Vitamin D Supplementation in Obese Sri Lankan Children: A Randomized Controlled Trial

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Abstract

**Background** Micronutrient deficiencies are identified among obese individuals. Vitamin D deficiency is prevalent in obese children, and is hypothesized to cause insulin resistance and metabolic abnormalities. This study aimed to determine the effect of vitamin D supplementation on obesity and related metabolic abnormalities among obese Sri Lankan children.

**Methods** A triple-blind randomized controlled trial was conducted among vitamin D deficient (< 20 ng/ml), obese children (n = 96). Children were randomly allocated to three intervention arms - treatment arm receiving weekly vitamin D$_2$ 50,000 IU; supplementation arm receiving 2500 IU weekly and control arm receiving placebo. Anthropometry, percentage fat mass (%FM) and blood pressure were assessed and fasting blood glucose, fasting insulin, lipid profile, ALT, AST, vitamin D, parathyroid hormone and hs-CRP and OGTT with 2-hour random blood glucose and insulin was performed at baseline and after 24 weeks of treatment. Ethics Review Committee of Faculty of Medicine, University of Colombo approved the protocol.

**Results** Waist circumference Z-score, %FM and serum calcium significantly improved across all three arms, ALT significantly improved in treatment and supplementation arms while, BMI Z-score, PTH and vitamin D significantly improved in the treatment arm. Biceps (p = 0.035) and subscapular (0.048) skin fold thickness, vitamin D (p = 0.004) and ALT (p = 0.012) significantly improved in the treatment arm.

**Conclusions** A strict dietary and physical activity regimen could improve some of the anthropometric, body composition and metabolic profiles, but high dose vitamin D, enhances those improvements. Therefore high dose vitamin D seems to potentiate management outcomes of obese children with vitamin D deficiency.

**Trial registration** The study was registered at the Sri Lanka Clinical Trials Registry (SLCTR/2015/017) on 12th September 2015 at https://slctr.lk/trials/slctr-2015-017.

**Background**
The incidence of NCD is increasing rapidly in developing countries than in industrialized countries. Obesity during childhood is a growing concern and the resulting socioeconomic and public health
burden is enormous. Contrary to the popular belief that obese individuals are adequately nourished, micronutrient deficiencies have been identified to be prevalent among obese across all age groups worldwide.

Vitamin D is an essential nutrient that plays an important role in calcium homeostasis and it also plays a crucial role in insulin secretion and maintaining glucose homeostasis via its endocrine mechanisms [1–3]. As much as identifying the magnitude of the problem of VDD among obese children, it is also important to identify the part it plays in insulin resistance among them. Obese individuals were noted to have VDD associated with other micronutrients in several studies, although the exact mechanism has not been revealed. Possible hypothesis for the lower 25-hydroxy vitamin D (25(OH)D) levels in obese children, includes decreased sun exposure due to sedentary lifestyle, poor quality diet and increased clearance of 25(OH)D due to being stored in adipose tissue [4].

Potential explanations for the association between low 25(OH)D level and impaired glucose tolerance have largely focused on direct effects of vitamin D on pancreatic β-cell insulin secretion. Vitamin D receptors and vitamin D-binding proteins are known to exist in pancreatic tissue, and calcium plays an important role in insulin secretion from β-cell [1, 5]. Other possible mechanism is the potential anti-inflammatory role of vitamin D. Since the immune-modulatory functions of vitamin D are incontestable, its deficiency in obese patients may coincide with enhanced systemic inflammation. In paediatric population, an association between low vitamin D level, and activation of pro-inflammatory, pro-diabetic and atherogenic pathways among the obese has been noted [6].

A study based on bi-directional genetic approach, has shown that a higher BMI leads to lower 25(OH)D. The effects of lower 25(OH)D increasing BMI are likely to be small. Therefore population level interventions to reduce BMI are expected to decrease the prevalence of VDD [7]. Furthermore it might justify use of vitamin D in an attempt of preventing or treating obesity and metabolic syndrome.

Vitamin D supplementation in obese children has shown beneficial metabolic effects. It has shown to reduce serum triglyceride, insulin and insulin resistance levels, but no improvement in cholesterol, fasting blood sugar and blood pressure compared to a placebo group[8]. Furthermore, vitamin D
supplementation has shown to improve BMI-SD scores, ALT and HbA1c levels in type 2 diabetes mellitus compared to type 1 diabetes mellitus [9]. Studies in adults have also shown that reduction in weight has improved the vitamin D status and the improvement is proportionate to the weight loss [10].

There are only few studies available on vitamin D status of Sri Lankan children. VDD using a higher cut off value (< 35 nmol/l) was seen in 26% and 25% of male and female children respectively in the Southern province of Sri Lanka [11]. A Similar study done more recently revealed VDD (< 20 nmol/l) in 29% of study population [12]. The prevalence of VDD (< 20 ng/ml) among obese Sri Lankan children was 75.2%, while insufficiency (20–30 ng/ml) was 21.3% [13].

In the light of ever increasing incidence of childhood obesity and associated higher prevalence of VDD, the current study was carried out to determine the effectiveness of vitamin D supplementation on obesity and related morbidities in children aged 5-15 years in an urban area of Sri Lanka.

Methods
This was a randomized controlled trial with three parallel arms of equal size. Obese children aged 5-15 years attending the Obesity Clinic of Lady Ridgway Hospital, Colombo, who are identified to be having VDD (defined as 25 (OH) D < 20 ng/ml or < 50 nmol/L) [14] at a previously conducted cross-sectional study, were invited to participate in this prospective triple-blind placebo-controlled therapeutic and supplementary study [13]. Children who were suffering from any chronic condition or were on long-term medication were excluded.

Sampling
Sample size was calculated to determine a statistically significant difference in insulin resistance in the treatment group and control group. According to the findings of Belenchia et al. the mean HOMA-IR levels in the treatment and control group following 6 months of Vitamin D supplementation was 3.49 (SD = 1.84) and 5.0 (SD = 1.82) respectively [15]. Using this effect size and an α error of 5% and a β error of 20% (power of 80%) the sample size required was calculated as, 26 per treatment arm. Allowing for a non-response rate of 25%, a sample size of 35 was recruited for each treatment arm. Children who were identified as having VDD (25 (OH) D < 20 ng/ml) in the prevalence study were
consecutively recruited until the required sample size was reached.

Data Collection Method
Baseline Assessment
Baseline assessment was conducted as part of the prevalence study, and data on socio-demographics, anthropometry (height, weight, WC, SFT), body composition, puberty and biochemistry were assessed. For the biochemical assessment, blood was drawn after a 12-hour overnight fast, for FBG, lipid profile, AST, ALT, hs-CRP (high sensitive C-Reactive Protein), serum insulin, 25(OH)D levels, serum Calcium and serum PTH levels, 2-hour RBG and insulin following OGTT [13].

Recruitment and Random Allocation
The study was conducted at the Obesity Clinic of Lady Ridgway Hospital, Colombo. Children recruited to the study were explained the study process and informed, written consent was obtained from the parents/guardians. Once registered for the trial, the subjects were randomly allocated using a predetermined, computer-generated, concealed simple randomization sequence in to one of the three treatment arms - vitamin D in treatment doses, vitamin D in supplementation dose or placebo (Fig. 1).

Principle investigator generated the randomization sequence and identical treatment packages were prepared for each participant according to the registration number. Allocation sequence of each participant was concealed from the recruiting officers who recruited and registered the participants, administered the treatment according to the registration numbers and conducted the data collection.

Intervention
Study subjects were followed up for 24 weeks with the respective treatments, structured dietary and physical activity interventions allocated to each treatment arm in the following manner:

Treatment Group: Structured diet + Physical activity + Vitamin D$_2$ treatment dose of 50,000 IU weekly

Supplementation Group: Structured diet + Physical activity + Vitamin D$_2$ supplementation dose of 2500 IU weekly.

Placebo Group: Structured diet + Physical activity + Placebo administered weekly

Dietary advice was given based on food based dietary guidelines produced by Ministry of Health [16]. Age-based portion size guide was given to parents and children and guidance was provided to them
on the amount and the quality of the food they should be consuming.

Physical activity advice included instructions to engage in 60 minutes of daily physical activity, which would help to burn calories and improve fitness. Activities such as brisk walking, swimming or indoor or outdoor physical activity were recommended. Whenever they could not perform them as a single activity, were advised to carry out as short intensive sessions each lasting 15–20 minutes to obtain a cumulative outcome of 60 min per day.

Treatment comprised one of the following: Therapeutic dose of 50,000 IU of vitamin D₂ (Ergocalciferol B.P., SGPharma®, Mumbai, India) weekly or a supplementary dose of vitamin D₂ 2500 IU weekly, or an identical placebo. All three were administered in the form of a powder, and the participants were instructed to take is dissolved in a glass of milk and take on same day of the week at a similar time of the day.

Follow up and compliance
Participants were requested to keep a written record of their compliance with the treatment and physical activity recommendations. Telephone calls were given during the initial period to reinforce compliance. The participants were followed up monthly at the obesity clinic, Lady Ridgway Hospital. During the follow up visits the subjects were assessed for compliance to treatment, dietary advice and physical activity schedule and for adverse effects of the treatment. At each visit height, weight, WC, FM and blood pressure were recorded. Apart from these evaluations, after 12 weeks of intervention assessment of serum calcium (to screen for hypercalcaemia) and urinary calcium to creatinine ratio (to screen for hypercalciuria) was done as a precautionary measure to detect any adverse response to vitamin D supplementation and no child showed adverse results.

Blinding
The study was designed as a triple-blinded trial. The study subjects were unaware of the treatment given. A blinded research officer carried out the assessment during each follow-up visit and at the end of 24 weeks. Statistical analysis of outcomes was conducted on a blinded data set identifying the three treatment arms using unrelated letters.

Outcome Assessment
Outcome assessment was conducted after 24 weeks of treatment. Primary outcome was the HOMA-IR
while other metabolic (FBG, 2-hour RBG, fasting insulin, 2-hour insulin, lipid profile, AST, ALT, hs-CRP, serum calcium, PTH), anthropometric (height, BMI and WC SD scores, SFT), body composition (%FM) parameters and systolic and diastolic blood pressure were defined as secondary outcomes. Anthropometric, body composition and metabolic parameters assessed at baseline were reassessed under the same conditions and according to the same standards specified. The laboratory assessments were performed in the endocrine laboratory of the Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Colombo. The trial was conducted from October 2015 to February 2017 and the study ended after completing follow up of 24 weeks according to the protocol.

Data analysis
Statistical analysis of data was conducted using SPSS-20 for windows. Intention-to-treat analysis was performed by substituting any missing values with latest available measurements. Baseline demographic, anthropometric, body composition and metabolic characteristics of the treatment, supplementary and control groups were compared using chi square test and One-way ANOVA test or relevant non-parametric tests. Between the three groups, the anthropometric, body composition and metabolic parameters at 24 weeks were compared using One-way ANOVA test or relevant non-parametric tests. Pre-post difference of the outcomes between the three groups was compared adjusting for their baseline values, using ANCOVA. Within the treatment and control groups the pre-post difference in the parameters were assessed using paired t-test or equivalent non-parametric tests.

Ethics considerations
The Ethics Review Committee of Faculty of Medicine, University of Colombo approved the protocol (EC-16-030). Informed written consent was obtained from the parents of all participants and assent was obtained from the participants above the age of 12 years. The Clinical Trials committee of the National Medicine Regulatory Authority (NMRA) of Ministry of Health approved the protocol. The study was registered at the Sri Lanka Clinical Trials Registry accessible at https://slctr.lk (SLCTR/2015/017). CONSORT guidelines are adhered to, in reporting the methods and findings of the trial.

Results
Participant follow up and adherence to therapy
Final sample of n = 96, was randomized into vitamin D treatment arm (n = 32), vitamin D supplementation arm (n = 33) and placebo arm (n = 31). Complete follow up data were available for 79 subjects, 27 in treatment, 25 in supplementation and 27 in placebo arms. Intention to treat analysis was performed after substituting the missing values with the last available values. Analysis of the baseline characteristics of the subjects lost to follow up showed that they were not significantly different from those who completed the trial.

Baseline characteristics
Table 1 shows the baseline characteristics compared between the three arms. Mean age was 9.8 years (SD 2.12) and 27 (28.1%) were girls. Age and gender distribution was similar in the three arms and there were no significant differences in mean systolic and diastolic blood pressure, anthropometry, body composition, serum vitamin D levels or other metabolic parameters between the three arms at baseline.
| Parameter                        | Supplementation (n = 33) | Treatment (n = 32) | Placebo (n = 31) | Sig.  |
|---------------------------------|--------------------------|--------------------|------------------|-------|
|                                | Mean (SD)                | Mean (SD)          | Mean (SD)        |       |
| Age (yrs)                       | 9.75 (2.26)              | 9.95 (2.02)        | 10.61 (1.83)     | NS    |
| Gender*                         | Male                     | Male               | Male             |       |
|                                | 22 (66.7%)               | 22 (67.7%)         | 25 (80.6%)       | NS    |
| Ethnicity*                      | Sinhala                  | Tamil              | Muslim          |       |
|                                | 23 (69.7%)               | 3 (9.1%)           | 7 (21.2%)        |       |
|                                | 7 (21.2%)                | 4 (12.9%)          | 7 (23.2%)        |       |
| BMI SDS                         | 2.83 (0.86)              | 2.72 (0.65)        | 2.66 (0.55)      | NS    |
| Height SDS                      | 0.64 (1.53)              | 0.66 (1.09)        | 0.50 (1.32)      |       |
| Waist Circumference SDS         | 3.22 (0.72)              | 3.04 (0.62)        | 2.91 (0.56)      | NS    |
| Percentage fat mass             | 43.31 (4.95)             | 41.89 (4.07)       | 41.94 (4.31)     | NS    |
| Systolic BP SDS                 | -1.33 (0.82)             | -1.29 (0.78)       | -1.41 (0.87)     | NS    |
| Diastolic BP SDS                | 0.86 (0.77)              | 0.64 (0.94)        | 0.68 (0.68)      |       |
| Vitamin D (ng/ml)               | 14.92 (3.92)             | 14.92 (3.04)       | 15.47 (2.78)     | NS    |
| Parathyroid hormone (pg/ml)     | 36.41 (26.47)            | 37.24 (18.13)      | 34.19 (15.37)    | NS    |
| Alkaline Phosphatase (IU/L)     | 262.79 (87.27)           | 243.0 (56.27)      | 270.29 (74.72)   | NS    |
| Serum Calcium (mg/dl)           | 9.64 (0.46)              | 9.72 (0.48)        | 9.71 (0.58)      | NS    |
| Fasting Blood glucose (mg/dl)   | 86.18 (8.62)             | 83.19 (5.28)       | 83.5 (6.17)      | NS    |
| 2-hour OGTT (mg/dl)             | 119.25 (21.08)           | 112.48 (18.84)     | 116.63 (21.84)   | NS    |
| Fasting Insulin (µIU/ml)        | 15.87 (8.96)             | 12.86 (8.58)       | 12.16 (8.47)     | NS    |
| 2-hour Insulin (µIU/ml)         | 118.6 (85.3)             | 109.1 (88.2)       | 11.9 (88.0)      | NS    |
| Total cholesterol (mg/dl)       | 198.27 (39.39)           | 183.94 (35.09)     | 176.98 (41.26)   | NS    |
| Triglycerides (mg/dl)           | 108.36 (46.76)           | 103.53 (57.79)     | 129.16 (67.57)   | NS    |
| HDL (mg/dl)                     | 38.48 (7.28)             | 41.53 (10.22)      | 41.55 (9.59)     | NS    |
| LDL (mg/dl)                     | 138.11 (33.62)           | 123.11 (32.24)     | 115.63 (29.11)   | NS    |
| AST (IU/l)                      | 31.88 (10.29)            | 38.38 (31.85)      | 31.45 (15.38)    | NS    |
| ALT (IU/l)                      | 33.72 (21.39)            | 39.22 (51.37)      | 29.42 (27.73)    | NS    |
| AST/ALT                         | 1.1764 (0.68)            | 1.39 (0.71)        | 1.38 (0.77)      | NS    |
| ALP (IU/L)                      | 262.79 (87.27)           | 243.0 (56.27)      | 270.29 (74.72)   | NS    |
| HS-CRP (mg/l)                   | 4.41 (3.53)              | 3.83 (4.36)        | 3.562 (3.13)     | NS    |
| Serum Ca (mg/dl)                | 9.64 (0.46)              | 9.72 (0.48)        | 9.71 (0.58)      | NS    |
| HOMA IR                         | 3.45 (2.12)              | 2.598 (1.79)       | 2.529 (1.81)     | NS    |

NS - Not statistically significant
* Number and percentage are given and one missing value was observed for ethnicity in treatment arm

Outcome measures

Anthropometric and metabolic parameters at baseline and after six months were compared within the
three treatment arms (Table 2). In the vitamin D treatment arm, BMI-SD score (2.72 to 2.52, p = 0.021), WC-SD score (3.04 to 2.75, p = 0.003), triceps SFT (27.36 to 24.88, p = 0.003) and %FM (41.89 to 38.75, p = 0.001) showed significant reductions from baseline to six months. Vitamin D levels (14.92 to 18.24, p = 0.003) increased while PTH (37.24 to 30.64, p = 0.006) and serum calcium (9.72 to 9.44, p = 0.013) levels decreased significantly. A significant improvement was seen in mean HDL (41.53 to 49.25, p = 0.000) and ALT levels (39.22 to 21.66, p = 0.036). Non-significant improvements from baseline to six months were seen in biceps skin fold thickness (19.69 to 17.75, p = 0.065), LDL levels (123.11 to 115.64, p = 0.075), AST (38.38 to 31.19, p = 0.075), AST/ALT ratio (1.39 to 1.55, p = 0.195) and ALP (243 to 231, p = 0.099) levels. Fasting blood sugar levels, in contrast, showed a statistically significant increase (83.19 to 90.23, p = 0.000) from baseline to six months.
### Table 2

Anthropometric and Metabolic Parameters at baseline and 6 months in the three intervention arms (n = 96)

| Parameter | Baseline Supplementation (n = 33) | Treatment (n = 32) | Placebo (n = 31) |
|-----------|----------------------------------|--------------------|-----------------|
| Mean (SD) | Mean (SD)                        | Mean (SD)          | Mean (SD)       |
| Height    | 0.64 (1.53)                      | 0.34 (1.38)        | 0.362 (1.09)    |
| BMI SDS   | 2.83 (0.86)                      | 2.70 (0.85)        | 2.72 (0.65)     |
| WC SDS    | 3.22 (0.72)                      | 2.89 (0.74)        | 3.04 (0.62)     |
| Triceps SFT (cm) | 26.93 (5.1) | 26.04 (5.8)        | 0.415 (2.76)    |
| Biceps SFT (cm) | 20.65 (5.2) | 20.56 (5.25)       | 0.725 (19.69)   |
| Subscapular SFT (cm) | 32.63 (8.6) | 33.5 (8.6)         | 0.567 (31.29)   |
| Suprailiac SFT (cm) | 31.08 (6.6) | 33.34 (6.6)        | 0.112 (31.23)   |
| SBP SDS   | -1.33 (0.82)                     | -1.26 (0.85)       | -1.29 (0.78)    |
| DBP SDS   | 0.86 (0.77)                      | 0.82 (0.716)       | 0.82 (0.645)    |
| Percentag e FM | 43.31 (4.95) | 41.29 (5.46) | 41.89 (4.688) |
| Vitamin D (ng/ml) | 14.92 (3.92) | 15.26 (3.675) | 14.92 (3.044) |
| PTH (pg/ml) | 3.61 (2.147) | 33.94 (21.94) | 0.325 (37.24) |
| FBS (mg/dl) | 86.18 (8.62) | 88.00 (7.79) | 0.291 (3.19) |
| OGTT (mg/dl) | 119.25 (21.08) | 119.91 (17.79) | 0.861 (124.8) |
| Fasting Insulin (µU/ml) | 15.87 (8.96) | 14.09 (7.97) | 0.198 (12.86) |
| 2-hour Insulin (µU/ml) | 118.60 (85.32) | 115.57 (97.49) | 0.847 (109.096) |
| Total choles terol (mg/dl) | 198.27 (39.39) | 201.64 (43.37) | 0.438 (183.94) |
| Triglycerides (mg/dl) | 108.36 (46.76) | 120.30 (53.79) | 0.176 (103.53) |
| HDL (mg/dl) | 38.48 (7.28) | 45.61 (12.1) | 0.005 (41.53) |
| LDL (mg/dl) | 138.11 (33.62) | 132.77 (39.81) | 0.176 (123.11) |
| AST (IU/l) | 31.88 (10.29) | 29.24 (8.25) | 0.075 (38.38) |
| ALT (IU/l) | 33.72 (21.39) | 25.97 (9.40) | 0.035 (39.22) |
| AST/ALT | 1.1764 (0.68) | 1.1957 (0.34) | 0.861 (1.39) |
| ALP (IU/L) | 262.79 (87.27) | 240.52 (66.28) | 0.008 (243.0) |
| hs-CRP (mg/l) | 3.41 (3.53) | 4.22 (3.7) | 0.687 (3.83) |
| Serum Ca (mg/dl) | 9.64 (0.46) | 9.44 (0.53) | 0.034 (9.72) |
| HOMA IR | 3.45 (2.12) | 3.099 (1.8838) | 0.264 (2.598) |

*Significance for the mean difference from baseline to 6 months - Paired samples T test **Significance between groups mean differences at 6 months - One way ANOVA test

BMI-Body mass index, WC-Waist circumference, SDS - SD score, SBP-Systolic Blood Pressure, DBP-Diastolic Blood Pressure, OGTT-Oral glucose tolerance test, PTH-Parathyroid hormone, FBS-Fasting Blood Sugar, HDL-High-density Lipoprotein, LDL-Low-density lipoprotein, AST-Aspartate transaminase, ALT-Alanine transaminase, ALP-Alkaline phosphatase, Hs-CRS-High sensitivity C reactive protein, FM-Fat mass, HOMA-IR-Insulin Resistance using homeostatic model
In the supplementation arm, mean WC-SD score (3.22 to 2.89, p = 0.006) and mean %FM (43.31 to 41.29, p = 0.000) showed significant reductions from baseline to six months. Mean HDL levels (38.48 to 45.61, p = 0.005) increased significantly while mean ALT levels (33.72 to 25.97, p = 0.035) showed a significant reduction. ALP levels (262.8 to 240.5, 0.008) and serum calcium levels (9.64 to 9.44, p = 0.034) showed a statistically significant increase from baseline to six months.

In the placebo-treated arm, statistically significant improvements from baseline to six months were seen in WC-SD score (2.90 to 2.69, p = 0.000), %FM (41.94 to 39.76, p = 0.008), HDL (41.55 to 48.65, p = 0.000), ALP (270.3 to 240.4, p = 0.008) and serum calcium (9.71 to 9.36, p = 0.013) levels. Subscapular SFT (29.45 to 32.54, p = 0.006) and FBG (83.5 to 92.9, p = 0.000) levels significantly deteriorated in the placebo arm during this period.

Baseline to six months difference in the anthropometric and metabolic parameters adjusted for the baseline levels, were compared between the three arms (Table 3). Statistically significant differences were seen in the baseline to six months difference in vitamin D levels, biceps and subscapular SFT and ALT levels between the three treatment arms. Baseline to six months increase in vitamin D levels in the treatment arm (3.33 ng/ml) was significantly (p = 0.004) higher than that of the supplementation (0.28 ng/ml) and placebo (0.46 ng/ml) arms. Reduction in biceps SFT in the treatment arm (-2.09) was significantly (p = 0.035) larger than the change in the supplementation arm (0.75). Similarly, the reduction in subscapular SFT in treatment arm (-1.54) was significantly (p = 0.048) larger than the change in the placebo arm (2.38). ALT levels in the treatment arm (-9.74) showed a significantly (p = 0.012) larger reduction compared to that of the placebo arm (-2.77).

Baseline to six months reductions of relatively larger magnitude in the treatment arm compared to supplementation and placebo arms were seen in BMI-SD score, triceps and suprailiac SFT, WC-SD score, percentage fat mass, serum parathyroid hormone, 2-hour insulin, LDL cholesterol, serum AST, AST/ALT ratio and HOMA-IR. However, these differences were not statistically significant.
Table 3
Difference in anthropometric and Metabolic parameters from baseline to six months in the three intervention arms after adjusting to baseline levels (n = 96)

| Parameter                      | Supplementation (n = 33) Mean Difference | SE  | Treatment (n = 32) Mean Difference | SE  | Placebo (n = 31) Mean Difference | SE  | Sig.* |
|--------------------------------|------------------------------------------|-----|-----------------------------------|-----|---------------------------------|-----|-------|
| Height SDS                     | 0.116                                    | 0.049 | 0.016                             | 0.049 | -0.052                         | 0.049 | 0.054 |
| BMI SDS                        | -0.062                                   | 0.054 | -0.135                            | 0.056 | -0.091                         | 0.055 | 0.650 |
| Triceps SFT (cm)               | -0.437                                   | 0.776 | -2.891                            | 0.801 | -1.520                         | 0.801 | 0.095 |
| Biceps SFT (cm)                | 0.752                                    | 0.780 | -2.091                            | 0.780 | -0.087                         | 0.806 | 0.035 |
| Subscapular SFT (cm)           | 1.384                                    | 1.101 | -1.535                            | 1.148 | 2.376                          | 1.160 | 0.048 |
| Supra-iliac SFT (cm)           | 2.256                                    | 0.968 | -0.213                            | 0.999 | 2.499                          | 1.015 | 0.110 |
| WC SDS                         | -0.222                                   | 0.072 | -0.325                            | 0.070 | -0.230                         | 0.069 | 0.521 |
| SBP SDS                        | 0.075                                    | 0.158 | 0.109                             | 0.161 | 0.192                          | 0.161 | 0.868 |
| DBP SDS                        | -0.047                                   | 0.120 | -0.181                            | 0.124 | 0.118                          | 0.124 | 0.240 |
| % FM                           | -1.639                                   | 0.533 | -2.536                            | 0.538 | -1.807                         | 0.538 | 0.458 |
| Vitamin D (ng/ml)              | 0.282                                    | 0.696 | 3.331                             | 0.718 | 0.463                          | 0.719 | 0.004 |
| PTH (pg/ml)                    | -1.004                                   | 2.224 | -6.370                            | 2.267 | -2.396                         | 2.311 | 0.224 |
| FBG (mg/dl)                    | 2.598                                    | 1.319 | 5.784                             | 1.403 | 0.841                          | 1.417 | 0.078 |
| OGGT (mg/dl)                   | 3.593                                    | 3.343 | 4.576                             | 3.290 | 3.328                          | 3.336 | 0.572 |
| Fasting Insulin (µIU/ml)       | -0.609                                   | 1.193 | -0.602                            | 1.200 | 0.551                          | 1.181 | 0.729 |
| 2-hour Insulin (µIU/ml)        | -1.996                                   | 12.872 | -25.056                           | 13.092 | -17.320                       | 13.09 | 0.444 |
| Total cholesterol (mg/dl)      | 5.223                                    | 4.731 | 1.662                             | 4.827 | 10.138                         | 4.936 | 0.470 |
| Triglycerides (mg/dl)          | 10.970                                   | 7.952 | 0.514                             | 8.234 | 10.369                         | 8.416 | 0.601 |
| HDL (mg/dl)                    | 5.285                                    | 1.594 | 8.082                             | 1.610 | 7.171                          | 1.607 | 0.460 |
| LDL (mg/dl)                    | -3.728                                   | 4.091 | -7.838                            | 3.999 | 0.340                          | 4.078 | 0.361 |
| AST (IU/L)                     | -2.832                                   | 1.315 | -3.481                            | 1.381 | -0.133                         | 1.358 | 0.188 |
| ALT (IU/L)                     | -4.732                                   | 1.602 | -9.740                            | 1.650 | -2.772                         | 1.696 | 0.012 |
| AST/ALT                        | -0.049                                   | 0.063 | 0.171                             | 0.065 | 0.015                          | 0.065 | 0.052 |
| ALP (IU/L)                     | -20.688                                  | 6.740 | -13.128                           | 7.113 | -21.082                        | 7.080 | 0.672 |
| HS-CRP (mg/L)                  | -0.184                                   | 0.433 | 0.239                             | 0.437 | 0.659                          | 0.445 | 0.408 |
| Serum Ca (mg/dl)               | -0.231                                   | 0.092 | -0.272                            | 0.095 | -0.337                         | 0.095 | 0.724 |
| HOMA IR                        | -0.051                                   | 0.245 | -0.209                            | 0.255 | 0.384                          | 0.246 | 0.220 |

BMI-Body mass index, WC-Waist circumference, SDS – SD score, SBP-Systolic Blood Pressure, DBP- Diastolic Blood Pressure, OGGT-Oral glucose tolerance test, PTH-Parathyroid hormone, FBS-Fasting Blood Sugar, HDL-High-density Lipoprotein, LDL-Low-density lipoprotein, AST-Aspartate transaminase, ALT-Alanine transaminase, ALP-Alkaline phosphatase, Hs-CRS-High sensitivity C reactive protein, FM-Fat mass, HOMA-IR-Insulin Resistance using homeostatic model, SFT-Skin fold thickness

*Significance tested using ANCOVA adjusting for the baseline levels of each parameter.

Adverse events and compliance to treatment

No adverse events were reported in the study population. Urinary calcium to creatinine ratio remained below 0.14 in all the participants. Compliance was found to be satisfactory in all the subjects who completed the trial.

Discussion

Vitamin D deficiency (VDD) and obesity are two global epidemics that are rising in the paediatric population with these two converging on each other where obesity has shown to a be strong risk factor for VDD and it emphasizes the need for more research to clarify the role of VDD on obesity and
its related metabolic risks[17]. Dietary and life style changes could prevent VDD in obese children. However, vitamin D supplementation is recommended for those with inadequate intake, for those with deficient vitamin D levels or for individuals who are symptomatic of VDD. Doses are based on whether VDD is being prevented or treated [17].

The 2014 AAP clinical guidelines on bone health promotion recommend supplementation for obese children with inadequate vitamin D intake or VDD [18]. Screening for serum 25(OH)D may not be necessary as supplementation is more cost-effective than screening in the presence of clinical manifestations of hypocalcaemia, especially in regions where VDD is high and also resources limited [19]. Both the AAP and Endocrine Society of America recommends children with VDD to be supplemented with either 2000 IU/day or 50,000 IU/week of vitamin D$_2$ or vitamin D$_3$, at least for six weeks, followed by a maintenance dose of 600–1000 IU/day[18, 20]. However, obese children may need higher doses. Ideally serum levels should be assessed annually[19] and doses adjusted accordingly [20].

Results of the baseline assessment of this study clearly showed that obese VDD children have associated changes such as increased levels of PTH and ALP while maintaining normal serum calcium levels. Treatment with vitamin D improved PTH and ALP levels and the reduction of calcium levels than baseline would be due to the removal of the PTH effect on bone resorption. Reduction in calcium and ALP level and non-significant reduction in PTH level was seen in the supplementation group as well supporting the same mechanism. However, the placebo group also has shown some improvement in these parameters, which could be due to reduction in the FM (as denoted by reduced WC and %FM) thus releasing more vitamin D to the circulation and bringing about its effects.

Therefore, this study shows that vitamin D treatment is beneficial for obese children over and above simple supplementation or dietary and physical activity interventions.

In recent years, significant relationships have been documented between VDD and insulin resistance, metabolic syndrome and related cardio-metabolic abnormalities [6, 21, 22]. Vitamin D has an important role in glucose and insulin metabolism [23]. Association between serum vitamin D levels and insulin resistance is seen even in children, and vitamin D supplementation is proposed for
children and adolescents to prevent insulin resistance, despite lack consensus on the dose [24]. However, evidence lacks as to whether vitamin D supplementation improves insulin sensitivity and reduces metabolic risk in children and adolescent [8].

In the current study the metabolic markers related to insulin resistance did not show any significant improvement in the vitamin D treatment or supplementation groups compared to the control. Similarly, the expected improvement was not seen in FBG and RBG in 2-hr OGTT. This lack of improvement could be due to the low dose of Vitamin D that was used in our study for treatment as well as supplementation. An Iranian study involving 10–16 year old obese children with metabolic syndrome were supplemented with 300,000 IU of oral vitamin D weekly for 12 weeks and observed a significant reduction in fasting insulin and HOMA-IR, although a significant reduction in FBG was not observed [8]. A study done in Australia involving overweight adults showed that there was no improvement in insulin secretion or insulin sensitivity at a dose, which was higher than what we used [25]. In another study carried out in Turkey involving 10–18 year old obese children did not show significant improvement in blood sugars, insulin or HOMA-IR after supplementing with vitamin D 2000 IU/day for three months [26]. This lack of improvement in insulin resistance and glycaemic parameters with vitamin D supplementation is highlighted in a systematic review as well [27].

Interestingly, our study showed significant improvement in ALT levels following treatment or supplementation with vitamin D, and this improvement in the treatment group was significantly higher than the other two. Pre-post difference in the AST/ALT ratio also was significantly higher in the treatment group compared to the other two. Improvement in hepatic markers is an important aspect in the management of obese children, with the increase in prevalence of non-alcoholic fatty liver disease, and our positive finding in this regard is important because there is minimal evidence reported on this association. There was no significant change shown in the liver enzymes in the Turkish study [26], and a recent review also highlights the need for further evidence to support this effect of vitamin D supplementation [28].

According to our findings, HDL showed a statistically significant increase in all three groups. Other fractions of lipid profile did not show any improvement while the total cholesterol levels in the placebo
arm significantly deteriorated from the baseline value. Except for triglyceride levels none of the other components showed any improvement in Iranian children [8]. Total and LDL showed a significant reduction in Turkish children who were supplemented with vitamin D, but there was no change in HDL or triglyceride levels [26]. Evidence on the effect of vitamin D on the lipid profile has been inconclusive as highlighted by a review of 19 randomized controlled trials [29]. In this background, our findings on the improvement of HDL denotes that diet and physical activity play a major role in lipid control, as much as vitamin D supplementation.

In the vitamin D treatment arm, BMI, WC, SFT and %FM improved significantly, while some of these parameters improved in the supplementation and placebo arms as well. Improvement in the biceps and subscapular SFT was significantly higher in the treatment arm. These findings show that vitamin D treatment has superiority over supplementation and placebo. However, diet and physical activity still play a part in improving the body composition of obese children. Overall, according to our findings vitamin D appears to play a part in insulin function and also in improving liver functions, which is likely to be mediated via insulin. This mechanism is quite plausible since non-alcoholic steatosis is considered as the hepatic component of metabolic syndrome. The current study was conducted at the Lady Ridgway hospital, which is the largest tertiary care children’s hospital in the country, and the Obesity Clinic is attended by a majority of children residing in the Western Province belonging to all ethnic groups. Hence the findings of the current study can be generalized to urban children of Sri Lanka. The low response seen in most outcomes in the current study could be due the low dose that we have used compared to the Iranian study, which is evident by the fact that even in the therapeutic group the mean vitamin D levels did not rise above the deficiency cut-off value of 20 ng/ml. Our sample size, although calculated for the primary outcome, may not have been adequate for the multiple secondary outcomes.

Conclusions
This study shows that vitamin D in combination with diet and physical activity helps to improve insulin function and liver derangements as well as anthropometric and body composition parameters in obese Sri Lankan children. As VDD prevalence is high in obese Sri Lankan children, it could be used as
an adjunct therapy in the management. More research is needed using higher and different dosage regimens to identify the optimum regimen of vitamin D.

**Abbreviations**

AAP
American Academy of Paediatrics

ALP
Alkaline phosphatase

**List Of Abbreviations**

AAP – American Academy of Paediatrics

ALP - Alkaline phosphatase

ALT - Alanine aminotransferase

AST - Aspartate aminotransferase

BMI - Body mass index

FBG - Fasting blood glucose

FM - Fat mass

HbA1c – Glycosilated haemoglobin

HDL - High density lipoprotein

HOMA-IR - Homeostatic model assessment of insulin resistance

HOMA-IR – Homeostatic Model of Insulin Resistance

hs-CRP - High sensitivity C reactive protein

IDF - International Diabetes Federation

LDL - Low density lipoprotein

NCD – Non Communicable Disease

OGTT - Oral glucose tolerance test

PTH - Parathyroid hormone

RBG - Random blood glucose

SDS - Standard deviation score

SFT - Skin fold thickness
SPSS - Statistical Package for Social Sciences.

VDD – Vitamin D deficiency

WC - Waist circumference

Declarations

1. **Ethics approval and Consent to participate**

Informed written consent was obtained from parents. The Ethics Review Committee of Faculty of Medicine, University of Colombo, approved the study protocol (EC-15-010)

2. **Consent for publication**

Not applicable

3. **Availability of Data and Material**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

6. **Competing Interests**

Authors declare that there are no competing interests.

5. **Funding**

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7. **Author contributions**

**VPW:** conceived, designed, carried out and supervised the study, and wrote the manuscript

**SGSA:** conceived, carried out the study, analyzed data and wrote the manuscript

**DBDLS:** designed the study, analyzed data and wrote manuscript

**NA:** designed, carried out the study and wrote the manuscript

**KMDLDK, JTNS, HDJ:** carried out the study and analyzed data and wrote manuscript

All authors read and approved the final manuscript.

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Figures
Children with Vit. D deficiency identified at the cross sectional study (n=96)

Baseline assessment (Fasting blood glucose, OGTT (2h RBG), lipid profile, ALT, AST, fasting & post-prandial insulin, 25 OH levels, serum Ca and serum PTH levels, body fat mass, BMI, hs-CRP) done

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**Random allocation**

**Vitamin D Treatment group (n=32)**

- 25(OH) Vitamin D 50,000 IU weekly + Dietary and Physical activity modification

  - Followed up 6/12
  - Completed follow-up n=27
  - Lost to follow-up n=5

**Vitamin D Supplement group (n=33)**

- 25(OH) Vitamin D 2500 IU weekly + Dietary and Physical activity modification

  - Followed up 6/12
  - Completed follow-up n=25
  - Lost to follow-up n=8

**Placebo Control group (n=31)**

- Identical placebo weekly + Dietary and Physical activity modification

  - Followed up 6/12
  - Completed follow-up n=27
  - Lost to follow-up n=4

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**Outcome Assessment after 6months**

Fasting blood glucose, OGTT (2h RBG), lipid profile, ALT, AST, fasting & post-prandial insulin, 25 OH levels, serum Ca and serum PTH levels, body fat mass, BMI, hs-CRP
Figure 1

Participant flow from recruitment to completion of trial

Supplementary Files
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