Journal of Steroid Biochemistry and Molecular Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Sex-specific expression of apolipoprotein levels following replenishment of vitamin D

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ARTICLE INFO

Keywords:
Vitamin D correction
Apolipoproteins
Cardiovascular disease
Sex-specific
Lipid metabolism

ABSTRACT

Numerous studies have been done to establish the relationship between vitamin D and lipids, yet a definitive causal link is not found. This interventional study aims to evaluate and compare levels of apolipoproteins among vitamin D deficient subjects at baseline and after they achieved full vitamin D status correction.120 Saudi adults with vitamin D deficiency [25(OH)D < 50nmol/l] were recruited and given 50,000IU cholecalciferol weekly for first 2 months, then twice a month for next 2 months, followed by daily 1000IU until month 6. Blood samples were taken at baseline and after 6 months. Serum 25(OH)D, lipid profile and apolipoproteins (A1, A2, B, C1, C2, C3, E and H) were analyzed using commercially available kits. Overall, serum 25(OH)D increased significantly (63.3 ± 16.5nmol/l at end of study vs. 32.5 ± 10.8 at baseline; $p < 0.0001$). In parallel, a significant increase in apolipoproteins C1, C2, C3 and E (all p-values < 0.01) and a significant decrease in apolipoprotein B ($p = 0.02$) was observed. Following, stratification according to sex, apolipoproteins C2 and C3 significantly increased only in males (p-values < 0.01) while apolipoprotein C1 significantly increased only in females ($p < 0.01$). In addition, apolipoprotein B significantly decreased only in females ($p = 0.002$). These results suggests role of vitamin D in modulation of circulating levels of lipoproteins. The sexual dimorphism observed in circulating levels of measured apolipoproteins following vitamin D correction may explain, in part, known sexual disparity in the events of cardiometabolic health.

1. Introduction

Numerous studies have associated vitamin D deficiency with several extra-skeletal health outcomes, including cardiovascular disease (CVD) [1–3]. The diverse effects of vitamin D includes improvement in insulin sensitivity [4] and suppression of inflammatory T cell activity [5]. An increased risk of both vitamin D deficiency and CVD have been shown in metabolically healthy overweight and obese individuals [6,7]. Shortage of vitamin D interventional studies targeting this group of otherwise healthy overweight and obese adults is highlighted [8]. The meta-analysis including previous observations also suggested that lipid fractions, especially high density lipoprotein cholesterol (HDL-C) and triglyceride levels significantly improved on vitamin D supplementation [9]. Although the lipid lowering effect of vitamin D is observed in several studies, a definitive causal link is yet to be discovered.

The importance of proteomics on various facets of nutritional research is gaining ground and our recent serum proteomic analysis [10] identified apolipoproteins mapped to pathways associated with lipid metabolism. Apart from the traditional lipid indexes like total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C; the non-traditional lipid-related markers, such as apolipoproteins, are increasingly investigated as independent risk factors for CVD events.

Apolipoproteins, the protein fractions of larger lipoprotein molecules (LDL-C, HDL-C), apart from rendering amphipathic properties to lipoproteins play an important function in their metabolism as cofactors, inhibitors or activators of certain enzymes in the lipid metabolism pathways, especially lecithin-cholesterol acyltransferase (LCAT) and lipoprotein lipase (LPL). Apolipoprotein B (apoB) and apolipoprotein

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https://doi.org/10.1016/j.jsbmb.2017.12.005
Received 9 July 2017; Received in revised form 30 November 2017; Accepted 12 December 2017

A1 (apoA1), respectively, have been associated with atherogenic lipid particles mainly LDL-C and anti-atherogenic particles such as HDL-C [11–13]. In a recent case-control study on subjects from 52 different countries, apo B/A1 ratio was a better indicator of CVD events than any cholesterol ratios like LDL-C/HDL-C or TC/HDL-C [14].

The lipid lowering activity of vitamin D might involve two pathways: through reduction in triglyceride formation by increasing hepatocellular calcium; and stimulation of apoA1, which helps in raising HDL-C through reverse cholesterol transport [15,16]. Many studies suggest a positive association between vitamin D and apoA1; however a cause and effect relation has not been established yet, as most of these studies are observational in nature. In contrast, the association between vitamin D and apoB has yielded conflicting results. While some studies suggest a significant negative correlation between vitamin D and apoB [17], others reported no correlation[18] and in contrast, some others reported a significant increase in circulating levels of apoB in females [19]. There is a limited knowledge on the relationship between vitamin D and other apolipoproteins, like apolipoproteins C, E and H. Additionally, the relationship between circulating levels of different apolipoproteins among themselves has been reported less. Clearly, additional studies are required to evaluate the ability of vitamin D to modulate circulating lipid levels at their apolipoprotein levels. In this regard, our interventional study aimed to compare and validate the different apolipoproteins among vitamin D deficient subjects after they achieved full vitamin D status correction.

2. Methods

2.1. Subjects, vitamin D supplementation and clinical assessment

A total of 200 male and female adult Saudi subjects with vitamin D deficieny (< 50nmol/l),randomly selected from an existing Riyadh 2 cohort [20], agreed to take part in this six month vitamin D interventional programme. At first visit, all the subjects were requested to answer a questionnaire that included demographic data, whether or not suffering from any chronic clinical condition or taking medication on a regular use, whether or not taking any vitamin D supplement etc. Out of these, 163 qualified for the intervention and the rest were excluded for non-compliance. Fresh vitamin D analysis revealed 43 with vitamin D levels in the sufficiency range and were left out. 120 subjects were thus enrolled in the programme and were advised by phone call to collect first packet of vitamin D supplement next day from their respective health care center. 500,00IU cholecalciferol (VitaD50,000®) was given weekly for first 2 months, then twice a month for next 2 months, followed by daily 1000IU (VitaD1000®) until month 6. The orientation and intervention was performed by qualified nutritionist, physician and nurses in respective health care center and all the procedures followed ethical principles advised in declaration of Helsinki. The intervention study was approved by Ethics Committee of College of Science, King Saud University (KSU), Riyadh.

Anthropometrics were measuremed at baseline and included height (cm), weight (kg), waist circumference (cm), hip circumference (cm), systolic and diastolic blood pressure (mmHg). Fasting blood samples were drawn at two points (baseline and after six months of intervention). The samples were sent to Prince Mutaib Chair for Biomarkers of Osteoporosis (PMCO), KSU, Riyadh, Saudi Arabia and were aliquoted and processed for specific laboratory analysis.

2.2. Biochemical measurements

25(OH) Vitamin D was analyzed using COBAS e-411 automated analyzer (Roche Diagnostics, Indianapolis, USA). PMCO is a participating laboratory for DEQAS (vitamin D External Quality Assessment Scheme). Fasting glucose and lipid profile was quantified using routine biochemical tests in Konelab (Thermo-Fisher Scientific, Finland). The limit of detection for the assay was 0.02 mmol/l, 0.1 mmol/l, 0.04 mmol/l and 0.02 mmol/l for glucose, TC, HDL-C and triglyceride assays respectively. LDL-C was calculated using Friedewald formula [21] as LDL-C (mmol/l) = TC(mmol/l) − HDL-C (mmol/l) − {Triglyceride(mmol/l)/2.2}.

The apolipoproteins: apoA1, apoA2, apoB, apoC2, apoC3 and apoE were quantified in Luminex multiplex (Luminexcorp, TX, USA) using MILLIPEX® MAP Human Apolipoprotein Magnetic Bead Panel (catalogue# APOMAG–62 K). The limit of detection was 0.86, 0.38, 1.91, 0.26, 0.57 and 0.49 (all in ng/ml) for apo A1, A2, B, C2, C3 and E respectively. The inter-assay and intra-assay variations for all these analytes is reported to be < 20% and < 10% respectively. Apolipoproteins C1 and H were quantified using ELISA kits supplied by abcam, UK (catalogue# ab108808 and ab108814 respectively). The limit of detection was 0.06 μg/ml and 0.6 ng/ml respectively. The intra-assay and inter-assay variations were respectively 4.5% and 7.2% (apoC1) and 4.7% and 7.3% (apoH). No significant cross-reactivity was observed among different apolipoprotein types. All the calibrators and controls supplied by the respective companies were tested routinely for best performance of the assays and the Quality Assurance department of KSU audits PMCO at regular intervals for highly reproducible research data.

2.3. Data analysis

Data was analyzed using SPSS version 16.0 (Chicago, IL, USA). For descriptives, normal continuous variables were presented as mean ± standard deviation and non-normal variables were presented as median (first quartile, third quartile).Nominal variables were presented as percentages (%).Appropriate statistical tests were employed to check differences in central tendency between baseline and 6-month data points (paired *t*-tests for normal continuous variable and Related-samples Wilcoxon signed rank test for non-nominal variables). Change in biochemical parameters (δ = 6-month − baseline) was normalized using formula NormδY = log10 {δY-min (δY) + 1) and these normalized values were used to run a correlation matrix (Pearson correlation). Significance was set at $p < 0.05$. For multiple correlations (correlation matrix between apolipoproteins), the significance was Bonferroni corrected and only p-value < 0.0007 was considered significant. Microsoft Excel 2010 was used to prepare figures.

3. Results

3.1. Table 1 General characteristics and change in biochemical parameters in all subjects

120 adult Saudi subjects with mean age of 40.5 ± 10.8 (mean ± S.D) were recruited, 87.5% of them were either overweight or obese (BMI > 25 kg/m2). At baseline, all the subjects were deficient in vitamin D levels (< 50 nmol/l). As expected with vitamin D supplementation, there was a significant improvement in circulating levels of vitamin D (63.31 ± 16.5 nmol/l at end of study vs 32.5 ± 10.8 nmol/l at baseline, *p*-value (< 0.0001). HDL-C increased (1.1 ± 0.5 mmol/l vs 1.02 ± 0.4 mmol/l, p = 0.045) while LDL-C decreased (3.12 ± 1.2 mmol/l vs. 3.30 ± 1.2 mmol/l, p = 0.054) at end of study compared to baseline. The analysis of changes in different apolipoproteins from baseline to end of study in all subjects reveals a significant increase in apolipoproteins C1{median (Quartile 1, Quartile 3) as 36.47 (32.6, 57.9) ug/ml vs. 34.01 (30.1, 49.3) ug/ml, p < 0.001}; C2 {136.8 (86, 205) ug/ml vs. 118.5 (67, 168) ug/ml, p = 0.023}; C3{180.9 (91, 294) ug/ml vs. 150.1 (67, 214) ug/ml, p = 0.002} and E {16.12 (7.9, 29.3) ug/ml vs. 13.29 (7, 21.8) ug/ml, p < 0.001}. In contrast in all subjects, apolipoprotein B decreased significantly from baseline to end of study {6.54 (4.3, 9.8) mg/ml vs. 7.58 (5.2, 9.6), p = 0.022}.