports of Buhr and Burtland (1989) that a decrease in alcohol intake lowers TC concentration. Also, El-Sayed and Al Bayyatti (2001) reported that alcohol increases serum TC concentration implying that students that consume alcohol regularly have risk of CAD (Lipid Research Clinics, 1984).

There was no significant difference with respect to HDL in smokers and non smokers. Buhr and Burtland (1989), had shown otherwise that smoking increases TC and decreases the HDL cholesterol concentration. The contrasts could be due to nutritional factors, which could play a role impacting on the pathways leading to the

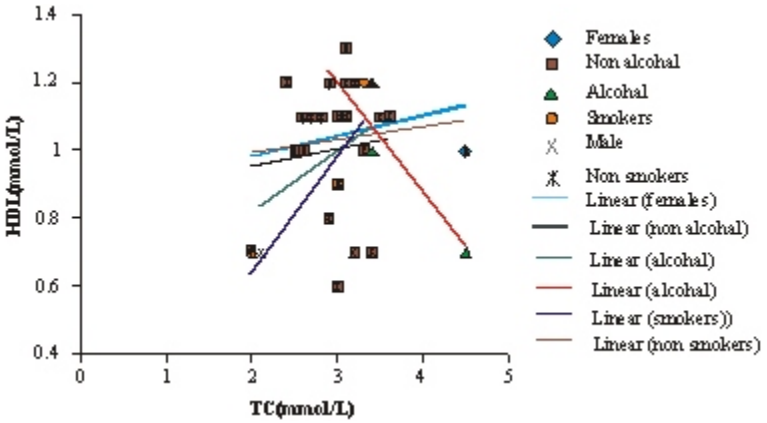

Fig 1: Relationship between High Density Lipoproteins (HDL) and Total Cholesterol (TC) in Students

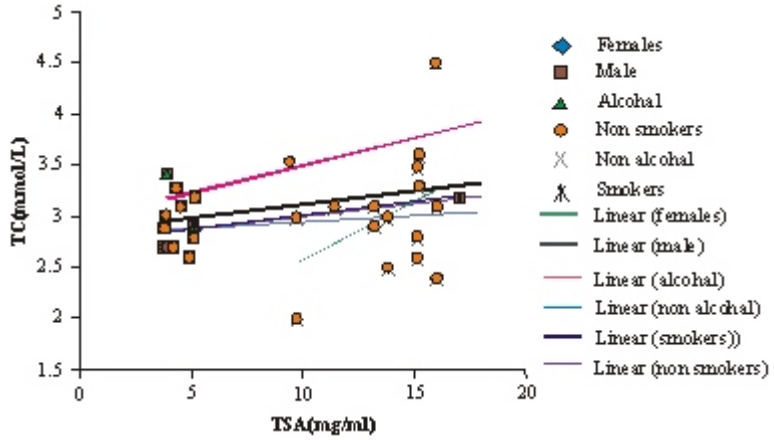

Fig 2: Relationship between Total Cholesterol (TC) and Total Sialic Acid (TSA) in students

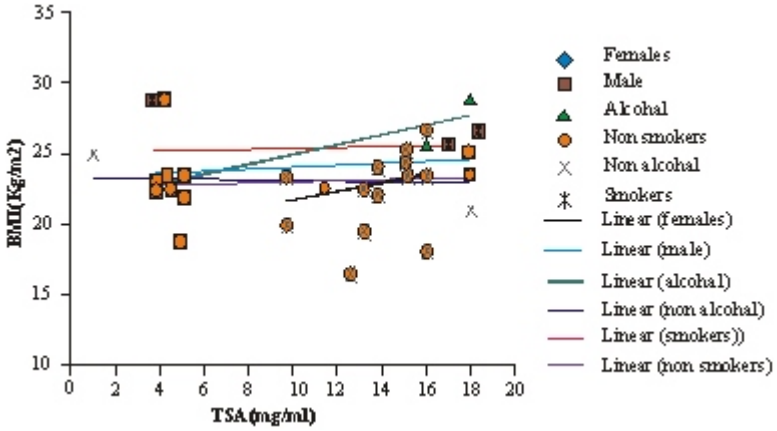

Fig 3: Relationship between Body Mass Indices (BMI) and Total Sialic Acid (TSA) in students

expression of lipids. Smoking has been shown to raise serum sialic acid concentration, and hence increases risk to CAD (Watt *et al.*, 1995). It has also been reported that smokers weigh less than non- smokers (Robert *et al.*, 1998). The absence of any significant differences may be due to moderate smoking by those who do. This then implies that smoking will not increase the risk of CAD in these students.

Relationship Between HDL, TC, TSA, FSA AND BMI: The increase in HDL observed as TC increases, doesn't agree with the report of Austin (1989), that high levels of TC and triglycerides are often linked with low HDL concentration, while Garry *et al.* (1992) reported that the higher the level of TC the greater the risk to CAD. However, studies by Ballantyne *et al.* (1978) showed that HDL concentration might increase with no significant

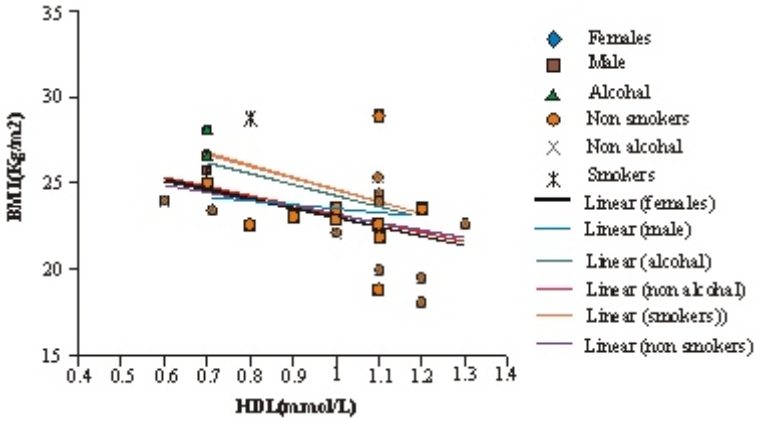

Fig 4: Relationship between Body Mass Indices (BMI) and High Density Lipoproteins (HDL) in students

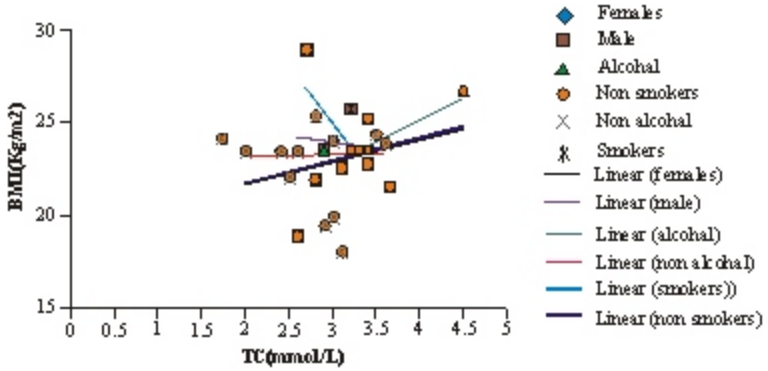

Fig 5: Relationship between Body Mass Indices (BMI) and Total Cholestrol (TC) in students

change in LDL cholesterol, the main carrier of cholesterol. The positive relationship obtained between serum concentrations of HDL and TC may be because most of these students take diets low in carbohydrate, which decreases TC concentration (Knopp *et al.*, 1997); and very low in fat, which decreases HDL (Sanders *et al.*, 1994). Also, they could have been on diets very high in carbohydrate and high in fat, thereby increasing TC and HDL. Some of them might have been taking contraceptive pills known to raise both TC and HDL. However, the negative relationship observed between HDL and TC in students that take alcohol shows that the question of whether alcohol increases HDL still persists (Ben *et al.*, 1991). This agrees with the report of Austin (1989), that high level of TC and triglycerides are often linked with low HDL concentration.

Also, increasing serum TC concentrations with a corresponding increase in TSA in all the students is in agreement with studies that state that an elevated level of TSA is linked with high TC levels (Wakabayashi *et al.*, 1992; Wu *et al.*, 1999) and has been linked to CAD (Rastam *et al.*, 1996)

Reports from the Nurses' Health Study showed a direct correlation between BMI and the occurrence of CAD (Grundy, 1995) in both men and women. This increase in risk was monotonic with 12% risk for every 1 kg/m^2 increase in BMI. The pathophysiologic mechanism underlying this is not well understood, however, the cardiovascular abnormality could be due to sodium retention and intravascular volume expansion, which induces an increase in venous vascular resistance (Grundy, 1995). Also, since increased serum TSA concentration has been linked to CAD. This could explain the positive correlation between BMI and TSA, whereas the negative correlation observed in students that do not take alcohol could possibly be because alcohol intake increases BMI, hence, these students will have lower BMI. Another reason why a positive relationship was obtained between BMI and TSA could be due to the fact that as BMI increases, volume of body fluids increases, and so sialic acid residues increase (Warren, 1959; Huttumen, 1966; Sillanaukee *et al.*, 1999; Schauer, 1982). In relation to HDL, the negative correlation with BMI may be due to the absence of HDL, to scavenge TC (Seidell *et al.*, 2001). Also, the positive correlation between BMI and TC in all students, except in smokers and males, may probably be due to the fact that males are more engaged in exercises (Robert *et al.*, 1998). However, Seidell *et al.* (2001) demonstrated that the general effect of BMI is to elevate TC.

In conclusion, it therefore means that females have higher risk to cardiovascular diseases than their male

counterparts, since they have higher serum TSA concentrations (Rastam *et al.* 1996; Wakabayashi *et al.*, 1992; Wu *et al.,* 1999). This study also shows that alcohol increases risk to cardiovascular diseases in these students, since those that take alcohol have significant TC concentrations and atherosclerotic indices compared to those that do not (Ben *et al.*, 1991). This study, thus, suggests that the students of Ahmadu Bello University, Zaria, should be more careful about their dietary intake, which has effect on BMI as well as lipid profile. This is because as we get older, we need to be concerned about how much fat we are putting on, because the faster the increase in body fat, the worse our lipid profile becomes. The take home message is that "Don't get fat, eat right and exercise more".

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