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Modulation of Cardiovascular Risk Factors (Haematological and Haemorrheological Parameters) Caused by Sucrose Diet

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ABSTRACT

The involvement of sucrose and its amount in the causation of cardiovascular disease is still controversial and inconclusive. The two latest reports of WHO/FAO and Institute of Medicine of Food and Nutritional Board (IOM of FNB) on optimal level of sucrose consumption are at least contradictory; therefore the need to clarify the effect of different concentrations of sucrose consumption on cardiovascular disease risk factor is expedient. Effect of sucrose consumption was assessed on twenty four male albino rats, four to six weeks old, 48-65 g, divided into five groups: G1 (control), G2 (10% energy supply from sucrose), G3 (20% energy supply from sucrose), G4 (30% energy supply from sucrose). The following parameters were determined: red blood cell count, white blood cell count, packed cell volume, blood and plasma viscosities; fibrinogen level and erythrocyte sedimentation rate. Analyses revealed that inclusion of sucrose at concentration of 20% energy supply significantly increased ($p < 0.05$) blood viscosity by 97.59%, plasma viscosity 16.48%, erythrocyte sedimentation rate 40.00%, plasma fibrinogen 13.63% and white blood cell count 6.59%, while no significant effect ($p > 0.05$) was observed on red blood cell count and packed cell volume. The study revealed that consumption of sucrose at twenty percent energy supply increased some selected haematological and haemorrheological parameters associated with cardiovascular disease.

Key words: Hematological, haemorrheological, sucrose, cardiovascular disease

INTRODUCTION

World Health Organization (WHO) reported that Cardiovascular diseases (CVD) contributed to one-third of all global deaths with developing countries, low-income and middle-income countries accounting for 86% of the disability-adjusted life years (DALYs) (WHO/FAO, 2003).

A lot of factors have been attributed to the etiology of cardiovascular diseases (Kaimkhani *et al.*, 2005; Lee *et al.*, 2003) of which dietary factor may have either positive or negative impact (Napoli *et al.*, 2006, 2007; Laleye *et al.*, 2007; Sivabalan and Menon, 2008;

Noroozi *et al.*, 2011). Sucrose a dietary factor which intake transcends virtually all ages has continued to be on a recurring list in the causation of cardiovascular and related diseases (Reiser *et al.*, 1989; Sivabalan and Menon, 2008). Though awareness on the reduction in sucrose consumption is gaining ground in developed countries yet it's safe level in respect of this disease is still controversial and inconclusive (WHO/FAO, 2003; Food and Nutrition Board, 2005).

Death from cardiovascular disease may be sometime sudden and without warning, however, there are some hematological and haemorrheological parameters that may serve as warning signals. Such as red blood cells count (Tonelli *et al.*, 2008; Kameneva *et al.*, 1998) white blood cells count (Madjid *et al.*, 2004; Brown *et al.*, 2001; Lee *et al.*, 2001); packed cell volume (Lowe *et al.*, 1993) blood viscosity (Wells, 1970) plasma viscosity (Koenia *et al.*, 1989) fibrinogen (Allen *et al.*, 2000; Ramsey *et al.*, 2005; Madkour *et al.*, 2006) and erythrocyte sedimentation rate (Sox and Liang, 1986).

Though literature is replete with the effect of sucrose on some traditional cardiovascular risk factors such as cholesterol and triglycerides (Reiser *et al.*, 1989; Frayn and Kingman, 1995; Parks and Hellerstein, 2000). However, there is paucity of data on the effect of sucrose on some of these hematological and haemorrheological parameters which are risk factor for cardiovascular diseases.

In view of the above we set to investigate the effect of various concentrations of sucrose consumption on these risk factors in order to establish relationship between sucrose consumption and the risk factors.

MATERIALS AND METHODS

Experimental animals: Thirty, 4-6 weeks old male albino rats weighting 48-65 g were purchased from physiology department Olabisi Onabanjo University Ago-Iwoye. The rats were acclimatized for two weeks in an individual metabolic cage, fed water and rat chow *ad libitum*.

Animal grouping and feeding: After acclimatization the animals were categorized into four groups fed for twelve weeks: G1 (control group fed rat chow only), G2 (fed rat chow+sucrose diet, twenty percent energy from sucrose), G3 (fed rat chow+sucrose diet, 20% energy from sucrose) and G4 (fed rat chow+sucrose diet, 30% energy from sucrose).

Animal sacrifice: After twelve weeks of feeding, the rats after fasted overnight, anaesthetized with diethyl ether placed back flat on a dissecting board the rats was opened up, blood collected by cardio puncture method using Needle and Syringe and the Needle was removed and the blood was gently pumped into an EDTA collection Bottle (except for fibrinogen which citrate bottle was used) properly mixed and processed for further analysis.

Sample preparation: Whole blood was used for blood viscosity erythrocytes sedimentation rate, white blood cell count, red blood cell count and packed cell volume while plasma which was prepared by centrifugation of the whole blood was used for the determination of plasma viscosity and fibrinogen.

Chemical reagents: All reagents used in this experiment were of analytical grade.

Analytical method: Packed cell volume was determined by method of Dacie and Lewis (1991), red blood cell counts was determined by method of Kasper and Wallerstein (1966) white blood cells

counts was determined by method of Booth and Hancock (1961) while plasma and blood viscosities were determined by modified method of Ugwu and Reid (1987). Erythrocyte sedimentation rate was determined by using Western green method (Westergreen, 1957) and plasma fibrinogen was determined by direct clot weight procedure as described by Ingram method (Ingram, 1961).

Statistical analysis: The data were analysed using one-way ANOVA, level of significance was assessed using Duncan Multiple Range Test at $p < 0.05$ (SPSS 14.0 software was used for data analysis).

RESULTS

As shown in Table 1, no significant difference ($p > 0.05$) was observed when red blood cell count of the control and the experimental groups were compared showing that intake of sucrose at these various concentrations had no effect on red blood cell count.

There was a significant difference ($p < 0.05$) in the white blood cell count of the control and other experimental groups. Similarly, differences ($p < 0.05$) existed between G4 and other groups. However, no significant difference was observed between G2 and G3.

No significant difference ($p > 0.05$) was observed in packed cell volume when the control (G1) group was compared with other experimental groups G2, G3 and G4. Also no significant ($p > 0.05$) difference exist between the various experimental groups indicating that sucrose at these various concentrations had no effect on PCV level.

Table 2 revealed a significant increase ($p < 0.05$) in blood viscosity level is observed when the control (G1) group is compared with other experimental groups G3 and G4 with an increasing percentage of 97.59 and 139.93 indicating a progressive increase in the blood viscosity level as the sucrose consumption increases. However, there is no significant ($p > 0.05$) increase in the blood viscosity of albino rats at G1 and G2 showing that consumption of sucrose at 10% of energy supply has no effect ($p > 0.05$) on blood viscosity.

A significant increase ($p < 0.05$) in plasma viscosity was observed when the control (G1) group was compared with G3 and G4 with an increasing percentage of 16.48 and 34.66 respectively indicating a progressive increase in the plasma viscosity as the sucrose consumption increased. However, there was no significant increase ($p > 0.05$) between G1 and G2 indicating that sucrose consumption had no effect on plasma viscosity at the level of 10% energy supply.

In table 3, a significant increase ($p < 0.05$) in fibrinogen level was observed when the control (G1) group was compared with other experimental groups G2, G3 and G4. However, there was no significant difference ($p = 0.05$) between the experimental groups that was G2, G3 and G4 showing that sucrose effects on plasma fibrinogen was not dose dependent.

Table 1: Effect of sucrose diet on haematological parameters

Rat group	Red blood		White blood		Packed cell	
	cell count (10^6 mm^{-3})	Difference (%)	cell count	Difference (%)	volume	Difference (%)
G1	6.41 $\pm$ 1.90 ^a	0.00	3475.00 $\pm$ 11.54 ^a	0.00	55.83 $\pm$ 2.73 ^a	0.00
G2	6.00 $\pm$ 0.90 ^a	-6.40	3692.16 $\pm$ 11.66 ^b	6.25	49.16 $\pm$ 2.14 ^a	-11.95
G3	5.90 $\pm$ 0.40 ^a	-7.96	3704.00 $\pm$ 11.54 ^b	6.59	48.66 $\pm$ 0.84 ^a	-12.84
G4	6.10 $\pm$ 0.60 ^a	-4.84	3958.66 $\pm$ 32.57 ^c	13.92	49.67 $\pm$ 0.62 ^a	-11.03

Values are expressed as Mean $\pm$ SEM for 6 rats per group, Mean values were compared using one way-ANOVA, Level of significance was assessed using Duncan multiple range test (DMRT) at $p < 0.05$, Values with different superscript are significantly different. G1: Control, G2 : 10% energy supply from sucrose, G3: 20% energy supply from sucrose and G4: 30% energy supply from sucrose

Table 2: Effect of sucrose diet on blood and plasma viscosities

Rat group	Blood viscosity	Difference (%)	Plasma viscosity	Difference (%)
G1	11.62±0.43 ^a	0.00	1.76±0.010 ^a	0.00
G2	12.38±0.51 ^a	6.54	1.86±0.008 ^a	5.68
G3	22.96±0.64 ^b	97.59	2.05±0.014 ^b	16.48
G4	27.88±0.66 ^c	139.93	2.37±0.016 ^c	34.66

Values are expressed as Mean±SEM for 6 rats per group, Mean values were compared using one way-ANOVA, Level of significance was assessed using Duncan multiple range test (DMRT) at $p \leq 0.05$, Values with different superscript are significantly. G1: Control, G2 : 10% energy supply from sucrose, G3: 20% energy supply from sucrose and G4: 30% energy supply from sucrose

Table 3: Effect of sucrose diet on fibrinogen and erythrocytes sedimentation rate

Rat group	Fibrinogen (mm dL ⁻¹) rate	Difference (%)	Erythrocyte sedimentation	Difference (%)
G1	3475.00±11.54 ^a	0.00	3.75±0.06 ^a	0.00
G2	3692.16±11.66 ^{ab}	12.43	4.16±0.56 ^b	10.93
G3	3704.00±11.54 ^b	13.63	5.25±0.05 ^c	40.00
G4	3958.66±35.27 ^b	13.99	0.29±0.06 ^d	67.73

Values are expressed as Mean±SEM for six (6) rats per group, Mean values were compared using one way-ANOVA, Level of significance was assessed using Duncan multiple range test (DMRT) at $p \leq 0.05$, Values with different superscript are significantly different. G1: Control, G2 : 10% energy supply from sucrose, G3: 20% energy supply from sucrose and G4: 30% energy supply from sucrose

A significant increase ($p < 0.05$) in ESR was observed when the control (G1) group was compared with other experimental groups G2, G3 and G4 with an increasing percentage of 10.93, 40.00 and 67.73 indicating a progressive increase in the ESR as the sucrose consumption increased.

DISCUSSION

Various hematological and haemorrheological parameters have been associated with cardiovascular diseases (Tonelli *et al.*, 2008; Rudnicka *et al.*, 2006; Madjid *et al.*, 2004; Raddino *et al.*, 2002). Some of these risk factors have been shown to be diet influenced (Adebawo *et al.*, 2007; Salau *et al.*, 2003; Ornish *et al.*, 1990). The results of this study revealed that inclusion of sucrose at concentration of 20% energy supply significantly increased ($p < 0.05$) blood viscosity, plasma viscosity, erythrocyte sedimentation rate, plasma fibrinogen and white blood cell count, while no significant effect ($p > 0.05$) was observed on red blood cell count and packed cell volume.

Studies have shown effect of dietary factors on RBC count (Adeniyi and Fasanmade, 2006; Abdelhalim and Alhadlaq, 2008). However, consumption of sucrose at 30% energy supply (G4) has no adverse effect on RBC count and PCV level.

The data in Table 1 also revealed that increase in sucrose consumption has no significant difference ($p > 0.05$) on PCV level. This is logical because the major factor determining PCV level is the concentration of red blood cell. It follows that the factor that affects RBC may affect PCV and it can be concluded that intake of sucrose at 30% energy supply has no effect on PCV level.

However Table 1 revealed increase in white blood cell count as sucrose consumption increased White blood cell has been associated with cardiovascular risk as increase in WBC increase the risk of the disease (Gillum and Mussolino, 1994) and it is affected by dietary factor (Ahamefule *et al.*, 2006; Ironkwe and Oruwari, 2011). It followed from the data above that inclusion of sucrose even at very low level of 10% energy supply from sucrose, increased white blood cells count and this implied that sucrose consumption may increase cardiovascular risk by increasing white blood cells. One of the possible mechanism may be as a result of effect of sucrose on phagocytic action of white

blood cells which high sucrose diet decreased and this may impose a feedback on the synthesis of more white blood cells to counter react the effect of sucrose.

Plasma viscosity increase which may be a step during atherosclerosis pathogenesis (Abu-Samak *et al.*, 2011) appeared to be on increasing trend as the sucrose consumption increased, significance difference ($p < 0.05$) observed in the control G1, when compared with G3 and G4 showing the effect of sucrose consumption on plasma viscosity, some of the factors increasing plasma viscosity are albumin, cholesterol and fibrinogen (Lowe *et al.*, 1980). However since no significant difference ($p > 0.05$) is observed in albumin (Salau, 2010) content it could be reasonably inferred that increase in plasma viscosity by sucrose consumption could be as a result of fibrinogen and cholesterol increase but not by albumin as revealed in the previous study (Salau, 2010).

In Table 2, the data showed that intake of sucrose at 10% energy supply has no effect on blood viscosity. However, when the sucrose intake increased to twenty and thirty percent energy supply at G4 and G5 there is a significant increase ($p < 0.05$) in blood viscosity. This could be as a result of increase in lipoproteins and fibrinogen. Thus it implies that sucrose may increase this cardiovascular risk factor when it supplies about 20% energy in the diet because high blood viscosity has been implicated in cardiovascular disorders. (Dintenfass, 1974; Lowe, 1986). Since no significant different in RBC which is a major component of blood it could be reasonably concluded that increase in blood, viscosity is as a result of increase in plasma viscosity.

A significant increase ($p < 0.05$) in fibrinogen is observed at the inclusion of glucose i.e., G2, 10% energy supply. However it appeared that increase in fibrinogen as a result of sucrose intake is not dose dependent. The influence of diet on fibrinogen has been shown to be as a result of its caloric value. High calorie diet may increase fibrinogen level (Ditschuneit *et al.*, 1995). Fibrinogen has been implicated in the etiology of cardiovascular disease. (Allen *et al.*, 2000; Krobot *et al.*, 1992). Some of the proposed mechanisms by which fibrinogen increases cardiovascular risk are: fibrinogen promotes fibrin formation and it is a major contributor to plasma viscosity, a condition that increases fibrinogen level as in case of sucrose consumption may affect cardiovascular risk factors.

Though there is paucity of data on effect of diet on sucrose on ESR. The effect of sucrose consumption appeared to be profound at 20% energy supply i.e., G3 and 30% of energy supply (G4) on ESR levels. From the ongoing, sucrose intake at level $\geq 30\%$ energy supply from sucrose may have a negative effect on ESR. Though no significant difference ($p > 0.05$) was observed in RBC count which is a major determining factor in ESR however increase in fibrinogen in this study could be responsible for the elevation of the ESR. As the sucrose intake increases which consequently increase fibrinogen level may lead to increase in the formation of rouleaux that causes the cell to settle more rapidly, consequently increasing Erythrocyte sedimentation rate.

CONCLUSION

From the ongoing the consumption of sucrose at a level greater than 10% energy supply has an adverse effect on cardiovascular risk factors with a more profound effect on haemorrhological factors rather than haematological factors. The major effect was observed in blood plasma which subsequently affects blood viscosity.

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