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# Research Article Possible Implication of Long Term Sucrose Diet on Integumentary Tissues' Minerals of Male Albino Rats

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## Abstract

**Background and Objective:** The role of sucrose in the biochemical derangement in many tissues/organs has been established. However, there is a dearth of information about its effect on mineral status in integumentary tissues such as hair, nail, and skin in male albino rats. This study investigated minerals response in some selected integumentary tissues and possible implications on their integrity and performance in rats fed sucrose diet. **Materials and Methods:** Eighteen male albino rats were grouped into three with varying concentrations of sucrose supplying energy at 0% (G<sub>1</sub>), 10% (G<sub>2</sub>) and 20% (G<sub>3</sub>), respectively. Animals were fed for six months and the hair, nail and skin were harvested for calcium, phosphorus and magnesium analyses. **Results:** Substantial increase was observed in phosphorus and magnesium, while a decrease was noted in calcium levels in tissues as sucrose consumption increased . **Conclusion:** We opined that the alterations in mineral levels occasioned by sucrose concentration in the diet might adversely affect the integrity and function of integumentary tissues.

Key words: Sucrose diet, minerals, toxicity, integumentary tissues, absorption, hypomagnesemia, sensation

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Data Availability: All relevant data are within the paper and its supporting information files.

#### INTRODUCTION

The involvement of a sucrose diet in the pathology of many diseases has been well established<sup>1-3</sup>especially when it supplies more than 10% energy content of the food. It prejudicial and harmful effect on various organs and tissues are also well documented. It achieves this through alteration of biochemical and physiological integrity of the cell and most often leading to changes in the histoarchitecture and morphological perturbations<sup>4,5</sup>.

Some of the organs/tissues affected are heart, liver, brain, blood, testes, bones and integumentary tissues<sup>6-10</sup>. Integumentary systems consist of organs and tissues necessary for protection. They include skin, hair and nails. Skin, part of the integumentary system, is the largest organ in the body in conjunction with other tissues (hair, nails, glands etc.) that help in protection, absorption, excretion, secretion, regulation and sensation.

Major minerals including calcium, phosphorus and magnesium that are necessary to maintain the biochemical and physiological integrity of the biological system (which includes integumentary tissues) could be affected by sucrose consumption<sup>11</sup>.

Fructose (a main component of sucrose) diminishes calcium transport<sup>12</sup>. A study showed that a twenty percent sucrose diet lowered calcium levels in bone and teeth while it increases the level in plasma and urine<sup>13</sup>. Also, the involvement of phosphate in glucose metabolism is established<sup>14</sup>. Moreover, it was noted in a study, that hypoglycemia-induced osmotic diuresis is mostly accompanied by magnesia which results in hypomagnesemia<sup>15</sup>. Furthermore, an increase in insulin results in the net shift of magnesium ions from the extracellular to the intracellular environs causing hypomagnesemia<sup>16</sup>.

The effect has also been seen in the integumentary tissues (skin, hair and nail). Sucrose's role has been seen in skin inflammation and slow wound healing<sup>17</sup>, microbial growth through pH reduction<sup>18</sup> etc. Glycosylation of proteins in integumentary tissues has also been attributed to hyperglycemia<sup>19-21</sup> as well as hair thinning, hair loss<sup>22</sup> and alopecia areata<sup>23</sup>.

The optimal amount of sucrose suitable for tissues and organs' health is still under debate. Unfortunately, many health authorities are singing discordant tunes on this issue<sup>24</sup>. Furthermore, most of the investigations carried out on sucrose to determine the optimal levels are carried out in a "one-concentration" approach such as 30% or 20% sucrose solution, a toxicological perspective rather than grading the sucrose consumption to mimic practical nutritional situation. Aside

from this, the consumption of sucrose is more of 'hidden form' in many solid and semi-solid foods such as bread, candies, diaries, etc. rather than solution form 5.

In view of the above, the study investigated the effect of various concentrations of sucrose on calcium, phosphorus and magnesium in the skin, hair and nails; and possible implication(s) on the integumentary system.

#### **MATERIALS AND METHODS**

**Chemicals:** All reagents used in this study were of analytical grade, purchased from Sigma-Aldrich Corporation (St. Louis, Missouri, USA). Calcium kits were purchased from Randox Laboratories (Crumlin, United Kingdom) while Magnesium and Phosphorus kits were purchased from Biosystems S.A. (Barcelona, Spain).

**Animals handling:** A total of 18 weanling male Wistar rats purchased from the Redeemer's University Animal House at Ede, Osun State, Nigeria were used for this experiment. The experiment started in June 2018 and lasted for seven months; terminating in January 2019. Animals weighing between 55-85 g were acclimatized for two weeks in individual metal cages where water and rat chow were given ad libitum. The care and handling of animals were under the guidelines established by the National Health and Medical Research Council.

Diet formulation: The pellets (rat chow) purchased from Ladokun Farms Limited, Ibadan, Oyo State, Nigeria were grounded into powdery form and then mixed with the granulated sugar at 10 and 20% energy ratios. The mixture was moistened with distilled water and re-pelletized with an industrial pelletizing machine and then dried in an oven at 60°C for two days to maintain 5% initial water content of the pellets. The pellets were stored in an airtight plastic container. The sucrose weight to energy equivalent was calculated based on the feed label. The gross energy per gram of rat chow according to Ladokun Feeds label was 3.65 kcal and the sucrose energy per gram weight is 3.94 kcal g<sup>-1</sup>. The resultant weight equivalent to 1kcal of rat chow and sucrose is 0.27 and 0.24 g, respectively. These factors were multiplied by the percentage of rat chow and sucrose required to compound different diets for each group, for their group label.

 G<sub>1</sub>: Rats in this group were placed on a commercial diet (rat chow) i.e. control group zero% energy supply from sucrose

- **G**<sub>2</sub> : Rats in this group were placed on an experimental diet consisting of 10% energy supplied from sucrose
- **G**<sub>3</sub> : Rats in this group were placed on an experimental diet consisting of 20% energy supply from sucrose

The rats were placed on respective diet ad libitum for 24 weeks. After the experiment, the rats were fasted overnight and sacrificed by cervical dislocation. The required organs and tissues were harvested i.e. skin, hair and nails.

#### **Biochemical analysis**

**Drying and ashing:** The various samples were weighed, transferred to the oven for drying at a temperature of  $105 \degree C$  for 12 hrs, later cooled in the desiccators and digested using HNO<sub>3</sub>, evaporated to dryness and water was added to dissolve. The sample was filtered and the filtrate collected for analysis.

**Mineral analysis:** The Calcium level was determined by a colorimetric method based on the methods of Sarkar and Chauhan<sup>25</sup> and Barnett *et al.*<sup>26</sup>. The Magnesium level was determined by a colorimetric method using a commercial kit as described by Barbour and Davidson<sup>27</sup> and Chromy *et al.*<sup>28</sup>, while the Phosphate content was determined by a colorimetric method as described by Gamst and Try<sup>29</sup> and Munoz *et al.*<sup>30</sup>.

**Statistical analysis:** The data were analyzed using a one-way Analysis of Variance (ANOVA) and (SPSS 14.0 software). Differences between means were assessed using Duncan Multiple Range Test. The level of significance was done at p<0.05 and values were expressed as mean $\pm$ SEM (6 replicates).

#### RESULTS

After feeding the rats for 24 weeks, chemical results were gotten. Significant changes were observed in some parameters ( $p \le 0.05$ ) while no significant changes were observed in others ( $p \ge 0.05$ ).

**Effect of sucrose consumption on minerals content of rat hair:** Figure 1 showed the effect of sucrose consumption on the minerals content of hair of rats fed with varying concentrations of sucrose diet. A significant decrease (p<0.05) was observed in calcium and magnesium levels whereas increase (p<0.05) was observed in phosphate and magnesium levels, though no significant difference (p>0.05) between calcium and magnesium levels when comparing G<sub>2</sub> with G<sub>3</sub>.









Also, no significant difference (p>0.05) was observed between magnesium levels in  $G_1$  and  $G_3$ .

#### Effect of sucrose consumption on minerals content of rat

**nail:** Shown in Fig. 2 is the effect of sucrose consumption on the minerals content of the nail of rats fed with varying concentrations of sucrose diet. A significant decrease (p<0.05) was observed in calcium level, whereas increase (p<0.05) was observed in phosphate and magnesium levels of experimental rats fed sucrose when compared with control. A significant increase (p<0.05) was also observed in calcium levels G<sub>3</sub> as compared with G<sub>2</sub>. However, phosphate and magnesium levels reduced (p<0.05) when G<sub>3</sub> as compared with G<sub>2</sub>.





Effect of sucrose consumption on minerals content of rat skin: Figure 3 depicted the effect of sucrose consumption on minerals content of the skin of rats fed with varying concentrations of sucrose diet. A significant decrease (p<0.05) was observed in calcium concentration, whereas increase (p<0.05) was observed in phosphate and magnesium levels in skin of experimental rats fed sucrose ( $G_2$  and  $G_3$ ) when compared with control ( $G_1$ ). Similarly, a significant increase (p<0.05) was observed in calcium levels in  $G_3$  as compared with  $G_2$ . However, phosphate level reduced (p<0.05) whereas magnesium level showed no significant difference when  $G_3$  was compared with  $G_2$ .

#### DISCUSSION

In the present study, the effect of sub-chronic exposure of rats to sucrose-rich diet on their integumentary tissues minerals was evaluated. In the hair, nail and skin, the observed trend was a significant increase in phosphorus and magnesium levels, concomitant with a decrement in calcium concentration values as sucrose consumption increased. Studies have established the effect of sucrose consumption on various organs and tissues integrity<sup>11,31</sup>, function and possible implications on their morbidities<sup>32</sup>. In the hair of sucrose-diet fed rats, calcium levels decreased while phosphorus and magnesium levels were elevated. Since the root hair is connected with the bloodstream, it is logical that hair components may partly indicate the blood constituents; thus, hair formation may be affected by the blood minerals

content<sup>33</sup>. Diet, especially refined carbohydrates (a class which sucrose belongs), has been implicated in the alteration of blood content and component<sup>34,35</sup>. One of the possible mechanisms of sugar affecting calcium may be through the overweight/obesity of an individual, a metabolic response of a prolonged sugar diet that has been linked to vitamin D production necessary for calcium absorption and bioavailability. This lowering effect of calcium level in the hair may lead to defective hair shape and loss and as calcium has been linked with hair keratinocyte differentiation<sup>36</sup>, one of the regulatory mechanisms of calcium is its inverse relationship with phosphate. The high level of phosphate is one of the metabolic responses to lower the calcium level. Studies have also shown the increasing effect of sucrose diet on phosphate levels<sup>37</sup>. Magnesium function has been observed in hair follicle synthesis<sup>38</sup> through its deficiency which has been linked to hair loss<sup>39</sup>. However, its high level has also been linked to various disorders such as dyslexia<sup>40</sup> and delinguency<sup>41</sup>. Summing it up, high sucrose may thus lead to hair loss, dyslexia and behavioral changes.

Furthermore, the effect of sucrose on minerals content of nails in the rats fed sucrose diet has followed a similar trend as observed in the hairs. Aside from blood connection, the nail could be a metabolic indicator of the bone due to its connection with phalanges thus; it can be influenced by physiology and pathophysiology status of the blood and bones<sup>42</sup>. Various mechanisms have been proposed on lowering the effect of calcium on bones<sup>31</sup>. This may cascade to the nails as the extension of phalanges. The decreased calcium levels and increase in magnesium and phosphate levels may lead to defects such as vertical striation<sup>43</sup>, transverse leukonychia<sup>44</sup> also hapalonychia and beau's line<sup>45</sup>.

The results from this study also revealed the effect of sucrose diet on the mineral level in the skin of male albino rats. Calcium levels decreased while phosphate and magnesium levels were elevated as noted in other integumentary tissues. Sucrose diet is known for increasing blood and urine calcium concentrations with a concomitant decrease in phosphate levels<sup>46</sup>. It was noted that diet high in calcium and carbohydrate could reduce serum phosphate due to the ability of calcium to bind phosphate and carbohydrate to cause intracellular shift due to insulin level increase; thus, giving more credence to the possibility of intracellular shift resulting in high phosphate in the cell<sup>47</sup>. Due to the importance of phosphate in carbohydrate metabolism, hypophosphatemia tends to portend a risk of pathologies such as insulin resistance, hyperinsulinemia and impaired glucose utilization<sup>48,49</sup>. Thus, the seemingly opposite effect of blood (high calcium/low phosphate) is what happens in other tissues (low calcium/high phosphate).

Changes in minerals' contents in integumentary tissues has been used as a biomarker for many diseases<sup>50,51</sup>. In a situation the disease is diet-related such as coronary artery calcification, it could be expediently inferred than the presence of such disease precursor such as sucrose diet in conjunction with the biomarkers in those integumentary tissues such as increased calcium, decreased magnesium levels in nails may indicate propensity towards the development of the disease<sup>52</sup>. Thus inclusion of sucrose diet may indicate susceptibility and vulnerability to cardiovascular disease in male albino rats fed long term sucrose diet. Such extrapolation to humans could be reasonably deduced7. The implication of this study is that long term consumption of sucrose diet may adversely affect the integumentary system's health which may consequently compromise its functions. Thus the study will be of veritable importance to nutritional scientists, dermatologists and other health and allied researchers. From the ongoing, sucrose consumption appears to be safer at a concentration of 10% or lesser. Consequently, adherence to WHO suggestion is recommended. It is worthy of note that this research work was based on animal models and precisely, male albino rats thus, extrapolation might have its attendant problems.

#### CONCLUSION

The study revealed that intake of sucrose especially at ten and twenty percent energy supply adversely altered the mineral status of the integumentary tissues by lowering calcium and increasing phosphorus and magnesium levels.

#### SIGNIFICANCE STATEMENT

This study highlighted the adverse effects of long term feeding of sucrose diet (concentrations of 10 and 20) in the integumentary tissues of male rats. This study also provided baseline data that will help other researchers explore other molecular mechanisms of adverse outcomes of sub-chronic diets. Possibly, a novel hypothesis involving impairment and imbalance after long term sucrose intake could be postulated.

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