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Original article

Vitamin D: Correlation with biochemical and body composition changes in a southern Brazilian population and induction of cytotoxicity in mesenchymal stem cells derived from human adipose tissue

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ABSTRACT

Studies have shown that metabolic disorders, serum inflammatory markers and weight gain (obesity) are correlated with vitamin D deficiency. Therefore, the present study correlated the serum calcidiol (s25 (OH)D_3) levels in a sample of individuals from southern Brazil with variables related to metabolic disorders, obesity and lifestyle habits and assessed the cytotoxic effect of calcitriol on adipose tissue-derived mesenchymal stem cells (ADSCs). The results showed a 79.23% prevalence of hypovitaminosis D in the study population and a correlation ($p < 0.05$) between a low serum vitamin D concentration and an elevated low-density lipoprotein cholesterol (LDL-c) level. Univariate linear regression analysis using 25 (OH)D_3 as a regressor showed a negative association ($p < 0.05$) with an indoor work environment ($\beta = -2.305$), increased body fat ($\beta = -0.095$), age ($\beta = -0.065$) and high-density lipoprotein cholesterol (HDL-c; $\beta = -0.109$). An *in vitro* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay performed with ADSCs using five calcitriol concentrations (15.625, 31.25, 62.5, 125 and 250 nM) indicated cytotoxic potential ($p < 0.05$) at the 62.5 nM concentration at 48 and 72 h and at the 125 and 250 nM concentrations at 24, 48 and 72 h. The results reported herein corroborate one another and suggest a key association between vitamin D deficiency and the development of obesity because ADSCs are involved in adipose tissue hyperplasia and differentiate into adipocytes that can sequester the bioavailable vitamin D necessary for homeostasis.

1. Introduction

Vitamin D has become a research study focus primarily because it modulates functions related to bone health [1,2], tumour development [3–5] and metabolic disorders [6–9].

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One of the various forms of vitamin D is vitamin D_3 (cholecalciferol), which can be produced through sun exposure (endogenous production) and ingested/supplemented. Therefore, vitamin D_3 may be the most studied vitamin in medicine because it is widely used as a supplement in hypovitaminosis D cases. Through a regulated process in the body, circulating vitamin D_3 is converted to the bioactive vitamin D hormone, which is best known as calcitriol ($1,25(OH)_2D_3$). For this purpose, vitamin D_3 undergoes a hydroxylation process that primarily occurs in the liver; this process is mediated by the 25-hydroxylase enzyme, which causes the conversion to calcidiol ($25(OH)D_3$). Subsequently, the second hydroxylation mostly occurs in the kidneys and is, in turn, mediated by the 1α-hydroxylase enzyme, which converts 25 $(OH)D_3$ into $1,25(OH)_2D_3$ [10–13]. However, optimal serum levels are not reached, even with the ingestion of vitamin D_3-rich foods [14]; thus, supplementation and sun exposure are needed, preferentially during the morning period [15–17].

Vitamin D deficiency, as assessed using the serum $25(OH)D_3$ level, has been reported in children, adults and elderly people from all continents [18–20]. Because deficiency occurs worldwide [21,22], further population studies are needed even in countries thought to have a high annual solar incidence [23–26]. In Brazil, vitamin D deficiency is most worrying because this condition affects different groups of study subjects, including young healthy individuals and young people with metabolic disorders (e.g., patients with metabolic syndrome) [27–30].

Systematic reviews have shown that metabolic disorders, including cardiovascular disease (with an increasing incidence), high serum lipid concentrations, serum inflammatory markers, changes in glucose metabolism and weight gain, are correlated with vitamin D deficiency [9,31–33]. These parameters are related to obesity progression and being overweight in humans. Adipose tissue expansion is mediated by hypertrophy of mature adipocytes or multipotent stem cell hyperplasia during adipogenic differentiation with a subsequent triglyceride accumulation in adipose tissue [34,35]. Recently, stem cells adjacent to adipose tissue have been shown to play key roles in the adipose tissue mechanisms underlying chronic inflammation; therefore, these cells may be a key factor in an adjunct treatment for obesity prevention [36]. Thus, studies on the genetic cascade of the inhibition of the proliferation and/or death of adipose tissue cells have provided new possibilities for the prevention of this disease [37–40].

Vitamin D has been reported to be a mesenchymal stem cell proliferation inhibitor and an inducer of apoptosis in various cell types, including adipocytes [37,38,41–43]. Therefore, the use of vitamin D, especially in its hormone form, may be a good strategy to prevent obesity.

Considering the above findings, the present study correlated the serum calcidiol ($s25(OH)D_3$) levels in a sample of individuals from southern Brazil with variables related to metabolic disorders, obesity and lifestyle habits and assessed the cytotoxic effect of 1,25 $(OH)_2D_3$, which is commonly found in one type of medication, on adipose tissue-derived mesenchymal stem cells (ADSCs).

2. Patients and methods

2.1. Population study

2.1.1. Inclusion criteria

Adult volunteers of both genders aged from 18 to 55 years who read and signed the informed consent form and lived for at least three months in the city of Londrina, Paraná state, Brazil (Latitude: 23°18′37″ South), were included in the research study. Only self-declared Caucasian individuals participated in the research study to follow the standards of similar studies [44,45] and to avoid effects from skin vitamin D production due to excess pigmentation. The study was submitted to the Ethics Committee on Research Involving Human Subjects of the State University of Londrina (Universidade Estadual de Londrina–UEL) and approved under opinion number 116/2011.

2.1.2. Exclusion criteria

Patients with cardiovascular, gastrointestinal or kidney diseases, thyroid disorders, diabetes, haemophilia, anaemia or cancer, individuals who did not reside in the municipality of Londrina for at least three months and those who were not self-reported Caucasians were excluded from the study. Individuals who reported the chronic use of medicines to treat diabetes mellitus or dyslipidaemia or who used multivitamins in the last six months were also excluded from the study.

2.1.3. Data on the initial and final sampling

The present study was announced in the Oswaldo Cruz Laboratory (Londrina – PR) to patients waiting for the collection of their routine exams. At that time, the researcher responsible for this work invited patients to include the evaluated exams at no additional cost and explained the importance of their contribution. A total of 370 patients demonstrated an interest in participating and authorised blood collection for vitamin D and other biochemical variable dosing.

2.1.4. General data and sample collection procedures

Biological material and data on anthropometric parameters were collected over a six-month period. The collections were performed two weeks after the beginning of spring and two weeks after the end of summer in 2013.

The experiment was performed with a double-blind design. The following materials were provided to each volunteer: a standard care questionnaire, wherein personal information (name, age, gender, address, ethnicity and use of medicines) was recorded, and the shortened and modified version of the International Physical Activity Questionnaire (IPAQ; [46]). The data recorded on the latter questionnaire were based on the objectives of the study and included daily sun exposure determined by workplace specifications, sunscreen use habits and whether vigorous/moderate physical activity was performed. Additionally, the volunteer was subjected to a rapid blood glucose test (Accu-Check Active®, Roche Diagnostics, Switzerland). If the result indicated values higher than 126 mg/dL, the subject was automatically excluded from the study and referred for medical evaluation. After this initial evaluation, the subjects were referred for biological material collection and anthropometric evaluation. The aforementioned procedures were performed in the morning from 08:00 to 10:00 AM to minimise externalities that could cause variations between the tested parameters.

2.1.5. Determination of biochemical variables

The $s25(OH)D_3$ values were evaluated using an automated chemiluminescence analyser (LIAISON®, DiaSorin Diagnostics, Italy). Free fatty acids, high-density lipoprotein cholesterol (HDL-c), triglycerides and glucose were assessed using the automated enzymatic-calorimetric method (ADVIA® 1650, Siemens, Germany). Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald equation [47].

2.1.6. Anthropometric evaluation

For the anthropometric examination, the waist circumference was measured using an inelastic tape measure (Incoterm®, Brazil). Bioelectric impedance analysis was performed using a MALTRON® BF-906 Body Composition Analyser (Maltron International, UK) to assess the percentage of body fat and the body mass index (BMI).