Exposure to Sun Rays: An Investigation of Serum Micronutrient Status in Wistar Rats

Ayobola A Iyanda
Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria

Abstract
Micronutrients are important elements/biomolecules in the body known for the vital roles they play in numerous metabolic processes in the body. Whereas, depleted serum levels of micronutrients are always associated with inadequate intake, altered levels can also result from increased oxidative stress. Ultraviolet rays of the sun are an example of an agent capable of inducing oxidative stress. Therefore the effect of ultraviolet rays of the sun on serum micronutrient levels in female Wistar rats is being investigated. Fourteen female Wistar rats of average weight of 235 g used for the study were divided into 2 groups of 7 rats each. Seven of the rats constituted the sun-exposed group and the other seven rats served as control. The control rats were kept in cages at ambient temperature of 26°C. Sun-exposed rats were left in an open field for 4 hours each day. The study lasted for a period of 6 weeks. Blood was collected from each rat by retro-orbital bleeding and the serum obtained was used for micronutrient levels. Serum levels of water and lipid-soluble vitamins were determined using High Performance Liquid Chromatographic technique (HPLC). Serum concentrations of trace elements were quantified using the Atomic Absorption Spectrometric method. Data were subjected to statistical analysis using the Student’s test. The level of statistical significance was established at P ≤ 0.05. Of all the micronutrients measured only thiamine, vitamin A, and pantothenic acid as well as minerals such as zinc, manganese and copper were significantly lower in sun exposed rats compared with control. From the results of this study, it seems apparent that sun-exposure is capable of altering serum micronutrient levels in a nocturnal animal species.

Keywords: micronutrients, wistar rats, sunrays, HPLC

Cite this article as: Ayobola A Iyanda. Exposure to Sun Rays: An Investigation of Serum Micronutrient Status in Wistar Rats. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (30); 2014; 1-5. DOI: 10.15272/ajbps.v4i29.465
INTRODUCTION

Vitamins and trace elements are important components in the body and are known for the vital roles they play in numerous metabolic processes in the body. Functions of some of micronutrients are as follows: hormone-like properties of vitamin D especially as regulators of mineral metabolism; antioxidants property of vitamins E and C as well as regulators of cell and tissue growth and differentiation (vitamin A). In addition, thiamine pyrophosphate takes part in synthesis of nucleic acids as a co-enzyme as well as participates in carbohydrate, and protein metabolism[1]. Vitamin B6 participates in histamine, free fatty acid, and amino acid metabolism. It also plays a vital role in the normal functioning of the central and peripheral nervous system, skin, and gastrointestinal tract. The minerals on the other hand are known for their roles as cofactors of diverse enzymatic processes[1].

Whereas, abnormal low serum levels of these micronutrients is always associated with inadequate intake, altered levels have also been recognized to be to linked increased oxidative stress. This is not far removed from the role of many micronutrients play as anti-oxidants. Free-radical generating agents have been reported to be either chemical or physical in nature. While therapeutic drugs, drugs of abuse and a variety of chemical agents have been documented for the free-radical generating properties, ultraviolet rays of the sun is a good example of physical agents capable of inducing oxidative stress. Its role in many diseases has been well documented.

As a predisposing factor, ultraviolet (UV) radiation has considerable influence on the incidence of skin cancer[2]. Australia is known to have the highest rates of skin cancer in the world[3-6]. Based on the knowledge of these high rates and the number of skin cancers that can possibly be prevented, National Goals and Targets for Australia[7] recommended decreased exposure to sunlight for individuals of all ages, and especially for those people at high risk of skin cancer. Some of people included in the high-risk group are those who have a large number of acquired melanocytic naevi and freckles, skin that burns and never tans, those who have dysplastic naevi and those who have solar kurtosis[4, 8-11]. Some of these conditions have been associated with altered serum micronutrient levels. The aim of this study is to determine whether sun exposure affect serum micronutrient levels, since ultraviolet B (UVB) of the sun is capable of generating free radical and many vitamins and minerals possess anti-oxidant properties.

MATERIALS AND METHODS

Experimental Animals and Animal Treatment: Fourteen female Wistar rats of average weight of 235 g were used for the study. The procedures were carried out in accordance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985). The animal experiment was carried out at the Experimental Animal Unit of Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The study period lasted for 6 weeks. Seven of the rats constituted the sun-exposed group and the other seven rats served as control. Each day, five days a week, exposure was for a period of 4 hours starting from 9:00 to 13:00. No form of sun-screening was employed to protect the sun-exposed group; rather they were left in an open field. The control rats were kept in cages at ambient temperature of 26°C. All experimental animals were supplied feed and water ad libitum. Blood was collected from each rat by retro-orbital bleeding, dispensed into anti-coagulant free bottle, and centrifuged at 3000 g for ten minutes. The serum obtained was stored at - 20°C until required for analysis.

Assessment of Serum Micronutrient Levels: Serum levels of vitamins namely; thiamine, riboflavin, niacin, folic acid, pantothenic acid, and vitamins A, B6, B12, C, D and E were determined using High Performance Liquid Chromatographic technique (HPLC). On the other hand, serum concentrations of Zn, Cu, Se, Mn, Co, Fe, Mo, and Cr, were quantified using the Atomic Absorption Spectrometric method. HPLC equipment was supplied by Waters® Corporation Milford, Massachusetts USA while Buck Scientific 205 (Atomic Absorption) was obtained from Buck Scientific, East Norwalk, Connecticut, USA.

Statistical analysis:

Data were expressed as mean ± SEM and comparisons between the groups were performed using the Student’s test. The level of statistical significance was established at P ≤ 0.05. All data were analyzed by means of the statistical package SPSS 15 (SPSS Sciences, Chicago, USA).

RESULTS

Results of the study are presented in Table 1 and 2 below. Data presented in Table 1 show that of all the trace elements examined i.e. zinc, copper, selenium, manganese and iron as well as molybdenum, chromium and cobalt only zinc, copper and manganese were significantly lower (p<0.05) in sun-exposed rats than control. Others were not significantly different (p>0.05). As presented in Table 2, while vitamin A, thiamine and pantothenic acid were significantly lower (p,0.05) in sun-exposed rats than in control, other like...
riboflavin, niacin, folic acid, and vitamins B6, B12, C, D and E were not significantly different (p>0.05).

<table>
<thead>
<tr>
<th>Element level</th>
<th>Control</th>
<th>Sun-exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn (µmol/L)</td>
<td>19.34±1.98</td>
<td>16.81±2.40*</td>
</tr>
<tr>
<td>Cu (µmol/L)</td>
<td>17.68±1.76</td>
<td>13.09±2.16*</td>
</tr>
<tr>
<td>Se (µmol/L)</td>
<td>1.23±0.05</td>
<td>1.27±0.05</td>
</tr>
<tr>
<td>Mn (nmol/L)</td>
<td>197.75±21.08</td>
<td>197.75±21.08*</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>140.96±6.19</td>
<td>143.33±7.05</td>
</tr>
<tr>
<td>Cr (nmol/L)</td>
<td>1.92±0.03</td>
<td>1.89±0.04</td>
</tr>
<tr>
<td>Mo (nmol/L)</td>
<td>18.32±1.98</td>
<td>19.01±1.49</td>
</tr>
<tr>
<td>Co (nmol/L)</td>
<td>3.57±0.17</td>
<td>1.63±0.20</td>
</tr>
</tbody>
</table>

Table 1: Serum trace element levels in sun-exposed and control Wistar rats
Results are expressed as mean ± standard error of mean. *p <0.05 is significant using Student’s t test, n=7

<table>
<thead>
<tr>
<th>Vitamin level</th>
<th>Control</th>
<th>Sun-exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>1.35±0.02</td>
<td>1.09±0.03*</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>36.84±3.64</td>
<td>39.00±2.91</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>21.41±2.92</td>
<td>23.20±1.98</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>204.61±5.30</td>
<td>208.46±2.16</td>
</tr>
<tr>
<td>Folic</td>
<td>26.02±1.96</td>
<td>24.90±2.11</td>
</tr>
<tr>
<td>Niacin</td>
<td>106.87±4.31</td>
<td>110.58±6.09</td>
</tr>
<tr>
<td>Thiamine</td>
<td>143.87±3.83</td>
<td>120.51±2.32*</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>53.80±3.02</td>
<td>55.04±3.97</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>1.86±0.02</td>
<td>1.47±0.02*</td>
</tr>
<tr>
<td>Cyanocobalamin (ng/L)</td>
<td>322±7.92</td>
<td>332±9.17</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>59.79±3.07</td>
<td>62.19±1.39</td>
</tr>
</tbody>
</table>

Table 2: Serum vitamin levels in sun-exposed and control Wistar rats
Results are expressed as mean ± standard error of mean. *p <0.05 is significant using Student’s t test, n=7

DISCUSSION
Ultraviolet radiation is a free-radical generating carcinogen that is capable of compromising skin appearance and function, and many micronutrients consisting of vitamins and minerals are important antioxidants. Interestingly, the ultraviolet action spectra of both the beneficial and harmful effects e.g. DNA damage, skin cancer, and vitamin D3 photosynthesis are similar. For vitamin D one of the micronutrients under consideration, the effects of sun exposure on its serum level are well documented. For example evidence exists that vitamin D3 insufficiency is strongest for frail elderly or inner-city ethnic minorities groups and that is as a result of inadequate sun exposure. But for fair-skinned teenagers and young adults, the sub-groups mostly affected by ultraviolet photo-damage, the effect is rather due to over-exposure to sun rays.

Many subcellular components of the skin as well as its structures have been linked with both beneficial and harmful effects of the ultraviolet rays, for Vitamin D concentrations, i.e. especially those of the inactive pre-hormone 25-hydroxyvitamin D [25(OH)D] that is frequently estimated in serum, its cutaneous photosynthesis within the irradiated epidermis seems to be an important factor that enhances its serum levels. Although, studies have shown that vitamin D obtained from diet or supplements can fully substitute for vitamin D synthesized in the skin.

The role of the sun (via vitamin D or free radical generation) in relation to many pathological conditions has been highlighted, some of the disorders being prostate cancer, rickets, basal cell carcinoma and squamous cell carcinoma. The causal role of UV irradiation in both nonmelanoma skin cancer and melanoma has been long been recognized by experienced clinicians and epidemiologists. This relationship has been repeatedly demonstrated since the 1920s from results of studies carried out on hairless mice and other animal models12. In addition, according to Gilchrest12 UV radiation is a proven carcinogen that has been linked with most of the estimated 1.3 million skin cancer cases in the United States each year13, which account for more than one-half of all human malignancies. The significant decrease in some of the micronutrients may not be unrelated to the ability of the sun rays to induce oxidative stress. Many micronutrients have well defined antioxidants roles.

For some conditions the relationship may not be clearly defined, but for squamous cell carcinomas in particular, the relation is direct, with more UV irradiation exposure leading to earlier onset and higher prevalence of cancers in both mice and humans12. Moreover, while for basal cell carcinomas, a definite
relationship has not been defined for many of the significantly different micronutrients, a direct cause-and-effect relation with UV irradiation has been observed in a mouse model\textsuperscript{[14]}. Although it seems that the dose-response relation between UV irradiation and melanoma is less obvious than for nonmelanoma skin cancer, at least in humans, studies have documented a cause-effect relation in multiple animal models\textsuperscript{[15, 16]}. Both melanoma and nonmelanoma skin cancer have been linked with micronutrient levels; with Bergomi et al.,\textsuperscript{[17]} confirming that abnormal intake or metabolism of Cu and Fe might be implicated in the etiology of melanoma.

What further suggests the possible relationship between skin disorders as well as many other conditions that had earlier been linked with sunrays and antioxidant depleted-sun exposure state is the fact that patients with the rare disease xeroderma pigmentosum, caused by a mutation in 1 of 8 DNA repair enzymes that are required to correct UV-induced DNA damage, develop nonmelanoma skin cancers and melanomas at least 1000 times as frequently as the general population beginning early in life, even when they attempt to avoid all sun exposure\textsuperscript{[12]}. Zinc is known for the vital role it plays in the DNA repair mechanism.

While in a nation like Nigeria excessive exposure to the sun occurs for economic reasons e.g. street trading; in Europe, Australia and USA, it is more for cosmetic reasons among the Caucasians. The public perception that sunbathing is pleasant and as well as the pronouncements of Coco Chanel in the 1920s that sun tanning was glamorous has encouraged the concept of a tan’s attractiveness and to motivate many people, especially teenagers and young adults, to attempt to tan their skin\textsuperscript{[18]}. This is despite recent observation and revised medical and scientific perception of a tan as a UV dose\textsuperscript{[21]}. The results of the present study seem to support what Gilchrest\textsuperscript{[12]} had noted, that it seems that mammals (humans) obtain ample vitamin D from a combination of diet, supplements, and incidental protected sun exposure.

The results of the present study seem to support earlier observation from a recent study\textsuperscript{[22]}, Sample of 93 healthy young adults recruited from the University of Hawaii and a Honolulu skateboard shop questioned the frequently suggested serum 25(OH)D sufficiency cutoff of 75 nmol/L. The subjects were recruited of a self-reported minimum outdoor sun exposure of 15 h (mean, 29 h) per week during the preceding 3 mo; 40% reported never using sunscreen, and the group overall reported an average of 22.4 h per week of unprotected sun exposure. While all these subjects were clinically tanned, nevertheless, the group’s mean 25(OH)D concentration, measured by 2 standard techniques (HPLC and radioimmunoassay), was 79 nmol/L, and 51% had a level below the suggested 75-nmol/L cutoff for sufficiency\textsuperscript{[22]}. The results obtained from the study of\textsuperscript{[22]} suggest that a public health goal of >75 nmol/L, not to mention >150 nmol/L, for the entire population might be unachievable by sun exposure. This means that diet plays an important role in preventing vitamin D deficiency.

Many other reports though have demonstrated that vitamin D was significantly increased as a result of sun exposure in many experimental models. In humans there is epidermal melanin, a large polymer that efficiently absorbs photons across the entire UV and visible light range, but the human skin contains not only melanin but other components like phototype a related but less well understood set of determinants. Even in places where exposure is for tanning purposes, it is known that phototype I or II skin burns readily with a first moderate UV exposure and then tans their skin especially teenagers and young adults, to attempt to achieve a tan’s attractiveness and to motivate many people, among the Caucasians. The public perception that sunbathing is pleasant and as well as the pronouncements of Coco Chanel in the 1920s that sun tanning was glamorous has encouraged the concept of a tan’s attractiveness and to motivate many people, especially teenagers and young adults, to attempt to tan their skin\textsuperscript{[18]}. This is despite recent observation and revised medical and scientific perception of a tan as a UV dose\textsuperscript{[21]}. The results of the present study seem to support what Gilchrest\textsuperscript{[12]} had noted, that it seems that mammals (humans) obtain ample vitamin D from a combination of diet, supplements, and incidental protected sun exposure. The results of the present study seem to support earlier observation from a recent study\textsuperscript{[22]}, Sample of 93 healthy young adults recruited from the University of Hawaii and a Honolulu skateboard shop questioned the frequently suggested serum 25(OH)D sufficiency cutoff of 75 nmol/L. The subjects were recruited of a self-reported minimum outdoor sun exposure of 15 h (mean, 29 h) per week during the preceding 3 mo; 40% reported never using sunscreen, and the group overall reported an average of 22.4 h per week of unprotected sun exposure. While all these subjects were clinically tanned, nevertheless, the group’s mean 25(OH)D concentration, measured by 2 standard techniques (HPLC and radioimmunoassay), was 79 nmol/L, and 51% had a level below the suggested 75-nmol/L cutoff for sufficiency\textsuperscript{[22]}. The results obtained from the study of\textsuperscript{[22]} suggest that a public health goal of >75 nmol/L, not to mention >150 nmol/L, for the entire population might be unachievable by sun exposure. This means that diet plays an important role in preventing vitamin D deficiency.

Many other reports though have demonstrated that vitamin D was significantly increased as a result of sun exposure in many experimental models. In humans there is epidermal melanin, a large polymer that efficiently absorbs photons across the entire UV and visible light range, but the human skin contains not only melanin but other components like phototype a related but less well understood set of determinants. Even in places where exposure is for tanning purposes, it is known that phototype I or II skin burns readily with a first moderate UV exposure and then tans their skin especially teenagers and young adults, to attempt to achieve a tan’s attractiveness and to motivate many people, among the Caucasians. The public perception that sunbathing is pleasant and as well as the pronouncements of Coco Chanel in the 1920s that sun tanning was glamorous has encouraged the concept of a tan’s attractiveness and to motivate many people, especially teenagers and young adults, to attempt to tan their skin\textsuperscript{[18]}. This is despite recent observation and revised medical and scientific perception of a tan as a UV dose\textsuperscript{[21]}. The results of the present study seem to support what Gilchrest\textsuperscript{[12]} had noted, that it seems that mammals (humans) obtain ample vitamin D from a combination of diet, supplements, and incidental protected sun exposure. The results of the present study seem to support earlier observation from a recent study\textsuperscript{[22]}, Sample of 93 healthy young adults recruited from the University of Hawaii and a Honolulu skateboard shop questioned the frequently suggested serum 25(OH)D sufficiency cutoff of 75 nmol/L. The subjects were recruited of a self-reported minimum outdoor sun exposure of 15 h (mean, 29 h) per week during the preceding 3 mo; 40% reported never using sunscreen, and the group overall reported an average of 22.4 h per week of unprotected sun exposure. While all these subjects were clinically tanned, nevertheless, the group’s mean 25(OH)D concentration, measured by 2 standard techniques (HPLC and radioimmunoassay), was 79 nmol/L, and 51% had a level below the suggested 75-nmol/L cutoff for sufficiency\textsuperscript{[22]}. The results obtained from the study of\textsuperscript{[22]} suggest that a public health goal of >75 nmol/L, not to mention >150 nmol/L, for the entire population might be unachievable by sun exposure. This means that diet plays an important role in preventing vitamin D deficiency.
variation in results obtained from studies carried out on human subjects in many regions of the world. Therefore, there is need to exercise care in extrapolating this study to human situation. Rather it may seem necessary to address the impact of sun exposure on human subjects bearing in mind the vital role micronutrients play in a number of physiologic processes. This is needful in Nigeria where dawn to dusk exposure to UVB is commonly experienced by street traders.

Although it has been identified that constitutively high epidermal melanin content protects persons with phototype VI skin, who often have African or aboriginal ancestry, from initial DNA damage, yet with longer and repeated sun exposures, such persons may suffer very substantial DNA damage that is eventually manifested as photoaging and skin cancer. Some of other high-risk groups who may respond to sun exposure differently from general population are those people who have a large number of acquired melanocytic naevi and freckles, those who have dysplastic naevi and those who have solar kurtosis as well as skin that burns and never tans[4, 8-11]. The effects of sunlight exposure on vitamin D3 synthesis are also decreased in individuals with darker skin pigmentation because of the presence of high concentrations of melanin in the stratum corneum that severely inhibits vitamin D3 production[24].

CONCLUSION
The results of this study suggest that exposure to sunlight is capable of altering the serum levels of various micronutrients at least in a nocturnal animal species like the Wistar rats.

REFERENCES