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Effects of vitamin D and L-cysteine cosupplementation on circulating bioavailable and total 25-hydroxy-vitamin D, the free/total testosterone ratio and inflammatory biomarkers in healthy vitamin D-deficient African Americans: a placebo-controlled double-blind clinical trial

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ABSTRACT

Background Subjects with metabolic syndrome and obesity have higher levels of inflammation with depression of the vitamin D (VD) hydroxylase/metabolising genes (*CYP2R1/CYP27A1/CYP27B1/VDR*) required to convert VD consumed in the diet into 25(OH)VD. Compared with total 25(OH)VD levels, measurement of bioavailable 25(OH)VD is a better method to determine the beneficial effect of VD.

Objective This study investigates whether cosupplementation with VD and L-cysteine (LC), which downregulates inflammation and upregulates VD-regulating genes, provides a better therapeutic benefit than supplementation with VD-alone in African Americans (AA).

Methods AA participants (men/women, aged 18–65 years; n=165) were block randomised into one of four groups and received daily, oral supplementation for 6 months with placebo, LC (1000 mg/day), VD (2000 IU/day) or VD+LC. Fasting blood collected at the baseline and final visits was analysed for total, free and bioavailable 25(OH)VD along with insulin, VD-binding protein (VDBP), sex hormone-binding globulin (SHBG), free and total testosterone, and inflammatory marker levels. Studies were carried out in THP-1 monocytes to elucidate the direct effect of LC and testosterone on VD-regulating genes.

Results Baseline data showed no differences in age, body mass index, calcium, liver or kidney function among the groups. Compared with levels in the group that received VD-alone supplementation, levels of neutrophil-to-lymphocyte ratio, C reactive protein, HOMA-IR, VDBP and HbA1c were significantly lower in the VD+LC group while the VD+LC group showed a significant increase in bioavailable 25(OH)VD in both sexes, total 25(OH)VD levels were significantly elevated in men but not in women treated with VD+LC. Blood levels of SHBG and free/total testosterone were elevated in the VD+LC group but not in the VD-alone group. LC and testosterone treatment significantly upregulated VD-metabolising genes (*CYP2R1/CYP27A1/CYP27B1/VDR*) and *SHBG* in THP-1 monocytes.

Conclusions VD cosupplemented with LC upregulates circulating bioavailable 25(OH)VD and reduces inflammation. Total 25(OH)VD levels were higher in men but not in women in the VD+LC group. This pilot study suggests that compared with supplementation with VD-alone, VD+LC cosupplementation could be a better approach to raising the total 25(OH)VD in men and the bioavailable 25(OH)VD in both sexes and lowering the inflammatory risk in the AA population.

Trial registration number NCT04939792.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ 70% of African Americans (AA) have vitamin D (VD) deficiency compared with 25% in the white population. AAs also have a higher incidence of diabetes, heart disease and other chronic diseases compared with the white population.

WHAT THIS STUDY ADDS

⇒ Current medical practice recommends supplementation with vitamin D alone to treat vitamin D deficiency. This study demonstrates that VD+L-cysteine cosupplementation is superior to supplementation with VD-alone in increasing bioavailable 25(OH)VD and reducing levels of inflammation in AA with VD deficiency.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These results give us a new approach to promote VD sufficiency and improve the health of the AA population.

INTRODUCTION

Many organs and tissues express vitamin D (VD) receptors, suggesting that VD affects essential physiological functions beyond maintaining bone health.[1–3] Epidemiological studies have revealed an association between VD deficiency and a greater incidence of chronic diseases, including diabetes and heart disease.[4 5] However, randomised controlled clinical trials have reported limited therapeutic success after supplementation with VD.[6 7] Thus, there is a dissociation between the benefits of VD supplementation and the prevention of chronic diseases in those with VD deficiency.[6] Why this disconnect exists remains a matter of debate.

Obesity and metabolic syndrome are associated with inflammation and impaired activity in the genes required for normal VD metabolism.[8–11] These include VD-hydroxylase genes (*CYP2R1/CYP27A1/CYP27B1/VDR*), which are required to convert consumed VD into 25-hydroxy-VD (25(OH)VD). The clinical trials investigating VD supplementation have failed to account for the impact of inflammation on the genes responsible for regulating VD metabolism.[6] This could have prevented the optimal conversion of consumed VD supplements into 25(OH)VD and impaired its metabolism in those individuals with decreased activity of VD-hydroxylase genes. L-cysteine (LC), an antioxidant, has been reported to lower both inflammation biomarkers and upregulate the VD-metabolism genes.[8 9 12] LC supplementation can restore glutathione biosynthesis in compromised cases, leading to an improved redox balance and reduced oxidative stress and inflammation.[13]

In clinical practice, VD deficiency is diagnosed by measuring the blood levels of total 25(OH)VD because it is a stable metabolite of VD; approximately 85%–90% is tightly bound to VD-binding protein (VDBP).[14 15] The total 25(OH)VD concentration is the sum of free 25(OH)VD, 25(OH)VD, which is loosely bound to albumin and 25(OH)VD bound to VDBP.[14 15] The free hormone hypothesis suggests that hormones not bound to high-affinity carrier proteins can easily diffuse through cell membranes to perform their biological activities.[14 15] Bioavailable 25(OH)VD is the sum of the free and albumin-bound VD. Various clinical studies have shown that bioavailable 25(OH)VD is a better biomarker of VD status and predictor of its health consequences than total 25(OH)VD.[16 17]

The African American (AA) population has both a higher incidence of VD deficiency and a higher incidence of diseases associated with 25(OH)VD deficiency.[18] This study conducted a randomised, double-blind, placebo-controlled, single-centre clinical trial to investigate whether VD+LC cosupplementation is superior to supplementation with VD-alone in increasing bioavailable 25(OH)VD levels and providing better therapeutic benefits in AA. In addition, the effects of LC and testosterone on VD regulatory genes were investigated using THP-1 monocytes.

MATERIALS AND METHODS

Subject enrolment

Figure 1 illustrates the overall inclusion/exclusion criteria for the recruitment and randomisation of the subjects included in the clinical trial. Inclusion criteria included AA adults aged: 18–65 years, willing to participate in five clinical visits, who demonstrated an understanding of the risks and benefits of the protocol, signed the informed consent form and were willing to complete standard health history questionnaires before and during the study. The study period ran from December 2022 to September 2023. Details on inclusion and exclusion criteria are given in online supplemental file. The clinical trial is registered at ClinicalTrials.gov (protocol #NCT04939792).

Placebo run-in period

During the placebo run-in period, all consented subjects were given placebo capsules to take orally (three capsules/day). The subjects were then block randomised equally into four separate groups (placebo, VD, LC and VD+LC) according to the biostatistician's algorithm. After randomisation and during the second visit, all subjects were given three containers marked with three different colours, each containing 70 capsules. Capsules contained either placebo, VD or LC, and the subjects were instructed to take one capsule from each container daily. Thus, subjects in (1) the placebo group took three placebo capsules, (2) LC group took two capsules of LC (500 mg) and one placebo, (3) VD group took two capsules of placebo and one of VD (2000 IU) capsule and (4) VD+LC group took two capsules of LC (500 mg) and one capsule of VD (2000 IU) daily for 6 months. Investigators remained blinded to the randomisation until all clinical visits and laboratory analyses had been completed. During the trial, subjects maintained their usual lifestyles. The study began with a sample size of 257 consented subjects, of whom 165 were enrolled successfully after meeting the inclusion and exclusion criteria. 49 subjects either dropped out or withdrew from the study after randomisation (visit 2).

Selection of VD and LC doses and purity of cholecalciferol and LC

Subjects with 25(OH)VD levels <30 ng/mL at baseline or randomisation were included in the data analyses. VD, LC, and similar colour and size matching placebo capsules were custom manufactured according to 21 Code of Federal Regulations Part 111 current good manufacturing practices regulations (West Coast Labs, Gardena, California, USA). The concentrations of VD and LC were checked and verified by the manufacturer, and again independently by another lab (Heartland Assays, Ames, Iowa, USA). The Unites States Food and Drug Administration has determined that an Investigational New Drug is not required for clinical trials using VD and LC.

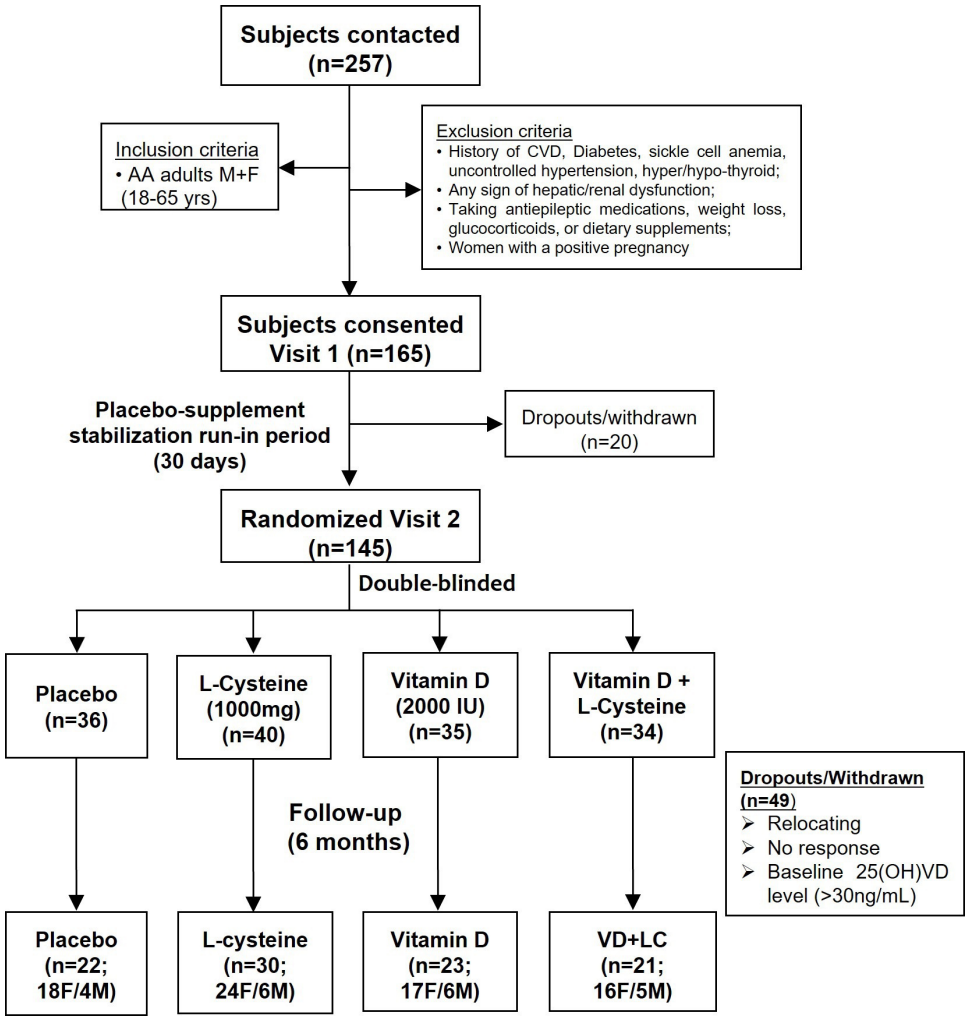

Figure 1 Flow chart showing recruitment and randomisation of study subjects. AA, African Americans; CVD, Cardiovascular disease; LC, L-cysteine; VD, vitamin D.

Blood collection and biochemical assays

Overnight fasting (8 hours) blood was collected from all subjects during each visit into precooled tubes kept in an ice bucket. The blood samples were sent to LSUHSC's clinical laboratory to analyse 25(OH)VD levels and various parameters (Hemoglobin A1C (HbA1c), complete blood count (CBC), comprehensive metabolic panel (CMP), glucose, albumin, calcium, liver enzymes and kidney function tests). The same laboratory/instruments were used during the entire clinical trial period. 25(OH)VD levels were tested using the chemiluminescence method (Siemens, Alpharetta, Georgia, USA). The clear plasma was separated from some EDTA tubes via centrifugation at 3000 rpm for 15 min and saved in several aliquots at −80°C.

Frozen plasma aliquots that had not been thawed previously were used for all biochemical assays (VDBP, insulin, sex hormone-binding globulin (SHBG), C reactive protein (CRP), total testosterone, parathyroid hormone and free testosterone). Details are given in online supplemental file. The Fibrosis-4 index (FIB-4) index for scaring of liver is calculated.[19] Bioavailable 25(OH)VD was calculated according to the formula given by Bikle.[14 15] The codes indicating which participant was assigned to which of the four groups were only opened after all laboratory analyses had been completed.

Cell culture treatment

Human THP-1 monocyte cells (1×10^6/mL) were maintained[20 21] and pretreated with different concentrations of LC (0–1000 µM/mL) and testosterone (0–10 ng/mL) for 24 hours. Cell viability was determined as described previously.[22] Following treatment, cells were harvested, and total RNA was extracted using an RNeasy Mini Kit (QIAGEN, Germany) and subjected to quantitative RT-PCR (online supplemental table S1).[22 23]

Statistical analysis

Data analyses were performed using Sigma Plot (V.14.5). Data were analysed statistically to compare the baseline (visit 2) and the final visit. The Wilcoxon signed rank, regression analysis, paired t-test and t-test were used to determine significant changes after supplementation for each treatment group. To check the normal distribution Mann-Whitney or signed ranked tests were used when the normality test of the data failed. Drop-outs and

Table 1 Baseline and 6-month post-treatment data for the treatment groups

	Placebo (n=22)		L-cysteine 1000 IU (n=30)		Vitamin D 2000 IU (n=23)		VD+LC (n=21)	
Characteristic	Baseline	Post-treatment	Baseline	Post-treatment	Baseline	Post-treatment	Baseline	Post-treatment
Age (years)	40.6±2.83	40.6±2.83	42.9±2.2	42.9±2.2	42.9±2.9	42.9±2.9	40±3.2	40±3.2
BMI (kg/m^2)	36.4±2	36.6±2	32.8±1.2	33.6±1.3	33±1.9	33.3±1.8	32.3±2	32±1.9
FIB-4 (score)	0.65±0.1	0.7±0.06	0.53±0.04	0.62±0.06	0.63±0.1	0.64±0.08	0.54±0.06	0.58±0.08
Glucose (mg/dL)	91.1±3.13	94.4±2.2	92.3±2.3	98±2.6	99±3.5	98.5±3	91±2.4	93.7±2
Creatinine (mg/dL)	0.91±0.04	0.93±0.04	0.9±0.03	0.9±0.03	0.86±0.03	0.89±0.04	0.86±0.03	0.86±0.03
Calcium (mg/dL)	9.34±0.08	9.2±0.07	9.3±0.06	9.2±0.07	9.4±0.09	9.3±0.07	9.4±0.08	9.2±0.11
Alkaline phosphatase (IU/L)	74.2±4.7	72.3±4.5	82.9±5.3	81.7±6.02	76.7±4.8	76.5±4.2	71.4±4.8	70.6±5.3
ALT (IU/L)	25.1±1.7	26±1.9	25±1.7	26±1.8	25.9±2	24±2	26.8±2.5	29.3±4.6
AST (IU/L)	19.7±1.7	21±1.6	17.4±0.9	20.2±1.4	18.3±1.5	18±0.95	20.4±1.7	21±2.9
Total 25(OH)VD (ng/mL)	18±1.5	21.7±1.6	15.8±1	18±1.3	17±1.4*	30.3±2.9#	16.4±1.6**	28.9±2.3##
Free 25(OH)VD (ng/mL)	4.3±0.73	3±0.39	2.2±0.38	2.44±0.23	3.42±0.59	3.6±0.48	3.71±0.6	3.3±0.4
Bioavailable 25(OH)VD	3.96±0.5	4.33±0.49	3.71±0.27	4.57±0.37	4.1±0.44	3.91±0.51	3.5±0.38^	5.13±0.62^^
Hb (g/dL)	13±0.3	12.7±0.3	13±0.22	12.7±0.2	13.4±0.23	13±0.22	12.9±0.4	12.8±0.4
PTH (pg/mL)	30.9±3.5	28±2.2	27.8±2.3	24±2	29.7±3.6*	26±3#	29.7±3.8	25±2

Data are presented as mean±SE.
* vs #, **vs ## and ^ vs ^^ p≤0.05.
ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; FIB-4, Fibrosis-4 index; Hb, haemoglobin; PTH, parathyroid hormone.

compliances were not included in the analysis. Data were analysed by separating the subjects based on their body mass index (BMI) as a covariate. All p values of ≤0.05 for a test were considered statistically significant.

RESULTS

A total of 96 subjects completed the study. Participants who dropped out of the study due to relocation to other states, or failure to keep follow-up visits (figure 1). There were 22 subjects (18F/4M) in the placebo-supplemented group (I), 30 subjects (24F/6M) in the LC group (II), 23 subjects (17F/6M) in the VD group (III), and 21 subjects (16F/5M) in the VD+LC group (IV). There were no significant differences in the baseline characteristics among the subjects in the four groups (table 1). Similarly, the baseline characteristics of the female and male subjects showed no significant differences (online supplemental tables S2 and S3). Among all four groups, there were no significant changes in BMI or FIB-4 after 6 months of treatment (table 1).

Figure 2A–C shows blood levels of total, free and bioavailable 25(OH)VD, respectively, at baseline and after daily supplementation with placebo, LC, VD or VD+LC. There was an increase in the total 25(OH)VD levels after supplementation with VD-alone and cosupplementation with VD+LC; however, there was no statistically significant difference in the total 25(OH)VD levels among the groups. Free 25(OH)VD levels were similar in all the groups. There was a significant increase in bioavailable 25(OH)VD in cosupplementation (VD+LC) subjects compared with those taking VD-alone. Figure 2D–F presents values as a percentage change of total, free and bioavailable 25(OH)VD at visit 5 (post-treatment) versus visit 2 (baseline) for each group, respectively. The fold-change in bioavailability levels was calculated by dividing the final visit value by the baseline for each subject in the four groups. Bioavailable 25(OH)VD was two fold higher in subjects taking VD+LC capsules compared with those taking VD-alone. When the study subjects were separated based on sex, both women and men demonstrated significant increases in total 25(OH)VD in both the VD+LC and the VD-alone groups (figure 2G,J). However, a significant difference between the groups was only seen in men. A significant increase in bioavailable 25(OH)VD was observed in both women and men, but only in the VD+LC cosupplemented group (figure 2I,L). Thus, cosupplementation with VD+LC is superior to supplementation with VD-alone in raising bioavailable 25(OH)VD levels in men and women while an increase in total VD was limited to men. Online supplemental tables S4 and S5 show similar effects of VD or VD+LC supplementation in the subjects, irrespective of BMI (≤30 vs>30).

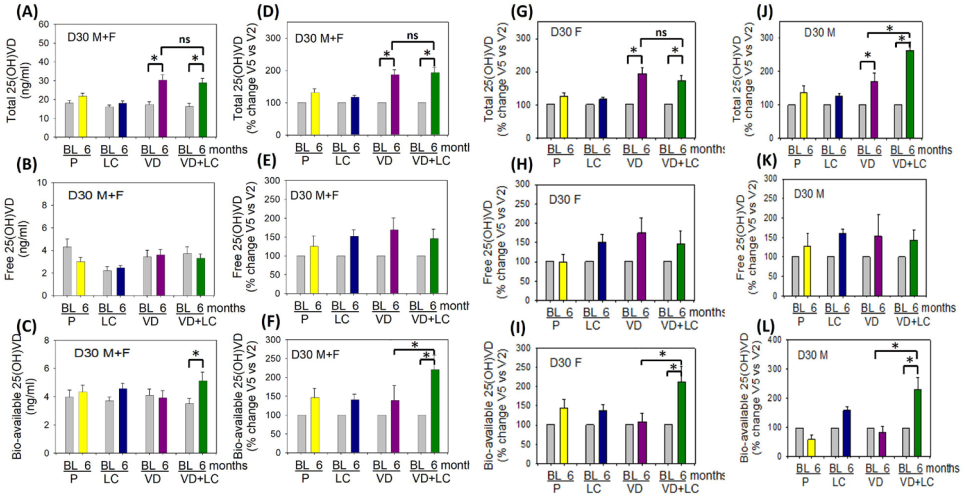

Figure 2 Effect of LC and/or VD supplementation on blood levels of total, free and bioavailable 25(OH)VD at baseline and post-treatment among the various treatment groups. *p≤0.05. BL, baseline; LC, L-cysteine; VD, vitamin D.

There was a significant decrease in VDBP in the VD+LC group compared with baseline but no change in with VD-alone group (figure 3A). The same trend was observed in the female and male groups (figure 3B,C). This suggests that cosupplementation was more effective at lowering VDBP compared with supplementation with VD-alone. SHBG is an anti-inflammatory protein implicated in increasing insulin signalling and sensitivity. The levels of SHBG were significantly increased in the cosupplementation compared with the VD-alone group. There was also an increase in SHBG in subjects supplemented with LC-alone (figure 3D–F). Similarly, there was a significant increase in the free/total testosterone ratio in the VD+LC co-supplemented group (figure 3G). This suggests that there was a significant decrease in VDBP and an increase in SHBG and the free-to-total testosterone ratio after VD+LC supplementation but not after supplementation with VD-alone. The increase in SHBG and free/total testosterone levels was significant among women (figure 3E,H) but not among men (figure 3F,I). One limitation of this study is the low enrolment of male participants.

The blood levels of neutrophil-to-lymphocyte ratio (NLR) were significantly lower in VD+LC-supplemented subjects compared with respective baselines before supplementation as well as with subjects supplemented with VD-alone (figure 4A–C). The CRP levels showed a decrease in the VD+LC cosupplemented group compared with supplementation with VD-alone, but this was not statistically significant (figure 4D). However, while there was a significant decrease in CRP in the male group (figure 4F), there was no significant change among the

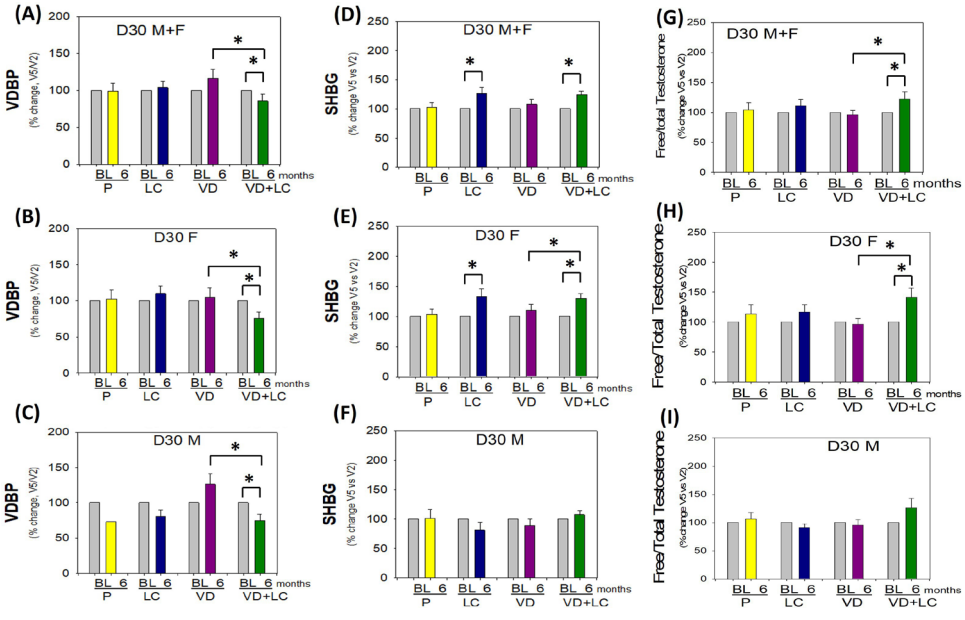

Figure 3 Effect of VD and/or LC supplementation on plasma levels of (A–C) VDBP, (D–F) SHBG and (G–I) the free/total testosterone ratio among the various treatment groups. *p≤0.05. BL, baseline; LC, L-cysteine; SHBG, sex hormone-binding globulin; VD, vitamin D; VDBP, VD-binding protein.

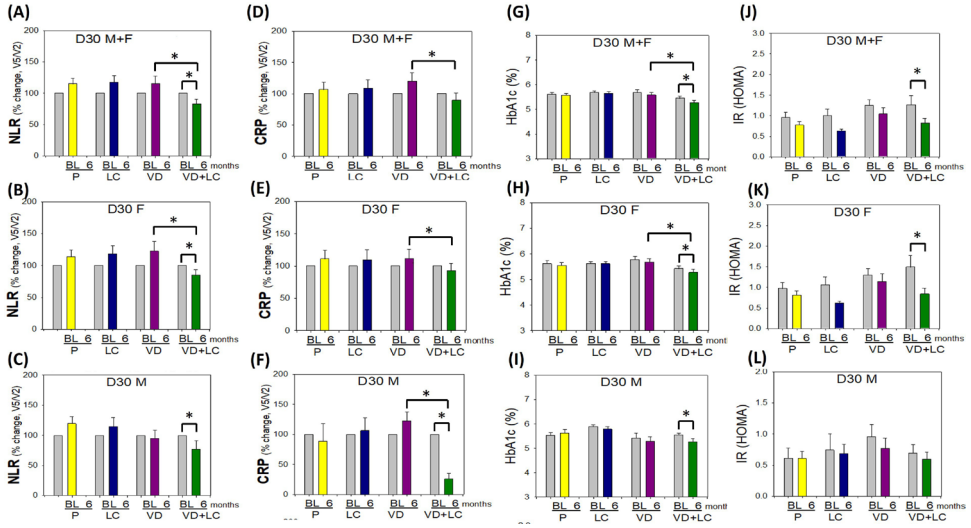

Figure 4 Effect of VD and/or LC supplementation on proinflammatory markers (A–C) NLR, (D–F) CRP, (G–I) HbA1c and (J–L) HOMA-IR among the various treatment groups. *p≤0.05. BL, baseline; CRP, C reactive protein; HbA1c, Hemoglobin A1C; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; LC, L-cysteine; NLR, neutrophil-to-lymphocyte ratio; VD, vitamin D.

women in the VD or VD+LC groups compared with the respective baselines (figure 4E). Similarly, HbA1c and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) were significantly decreased in the VD+LC group compared with the VD-alone group (figure 4G,J). Women supplemented with VD+LC showed a significant decrease in HbA1c and HOMA-IR levels; however, the decrease observed in the VD-alone group was not significant (figure 4H,K). Supplementation with VD-alone or VD+LC had no significant effect on HOMA-IR in the male group (figure 4L). Overall, cosupplementation provides a better anti-inflammatory effect than supplementation with VD-alone in AA subjects.

Figure 5 illustrates the effect of LC and testosterone treatment on THP-1 monocytes and their relative mRNA expression of VD metabolism and testosterone-related genes. LC treatment upregulated the expression of the VD-hydroxylase genes *CYP2R1*, *CYP27A1*, *CYP27B1* and

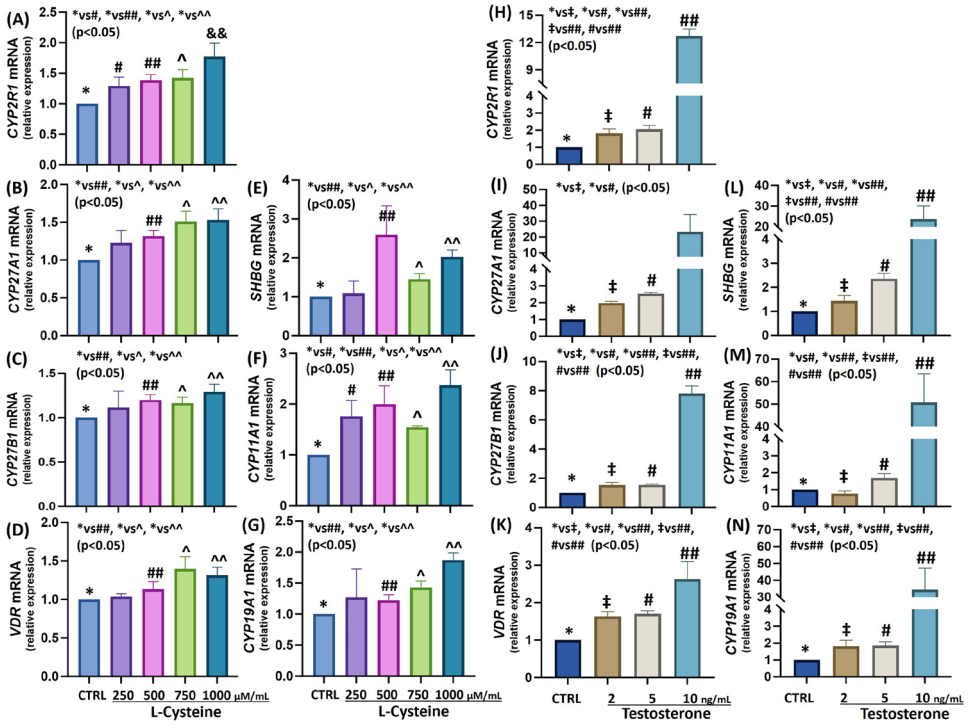

Figure 5 The effect of LC and testosterone treatment on THP-1 monocytes and the relative mRNA expression of VD-metabolism and testosterone-related genes. Data are presented as mean±SD (n=4). CTRL, Control; LC, L-cysteine; VD, vitamin D.

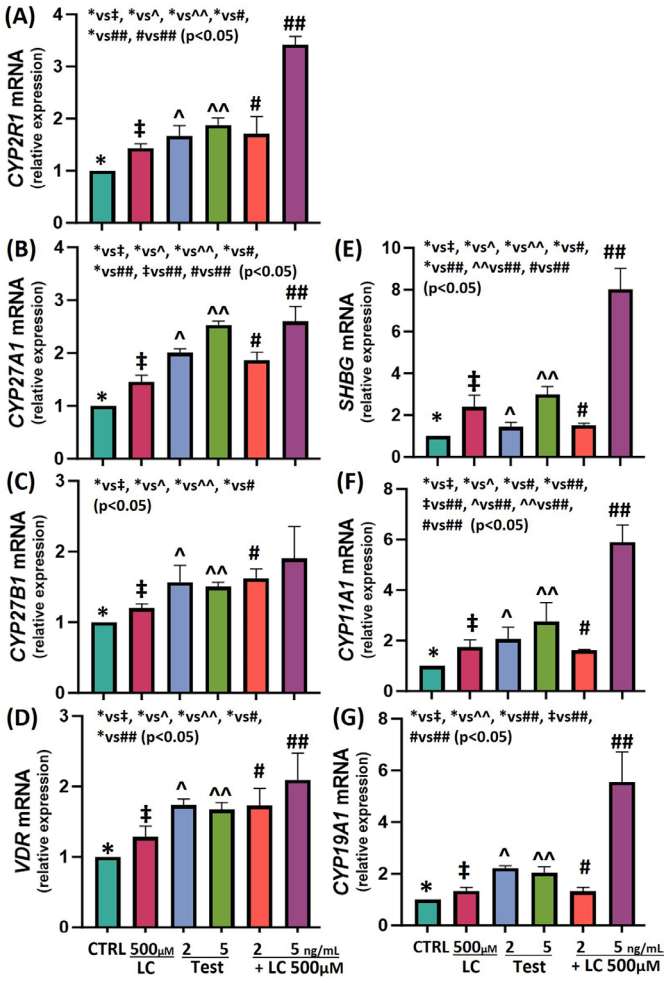

Figure 6 The effect of combined treatment of LC and testosterone on THP-1 monocytes and the relative mRNA expression of (A–D) VD-metabolism and (E–G) testosterone-related genes. Data are presented as mean±SD (n=4). CTRL, Control; LC, L-cysteine; VD, vitamin D.

VDR (figure 5A–D). LC also upregulated the expression of *SHBG* and testosterone biosynthesis genes (*CYP11A1/CYP19A1*) (figure 5E–G). Testosterone treatment per se upregulated VD metabolism genes (figure 5H–K) and the *SHBG*, *CYP11A1* and *CYP19A1* genes (figure 5L–N).

Figure 6 shows that in the presence of LC, the efficacy of testosterone increases by upregulating VD-hydroxylase genes as well as VDR (figure 6A–D). Similarly, LC cosupplementation further upregulates the SHBG and testosterone biosynthesis genes (figure 6E,F). The results suggest a synergistic effect between LC and testosterone, leading to an increased expression of genes related to VD metabolism and testosterone biosynthesis, along with the production of SHBG in monocytes.

DISCUSSION

VD deficiency and inflammation are more prevalent in obese subjects and those with metabolic syndrome.[8–11] Recent studies have shown that obesity and metabolic syndrome are associated with the downregulation of VD-metabolism genes, including the VD-hydroxylase genes (*CYP2R1/CYP27A1/CYP27B1/VDR*).[8–11] These genes are required to convert consumed VD into 25(OH)VD to enable its metabolic action.[10 11] Recent studies report that LC can upregulate the VD-metabolism genes.[13 20–22] This study investigates whether simultaneous supplementation of LC along with VD provides a better therapeutic effect in the optimisation of 25(OH)VD and lowers inflammation levels.

Bioavailable 25(OH)VD

The free hormone hypothesis suggests that serum bioavailable 25(OH)VD rather than total 25(OH)VD may be a better biomarker of VD status and predictor of its health consequences.[16 17 24] This study observed that following supplementation, there was a significant reduction in VDBP in subjects supplemented with VD+LC compared with baseline. In contrast, a modest increase in VDBP was seen in subjects supplemented with VD-alone (figure 3A). It appears that LC supplementation decreases VDBP biosynthesis and/or secretion in the blood, which may increase the pool of bioavailable 25(OH)VD on hand for metabolic action.

LC and inflammation

LC is an anti-inflammatory molecule and upregulates the expression of VD-hydroxylase/metabolism genes.[1 8 9 12] Animal studies have shown that consumption of VD and LC more effectively raises blood levels of 25(OH)VD (treating VD deficiency) and lowers insulin resistance and inflammation compared with taking VD-alone.[20–22] This clinical trial observed that compared with supplementation with VD-alone, VD+LC cosupplementation significantly increased bioavailable 25(OH)VD in AA subjects. LC can detoxify oxygen radicals and protect against cellular damage[12] and also increase serum testosterone levels.[25 26] The NLR in circulating blood is widely used as an index to monitor the balance between systemic inflammation and immunity and its elevated levels are associated with metabolic syndrome and immune dysfunction conditions.[27 28] Low levels are linked with inflammation and VD deficiency.[29] Compared with VD-alone, VD+LC showed greater efficacy in reducing levels of proinflammatory biomarkers (NLR, CRP, HbA1C and HOMA-IR) and increasing SHBG, an effective anti-inflammatory molecule.

Sex hormone-binding globulin

Traditionally, SHBG is considered a binding protein that transports testosterone and estradiol to target tissues and regulates their free concentrations. However, SHBG also influences biological actions independent of total or free testosterone.[30–32] SHBG prevents sex-steroid deficiency by increasing its absorption, half-life and steroid biosynthesis.[33] Studies have shown that transgenic mice that overexpress human SHBG transgenes circumvent metabolic syndrome, inflammation and type 2 diabetes.[34 35] This study observed a significant increase in circulating

SHBG in subjects supplemented with VD+LC but not in those supplemented with VD-alone. Further, LC cosupplementation decreases inflammation by lowering NLR, CRP, HbA1C and HOMA-IR and elevating SHBG levels in the blood. Increased SHBG levels can upregulate insulin signalling pathways, improve glucose metabolism and contribute to the decrease in HbA1c and the proinflammatory biomarker CRP and the NLR.

This study did not demonstrate any difference in 25(OH)VD levels between the subjects supplemented with VD-alone vs VD+LC-supplemented subjects. However, when results were separated based on sex, a significant increase in both total and bioavailable 25(OH)VD was observed in male subjects but not in female subjects in the VD+LC supplemented group, a finding not observed in the group supplemented with VD-alone. The exact mechanism by which LC decreases VDBP and increases bioavailable 25(OH)VD and whether it is related to the sex-specific increase in total 25(OH)VD in men is not clear. One hypothesis is that LC is an antioxidant and thus helps protect against oxidative damage and reduce inflammation. This can decrease VDBP biosynthesis or secretion from the liver, similar to the decrease in CRP observed in the VD+LC group. Other studies also report an association between increased VDBP levels and decreased levels of bioavailable VD.[36] This indicates that lower levels of VDBP can increase the loosely bound bioavailable VD pool in the circulation by decreasing circulating VD binding to the high-affinity VDBP.

Cell culture study

In this clinical trial, we observed an increase in total VD in men, but not women, after cosupplementation with VD+LC but not VD-alone, which is interesting. This led us to question whether testosterone in men upregulates the VD-metabolism genes, thus increasing total VD. The link between testosterone in men and VD, and the direct effect of LC and/or testosterone treatment on VD-metabolism gene expression, was examined in THP-1 monocytes. It is believed that cell culture studies offer a direct insight into the effects of treatment in a controlled environment, thus providing valuable information on the interplay between testosterone and VD levels. Cell culture studies have shown that testosterone treatment of monocytes upregulates VD-metabolism genes, which can contribute to elevated VD levels in men compared with women. In addition, previous studies have reported that the testis also has additional high levels of *CYP2R1*, which can promote VD hydroxylation in men.[37]

Additionally, LC and/or testosterone treatment in THP1 monocytes upregulated the expression of *SHBG* and testosterone regulatory genes. *CYP11A1*, a member of the cytochrome P450 side chain cleavage enzyme, plays a key role in the male hormone testosterone synthesis pathway and occupies a regulatory place in the biosynthesis of steroids. The overexpression of VDR significantly enhances *Cyp11a1* expression and testosterone concentration.[38] The concentrations of LC and testosterone used were similar to those found in the bloodstream. Thus, the in vitro results hold significant physiological importance and contribute to the understanding of our findings in this clinical study.

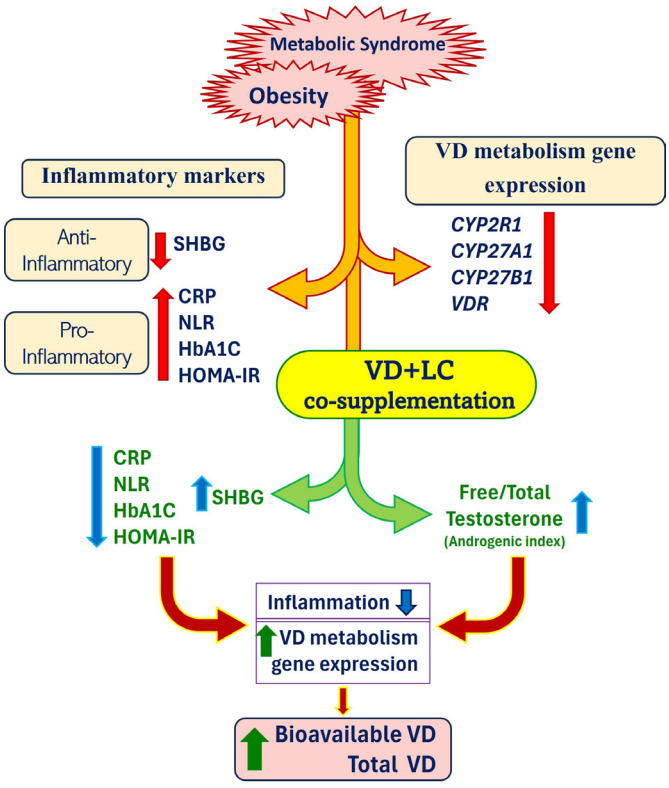

Figure 7 Summary of the clinical trial suggesting that cosupplementation with VD+LC reduces proinflammatory markers and increases SHBG and the free/total testosterone ratio, resulting in the upregulation of the VD-hydroxylase genes and an increase in the bioavailable and total VD. CRP, C reactive protein; HbA1c, Hemoglobin A1C; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; LC, L-cysteine; NLR, neutrophil-to-lymphocyte ratio; SHBG, sex hormone-binding globulin; VD, vitamin D.

However, supplementation with LC alone did not result in higher VD levels in men versus women. This may suggest that adequate levels of VD are required to optimise the effect of testosterone in VD-deficient men. Interestingly, the free/total testosterone ratio[39 40] levels were elevated in the cosupplementation group. This suggests that the upregulation of circulating free testosterone can help promote VD sufficiency. Overall, this pilot study suggests that cosupplementation with LC could lower VDBP and inflammatory biomarkers and upregulate bioavailable 25(OH)VD (in both sexes) and total 25(OH)VD in men mediated by an increase in free testosterone (figure 7). Thus, cosupplementation with VD+LC could provide a better therapeutic benefit compared with the current practice of supplementation with VD-alone.

Strengths and limitations

This study has many strengths along with its limitations. The strengths include a large AA population with a similar socioeconomic background, a broad age range,

rigorously adjudicated safety endpoints and high rates of follow-up with adequate compliance. In this trial, we used block randomisation, which allowed us to prevent any ambiguity or differences between supplemented groups. As a result, each group had an equal distribution of subjects, and each week we had an almost equal number of subjects from each group. This allowed us to investigate in parallel the combined and separate effects of VD and LC intake without any seasonal bias. At baseline, all subjects underwent a placebo run-in period for stabilisation. In addition, the baseline and the final clinical visits for all subjects occurred within 90 days. Therefore, there was a limited scope for seasonal variation among the groups.

After randomisation (visit 2), 49 participants dropped out at various stages of subsequent visits, which is considered one of the limitations of this trial. However, the number of dropouts was evenly distributed among the four groups (placebo, n=15; LC, n=11; VD, n=10; VD+LC, n=12). The primary reasons that participants dropped out were relocation to other states or loss of contact. Another study limitation is the lack of dietary consumption information. However, any variations in dietary consumption should not have had any effect because the subjects were prescribed high doses of VD and LC. Although exposure to sunlight does lead to the production of a percentage of VD, ultimately geography did not play a major role in sun exposure in our subjects since all subjects were from the same region and block randomisation ensured similar sun exposure to subjects in each group. Therefore, we do not believe that any seasonal/geographical variations may have skewed the results. The small sample size of men and the absence of a control group from the white population is another limitation of this trial. However, this study has a placebo run-in period (30 days) for all the subjects in each group, in addition to the placebo-only group, which served as our control.

CONCLUSIONS

This pilot study suggests that compared with supplementation with VD-alone, VD+LC cosupplementation could be a better approach for increasing the levels of total 25(OH)VD in men and the bioavailable 25(OH)VD in both sexes while reducing inflammation among AA (figure 7). Further clinical trials are needed to investigate whether LC cosupplementation with VD could be used to successfully treat VD deficiency.

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