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RESEARCH ARTICLE

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High sucrose diet modulates Calcium status in male albino rats: Possible implication on cardiovascular disease and dental caries

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ABSTRACT

Effects of various concentrations of sucrose diet were assessed on calcium status of thirty weanling male albino rats randomly divided into five equal groups as follows: G1 (baseline group); G2 (control group; rat chow only); G3, G4 and G5 (experimental groups; with energy supply from sucrose at 10, 20 and 30 percent respectively). The experimental groups were fed for twelve weeks; standard laboratory procedures were used to determine feed and nutrient intakes; feaces and urine were taken for calcium estimation, the rats were fasted overnight, anaesthetized with diethyl ether and blood was collected using cardio puncture method. Bones and teeth were removed washed, dried and ashed. Plasma was collected by centrifugation, urinary and plasma calcium was assessed by enzymatic kits while feacal, bone and teeth calcium were assessed using standard laboratory methods. Analyses revealed that inclusion of sucrose at concentration of 20 percent of energy supply significantly increased ($p<0.05$) plasma calcium, 59.54 percent and urinary calcium, 125.72 percent. Significant decrease ($p<0.05$) was observed in faecal calcium, 9.54 percent; bone calcium, 3.21 percent and teeth calcium, 15.98 percent. The study revealed that consumption of sucrose at twenty percent energy supply may reduce teeth and bone calcium which may affect their integrity; increased plasma calcium which may affect cardiovascular risk factors in albino rats. Consequently, current recommendation of WHO/FAO stating that sucrose should not supply more than ten percent of energy intake rather than upper limit of twenty five percent set by IOM of Food and Nutrition Board must be adhered to.

Key words: Sucrose diet, calcium status, dental carries, cardiovascular disease, rats.

INTRODUCTION

Calcium is the most abundant cation in human tissues and serves many roles among which are its importance in bone calcification, metabolic regulations and signalling. Its status depends on intake, distribution in various tissues such as bones and teeth; excretion in urine, disposal in feaces and the amount present in blood or serum.

Plasma calcium is believed to reflect the dietary calcium [1] Plasma calcium has been linked with cardiovascular risk. [2], myocardial infarction [3], serum cholesterol, HDL cholesterol and triglycerides [4]. Its homeostasis depends on the urinary calcium loss, absorption from food, feacal loss and mobilization of from calcium stores such as teeth and bone among other factors.

The alterations in calcium balance in humans have also being linked to weak bones and changes in bone composition [5, 6]. Similarly, tooth formation and integrity is affected by sucrose diet [7]. Since the synthesis of cementum and enamel requires calcification, thus any compromise in calcium status would affect its integrity
Different dietary factors have been shown to affect calcium status among which sucrose has been on a resounding note [5].The amount of sucrose required for optimal health is still controversial and inconclusive, while WHO/FAO recommends 10% energy supply from sucrose in the diet[8]; Food and Nutrition Board of Academy Sciences concluded that 25% energy supply from sucrose may not have adverse effect on humans 'health [9].

In view of the above we set to investigate the effect of different concentrations of sucrose diet on calcium status and possible implication on cardiovascular, teeth and bone health.

MATERIALS AND METHODS

Experimental Animals

Thirty (30) weanling male Wister rats from purchased from physiology department, of Olabisi Onabanjo University were used for this experiment. The rats weighing 57-63g were acclimatized for 2 weeks in cages where water and rat chow were given *ad libitum*.

High sucrose diet were prepared as described by [10], aanimals were randomly divided into five (5) groups:

Group 1 (G1, baseline).
Group 2 (G2, normal control)
Group 3 (G3, test group1)10% energy supplied from sucrose.
Group 4 (G4, test group 2) 20% energy supply from sucrose.
Group 5 (G5, test group 3) 30% energy supply from sucrose.

After the twelve-week period, the rats were sacrificed after fasted overnight, blood withdrawn from the rats by cardiac puncture, centrifuged at 3,000rpm for20 min and the plasma was collected.

Biochemical analysis

Plasma and urinary calcium was analysed using Randox kit purchased from England, while faecal dropping, bones and teeth of the rats were analysed for calcium using the method of Belcher and Nutler [11].

Statistical analysis

All data were analyzed using one-way ANOVA, $p<0.05$ was considered significant. SPSS 14.0 was used for data analyses.

RESULTS

Table 1: Plasma Calcium in Experimental and Control Male Albino Rats

Group	Plasma Ca^{2+} mg/dl	% Difference from Control
G1	6.465 ± 0.30^{a}	
G2	8.302 ± 0.43^{b}	0.00
G3	10.193 ± 0.45^{c}	22.78
G4	13.245 ± 0.74^{d}	59.54
G5	15.703 ± 0.34^{e}	89.15

Values are expressed as mean ±SEM for six (6) rats per group. Values with different superscripts are significantly different ($p<0.05$).

Table 2: Urinary Calcium Excretion in Experimental and Control Male Albino Rats

Group	Urinary Ca^{2+} (mg/dl)	% Difference from Control
G1	2.61 ± 0.57^{a}	
G2	2.94 ± 0.49^{a}	0.00
G3	6.55 ± 0.58^{b}	122.66
G4	6.64 ± 0.57^{b}	125.72
G5	7.16 ± 0.66^{b}	144.19

Values are expressed as mean ±SEM for six (6) rats per group. Values with different

. **Table 3: Feacal Calcium in Experimental and Control Male Albino Rats**

Group	Feacal Ca^{2+} (mg/100g)	% Difference from Control
G1	16.09 ± 0.61^{a}	
G2	22.02 ± 0.61^{c}	0.00
G3	19.71 ± 0.63^{b}	-10.49
G4	19.92 ± 0.72^{b}	-9.54
G5	19.61 ± 0.56^{b}	-10.92

Values are expressed as mean ±SEM for six (6) rats per group. Values with different superscripts are significantly different (p<0.05).

Table 4: Bone Calcium In Experimental and Control Male Albino Rats

Group	Bone Ca^{2+} (mg/g)	% Difference from Control
G1	61.50 ± 1.20^{a}	
G2	90.00 ± 0.28^{d}	0.00
G3	88.07 ± 0.55^{cd}	-2.14
G4	87.11 ± 0.60^{bc}	-3.21
G5	85.06 ± 0.75^{b}	-5.49

Values are expressed as mean ±SEM for six (6) rats per group. Values with different superscripts are significantly different (p<0.05).

Table 5: Teeth Calcium in Experimental and Control Male Albino Rats

Group	Teeth Ca^{2+} (mg/g)	% Difference from Control
G1	55.75 ± 0.52^{a}	
G2	89.39 ± 0.97^{e}	0.00
G3	82.72 ± 0.61^{d}	-7.46
G4	75.11 ± 0.63^{c}	-15.98
G5	69.06 ± 0.57^{b}	-22.74

Values are expressed as mean ±SEM for six (6) rats per group. Values with different superscripts are significantly different (p<0.05).

Table 1 shows the plasma calcium level of albino rats consuming various concentration of sucrose. A significant increase ($p<0.05$) in plasma calcium level was observed when the experimental groups (G3, G4 and G5) were compared with the control (G2) with an increasing percentages of 22.78, 59.54, and 89.45 respectively indicating a progressive increase in the plasma calcium level as the sucrose consumption increased.

Shown in table 2 is the effect of sucrose consumption on urinary calcium level in male albino rats. A significant increase ($p<0.05$) in urinary calcium level was observed when the experimental groups (G3, G4 and G5) were compared with the control group G2. However, there was no significant difference ($p>0.05$) between the experimental G3, G4 and G5 showing that sucrose intake on urinary calcium level was not dose dependent.

Table 3 showed the effect of sucrose consumption on feacal calcium. A significant decrease ($p< 0.05$) in feacal calcium level was observed when the experimental groups (G3, G4 and G5) were compared with the control group (G1). However, there was no significant difference ($p>0.05$) between the experimental groups G3, G4 and G5 when compared with each other showing that the effect of sucrose intake on feacal calcium was not dose dependent.
Table 4 showed the effect of sucrose consumption on bone calcium. A significant decrease ($p<0.05$) in bone calcium level was observed when the experimental groups (G3, G4 and G5) were compared with the control group (G2), with a percentage decrease of 3.21 and 5.49 respectively.

There was no significant ($p>0.05$) difference between G2 and G3, as well as in G3 and G4, and G4 and G5 respectively. However there was a significant difference ($p<0.05$) between G1 and G2.

Table 5 depicts the teeth calcium level of rats consuming various concentrations of sucrose. A significant decrease ($p<0.05$) in teeth calcium level was observed when the experimental groups (G3, G4 and G5) were compared with the control group(G1), with a decreasing percentage of -7.46, -15.98, and -22.74 respectively, indicating a progressive decrease in the teeth calcium level as the sucrose consumption increased.

DISCUSSION

Various investigators have established the relationship between sucrose intake and calcium status [5 12, 13]; and the link in causation of dental caries and cardiovascular disease [2, 14]. However, the optimal level of consumption is

still controversial and inconclusive [10]. This study demonstrated the adverse effect of consuming high sucrose diet (more than 10% energy supply from sucrose) on plasma calcium, bones and teeth integrity

Changes in plasma calcium levels that were observed in rats fed with different concentrations of sucrose diet shows that the higher the sucrose intake, the higher the plasma calcium as evidenced in this study (Table 1). A progressive increase of 22.78% was observed in the group fed with 10% percent energy supply indicating that introduction of sucrose into the diet of the rats increases the plasma calcium and the effect continued until about 90% increase in plasma calcium was observed with the group fed with 30% energy supply from sucrose. This result is in consonance with the previous reports [5, 15, 16], that high sucrose diet leads to alteration in calcium balance in humans. It was also shown that calcium homeostasis depends on urinary calcium loss and absorption from food which is affected by the type of diet as reported previous investigators [1].

One of the mechanisms suggested on how sucrose rich diet alters serum calcium is by increasing urine calcium levels and also increasing serum insulin levels and this may be the reason for the slight fall in blood glucose of rat fed with a high sucrose diet 43.00±1.6mg/dl when compared with control 50.8±1.15mg/dl [13]. The calciuric effect of sucrose is thought to be the result of reduced tubular reabsorption [12, 15].

The result demonstrated clearly that sucrose intake increased plasma calcium which may eventually increase urinary calcium excretion. Increase in sucrose consumption above 10% percent energy supply may have a detrimental effect on the plasma calcium level as already shown that about 90% increase in plasma calcium is observed when sucrose intake increased from 0% to 30% energy supply. This study therefore supported other reports which discouraged the intake of sucrose at high level and also corroborated the fact that when sucrose supply is more than 10% of energy requirement which is the FAO/WHO's joint committee recommendation, it may have an adverse effect on plasma calcium. Since serum calcium has been associated with cardiovascular risk factor, it could be concluded that sucrose rich diet which increases serum calcium may eventually increase cardiovascular risk [9, 10].

This study revealed that increase in sucrose consumption also increased urinary calcium. Increase in urinary calcium of about 144% was observed when G5, 7.16±0.66mg/dl was compared with control group (G2) 2.94±0.49. One of the mechanisms proposed on how sucrose diet increases urinary excretion is reduction in tubular reabsorption [5, 15]. A substantial increase in urinary calcium caused by sucrose rich diet has been suggested to increase the risk of developing bone loss associated with aging [5, 13].

The faecal calcium depends majorly on the absorption from diet which is as a result of intake of calcium with a meal [17]. Increase in faecal calcium observed in G2 (22.02 ± 0.61) when compared with the baseline, G1 (16.09 ± 0.61) shows that as the rats grow older, there was a decrease in absorption of calcium which was reflected in the faecal calcium. However, decrease in faecal calcium observed in (Table 3) shows that inclusion of sucrose in the diet increases calcium absorption, though not dose dependent. This is contrary to the report of Tjaderhane and Larmas, [18]. The possible explanation for this could be that increase in urinary excretion of calcium may drive absorption and bone reabsorption as shown in Tables 4 and 5 where sucrose consumption decreases bone calcium and teeth calcium.

The study revealed that inclusion of sucrose in the diets of the rats increases the calcium absorption and decreases faecal calcium. This mimics hypercalcuria condition as explained by previous investigators [19, 20].

A downward trend observed in bone calcium in this study indicated that as sucrose consumption increases, bone calcuim reduces, though the only significant reduction ($p<0.05$) was observed in G4 and G5 compared with control G2 (Table 4). The reason is that calcium is not stored as such.The mineralization of bone requires active metabolic work, i.e. a composite of a protein matrix encrusted with mineral cystal [17]. This decrease in protein content of the diet as sucrose content increase [10] might lead to decrease in available protein for calcium metabolism . It appears that bone calcium is easily affected by protein status of food. The synergistic effect of decrease in tubular reabsorption as reported by Ericsson et al[5] may induce bone resorption in order to raise serum calcium. Also as reported by Broadus *et al* [20], higher protein intake is needed to promote calcium absorption.The reduction in calcium level as shown in Table 3 and protein level with the combined effect of reduction in tubular reabsorption may cummulatively reduce bone calcium which may later be responsible for osteoporosis [17].

Tooth decay or dental caries is believed to be caused by the presence of sucrose in the diet thereby encouraging bacteria that causes tooth decay [21, 22, 23]. However, recent studies have shown that tooth decay could be more of calcium metabolism which make teeth more vulnerable to bacterial attack and as it has been shown that high sucrose diet affects teeth development and integrity [13] by lowering calcium availability to the teeth.

The result obtained in this study showed a decrease in teeth calcium content as sucrose intake increased. Intake of sucrose at about 10% supply of energy decreased teeth calcium by 7.46% while 20 percent and 30 percent sucrose supplies of energy reduced teeth calcium by 15.98 and 22.74% respectively. Thus it appeared that the teeth are more vulnerable to calcium depravity than bones.

CONCLUSION

High sucrose diet altered calcium status by increasing plasma and urinary calcium levels; decreasing feacal, bone and teeth calcium contents, which may adversely affect cardiovascular, bone and dental health.

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APPENDIX

Appendix 1: Commercially produced stock diet

Food constituents	Per 100g Weight
Energy [kcal/100g]	**365**
Carbohydrate [%]	**62.0**
Protein [%]	**21.0**
Fat [%]	**3.5**
Dietary fibre [%]	**6.0**
Calcium [%]	**0.8**
Phosphorus [%]	**0.8**
Moisture [%]	**5.0**
Salt Antioxidant & Antibiotics	**0.9**

Analysis of this diet as revealed by the manufacturer [Ladokun feed Nigeria Limited Ibadan Nigeria] showed the following constituents

Appendix 2:**Raw ingredients of commercial diet**

1 Maize
2 Wheat middling
3 Groundnut cake
4 Bone meal
5 Oyster shell
6 Salt
7 Antioxidants and antibiotics

Appendix 3: Dietary calcium (%) content in 100g feed

	SUCROSE CONTENT	CALCIUM CONTENT	TOTAL CALCIUM (%)
G1	**-**	**100 (0.008)**	**-**
G2	**-**	**100 (0.008)**	**-**
G3	**9.332 (0)**	**90.668 (0.008)**	**0.73**
G4	**18.804 (0)**	**81.196 (0.008)**	**0.65**
G5	**28.418 (0)**	**71.582 (0.008)**	**0.57**