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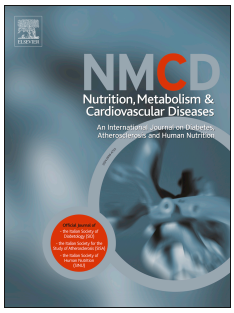

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Effect of vitamin D supplementation on microvascular reactivity in obese adolescents: A Randomized Controlled Trial

Short title: Vitamin D and vascular health in obese adolescents

Key words: Vitamin D / microcirculation / macrocirculation / obese children

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ABSTRACT

Background and Aim: Childhood obesity is associated with vitamin D (VD) deficiency and vascular dysfunction. Considering evidence indicates that VD may improve vascular function, this study, for the first time, assessed the effect of VD supplementation on microvascular reactivity in obese adolescents (OA). **Methods and Results**: This randomized controlled trial included 26 OA, receiving fruit juice with (n=13) or without VD (4000 IU/d; n=13) over a 3-month lifestyle program, as well as 23 normal-weight adolescents (controls). The primary outcome was the pre-to-post-program change in microvascular reactivity determined by laser speckle contrast imaging with acetylcholine and sodium nitroprusside iontophoresis. Changes in 25 hydroxyvitamin D (25(OH)D), flow-mediated dilation (FMD), nitrate-mediated dilation (NMD), insulin resistance (HOMA-IR) and inflammatory markers (C-reactive protein [CRP]) were monitored. At inclusion, in comparison to controls, OA exhibited lower total and free 25(OH)D, impaired microvascular responses, and impaired FMD, but similar NMD. After the lifestyle program, total and free 25(OH)D increased in all OA, with a greater increase in those receiving VD supplements. HOMA-IR and CRP decreased in all OA. Neither FMD nor NMD were altered in either group. Endothelium-dependent microvascular reactivity only increased in the VD-supplemented group, reaching values comparable to that of controls. Similar results were found when analyzing only OA with a VD deficiency at baseline. **Conclusion**: VD supplementation during a lifestyle program attenuated microvascular dysfunction in OA without altering macrovascular function. **Registration number for clinical trial:** NCT02400151.

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INTRODUCTION

The prevalence in paediatric obesity is a world-wide health issue that, while already burdening public health systems now, has the potential to put an enormous strain on global health resources in the not-so-distant future [1]. Indeed, obesity can have serious health consequences, especially for the vascular system, throughout childhood and adulthood [2]. Previous research has found that childhood obesity is associated with the early signs of endothelium-dependent and -independent vascular dysfunction, not only in the macrocirculation [3–6], but also in the microcirculation [7–9]. Interestingly, the microcirculation is increasingly recognized as an independent site in the development of vascular diseases [10]; collectively highlighting the need to evaluate changes in microvascular health in response to an array of lifestyle and dietary factors across all age groups.

In addition to being linked with vascular dysfunction, obesity in children is also associated with a lower concentration of vitamin D [11–13]. A vitamin D deficiency can be multifactorial, resulting from limited exposure to sunlight, low consumption of vitamin D-rich food products, larger vitamin D sequestration and/or altered metabolism in adipose tissue [14]. Interestingly, Vitamin D may have a direct role in promoting normal vascular function. Indeed, this vasoprotective property is supported by evidence demonstrating the presence of a vitamin D receptor and 1-α hydroxylase in vascular endothelial cells and vascular smooth muscle cells [15]. Moreover, there is evidence that nitric oxide synthase activity and nitric oxide production is decreased in experimental models where the endothelial vitamin D receptor is lacking [16].

Consistent with this position, vitamin D deficiency in adults has been associated with macrovascular endothelial dysfunction in cross-sectional studies [17,18]; and with microvascular endothelial dysfunction in nephropathy patients, with or without diabetes [19–

21]. However, observational studies evaluating the association between vitamin D deficiency and vascular dysfunction in obese children were not concordant [22–26]. Additionally, randomized clinical trials examining the macrovascular effect of vitamin D supplementation in adults have been inconclusive so far [27,28]. In obese children, only Javed *et al.* [25] and Rajakumar *et al.* [12] have reported that vitamin D supplementation has no impact on macrovascular endothelial function. In a recent meta-analysis in adults [29], based on only 3 trials [19,30,31], there was a small, significant positive-effect of vitamin D supplementation alone on microvascular function (SMD, 0.43; 95% CI, 0.09–0.76; P=0.01). To date, however, no study has investigated the effect of vitamin D on microvascular function in obese children.

While multidisciplinary weight loss interventions are recommended for paediatric obesity management and for the effective improvement of endothelial function in obese children [32], the potential synergistic effect of vitamin D supplementation combined with a weight-loss program remains unexplored in obese adolescents. Subsequently, the primary aim of this exploratory, double-blind, randomized controlled trial was to investigate the impact of vitamin D supplementation on microvascular reactivity in obese adolescents enrolled in a lifestyle program. Secondarily, this study also aimed to assess if the combined vitamin D-lifestyle intervention mediated any changes in macrovascular reactivity, insulin resistance and inflammatory status.

Materials and Methods

Study Population

The VIDADO trial (NCT02400151) is a clinical study designed to examine the effect of a lifestyle program, combined or not combined with vitamin D supplementation, on fat mass

and cardiovascular-metabolic risk factors in obese adolescents. Twenty-six obese adolescents, 12 to 17 years old, were enrolled in a paediatric weight management clinic in the south of France. Allowing for a case-control study, 23 puberty-matched, normal-weight volunteers were also recruited into the study. To avoid seasonal variations, all adolescents were assessed in either the period April 2015 to July 2015 (1st group) or in the period April 2016 to July 2016 (2nd group). All participants were asked to complete a questionnaire to assess their usual pattern of dietary intake by using a food-frequency questionnaire containing calcium and VD related nutrients. Written, informed consent was obtained from all adolescents and their parents prior to entering the study. The study protocol was conducted in accordance with the declaration of Helsinki.

Body mass index z-scores were calculated. Values greater than 3 defined severe obesity. Exclusion criteria for this study were: history or presence of premature cardiovascular or metabolic disease in a first-degree family member, a current smoking status, regular intake of any medication, pubertal status (less than Tanner stage 2) and current vitamin D supplementation. For obese adolescents, there was specific exclusion criterion, namely weight loss greater than 5% of total body weight during the previous three months. Normal weight controls were excluded if their body mass index was above the 90th percentile for gender and age. To minimize the potential moderating effects of physical activity, adolescents who participated in extra-school sport activities more than three hours per week were also not included.

Obese adolescents were randomized into two groups, those who received 200 mL of fruit juice every morning without a vitamin D supplement (n=13) and those who received 200 mL fruit juice every morning with a vitamin D supplement (n=13; 4000 IU/d of Vitamin D_3 UVEDOSE 100000 IU/2 mL, Crinex laboratory, France) [33]. The hospitalized adolescents were instructed to drink the fruit juice each morning during breakfast, witnessed by the

dietician; ensuring 100% compliance to the study intervention. All obese adolescents entered a three-month lifestyle program. Two of the obese adolescents who were in the group receiving vitamin D supplements left the clinic before the end of the lifestyle program for personal reasons and were, subsequently, removed from the study (at three months, n=11). Throughout the clinic-based lifestyle program, obese adolescents received both standard and personalized meals prescribed by dieticians on a daily basis. The meals were based on a balanced distribution of macronutrients: carbohydrates (55%), proteins (15%) and lipids (30% total, with less than 10% saturated fat). Their total daily food intake was calculated to enable them to reach a negative energy balance of 500 kcal/day. During the three-month lifestyle program, aerobic training consisted of 180 minutes per week (three sessions of 60 min about 300 kcal per training session) of supervised moderate-to-vigorous intensity exercise as previously described [34].

Demographic, clinical and biological parameters

Measurement of anthropometrics and biological blood markers were performed at baseline, prior to the beginning of the lifestyle program, in both obese adolescents and normal weight controls, as well as after the three-month program in obese adolescents only. To account for the effect of sun exposure, measurement of 25-hydroxyvitamin D (25(OH)D) concentrations was also performed after the three-month program in normal-weight controls. Body composition was assessed by dual-energy x-ray absorptiometry. Blood samples were collected to assess the levels of fasting plasma glucose, plasma insulin, high-sensitive C-reactive protein (CRP) and 25(OH)D concentrations. Serum total 25(OH)D concentrations were determined by the electrochemiluminescence immunoassay (ECLIA) method, using Roche Diagnostics kits (Roche Elecsys vitamin D total). The lower limit for detection using this method is a concentration of 7.5 nmol/L. The coefficient of variation for each 25(OH)D

concentration intra-assay was <7%. Serum Free 25(OH)D was quantified by ELISA (DIAsource ImmunoAssays, Louvain-La-Neuve, Belgium). Concentrations of serum CRP were measured according to the manufacturer's specifications using commercially available ELISA kits purchased from R&D systems (Minneapolis, USA). Insulin resistance was estimated by the calculation of the homeostasis model assessment of insulin resistance (HOMA-IR) index. Blood pressure was measured on the left arm by an automated system (Dinamap, GE Medical Systems, Milwaukee, USA).

Vascular measurements

Macro- and microvascular assessments were performed at baseline, prior to the beginning of the lifestyle program, in both obese adolescents and normal weight controls; and after the three-month program in obese adolescents only. The adolescents presented to the vascular assessments at least three hours fasted, having abstained from caffeine consumption and strenuous exercise for the 24 hours prior. Vascular assessments were conducted after the adolescents rested in a supine position for 20 minutes, in a room maintained at 22-24°C. Indeed, the adolescents remained in a supine position throughout the micro- and macrovascular reactivity assessments. All vascular measurements and off-line analyses were performed by two observers (A.V. and C.M.) blinded to group allocation.

Assessments of microvascular reactivity were performed by one investigator (C.M.). Prior to starting the assessments, the arm was immobilized with a vacuum cushion. Changes in cutaneous microvascular blood perfusion were recorded on the ventral face of the forearm skin using a laser speckle contrast imaging system with a laser wavelength of 785nm (PeriCam PSI System®, Perimed, Järfälla, Sweden). Briefly, laser speckle contrast imaging calculates microvascular blood perfusion by detecting the random speckle pattern generated by the laser light illuminating the moving red blood cells in the skin tissue. The sampling

frequency was 18Hz and the distance between the laser head and skin surface was fixed at 15cm (Mahé et al., 2011). The size of the region of interest was approximately 35mm² and it was positioned in the middle of the electrode. The perfusion values from the skin were calculated using the manufacturer's software (PimSoft 1.2.2.0®; Perimed, Järfälla, Sweden).

Microvascular reactivity was challenged using transdermal iontophoresis of acetylcholine and sodium nitroprusside, allowing for the assessment of endothelium-dependent and endothelium-independent vasodilation, respectively. Transdermal iontophoresis is a non-invasive method that can move vasoactive drugs, electrically charged molecules, through to the skin blood vessels by use of a device emitting a weak electrical current (PF 751 PeriIont systems, Perimed, Järfälla, Sweden). As recommended by Loader *et al.* [36], acetylcholine was administered with an anodal current of 0.02 mA for 200s and sodium nitroprusside was administered with a cathodal current of 0.02 mA for 400s concurrently at separate sites. Basal cutaneous blood perfusion was measured by laser speckle contrast imaging for two minutes prior to the beginning of acetylcholine and sodium nitroprusside iontophoresis (200 µl of 1% solution dissolved in sodium chloride). Indeed, cutaneous blood perfusion was also recorded throughout iontophoresis until there was a plateau in the vascular response to the vasoactive drug being administered. Peak cutaneous blood perfusion values were averaged over a 10 second period at the maximal plateau. The cutaneous blood perfusion responses were expressed in conventional perfusion units (PU) and as the maximum percentage change from baseline.

Macrovascular reactivity was assessed at the brachial artery by the same investigator (A.V.) across all assessments, according to the International Brachial Reactivity Task Force Guidelines and the expert consensus 2019 [37,38]. Brachial artery measurements were captured using high-resolution vascular ultrasonography (MyLab30, Esaote SpA, Firenze, Italy) with a 10-MHz multi-frequency linear probe. B-mode images and Doppler signals were

continuously and simultaneously recorded for off-line analysis. All results were calculated as the average of five consecutive measurements. To challenge macrovascular endothelium-dependent reactivity, flow mediated dilation (FMD) was performed concurrently to the ultrasonography recordings. Briefly, a pneumatic cuff was put on the right forearm below the elbow. The ultrasound probe was placed approximately midway between the antecubital and axillary regions before the brachial artery diastolic lumen diameter was measured. The cuff was then inflated to 250 mm Hg for five minutes before the cuff was rapidly deflated to induce post-ischemic hyperemia, at which point in the assessment the brachial artery diastolic lumen diameter was measured again.

Fifteen minutes after FMD concluded, baseline measurements of the brachial artery diastolic lumen diameter were repeated before 0.4 mg of isosorbide dinitrate (Isocard, Schwarz Pharma, Monheim, Germany) was given sublingually to assess endothelium-independent vasodilation (i.e. nitrate-mediated dilation, NMD). This procedure has been previously described in detail elsewhere [5,39]. Both FMD and NMD were expressed as the percentage change in peak diastolic brachial artery lumen diameter after reactive hyperemia and exogenous organic nitrate administration, respectively, relative to the baseline diastolic diameter. Time-averaged mean blood flow velocity and blood flow were determined. Shear rate (s^{-1}) was calculated as 4 × time-averaged mean blood flow velocity/mean brachial diameter, to estimate peak shear stress [40]. Within-subject coefficient of variations in our laboratory at rest were 1.8% for arterial diameters and 12.7% for FMD [41].

Statistical analyses

Statistical analyses were performed with SPSS® software (IBM Corp, Armonk, NY). The primary outcome variable was the percentage change in microvascular blood perfusion in response to acetylcholine iontophoresis. The sample size was calculated by considering a type

I error rate of 5% (2-tailed) and a power of 90% based on the results from Dreyer *et al*. [19]. Calculations indicated that a sample size of eight participants was required in each group to achieve statistical power. Due to the small sample size (<30), non-parametric statistical tests involving median comparisons were performed. Firstly, Mann-Whitney Tests were performed to check for differences between obese adolescents and normal weight controls at baseline. Additionally, Mann-Whitney tests were performed to check for baseline differences between the group of obese adolescents receiving vitamin D supplements and those who were not. Wilcoxon Signed-Rank tests were used to compare the effect of the lifestyle program combined with vitamin D supplementation to the effects of the lifestyle program alone. Spearman correlations were used to identify associations between baseline values and any change in vascular variables and 25(OH)D. Accordingly, the change in outcomes between groups was compared using an alternative non-parametric ANCOVA [42] using ranked change from baseline variability and ranked the baseline HOMA-IR values. For follow-up analysis, a subgroup analysis with only the 18 obese adolescents deficient in vitamin D at baseline was also performed.

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RESULTS

The main clinical characteristics of the adolescents are presented in Table 1. At baseline, as expected, obese adolescents had a significantly higher body mass index, body mass index-Z score and fat mass than normal weight controls. Obese adolescents also had significantly lower total and free 25(OH)D concentrations, higher systolic and lower diastolic blood pressures, a greater HOMA-IR index and higher CRP levels than normal weight controls. Vitamin D deficiency (defined by 25(OH)D concentration < 50 $nmol.L^{-1}$) [43] was detected in

73% (18/26) of the obese adolescents and in only 22% (5/23) of the normal weight controls. A 3-day dietary recall and food frequency questionnaire was administered to quantify the adolescent's vitamin D food sources (e.g. egg yolk, fatty fishes, fortified dairy products and mushrooms). The median intake was 1.6 microg (min: 0.82 max: 7.41). Only, three obese adolescents had an intake up to 5 microg, equivalent to 200 UI, which is three times less than the recommendation from the Institute of Medicine (600 UI); confirming inadequate dietary intake of VD in obese adolescents. Between the two subgroups of obese adolescents, those who were not receiving vitamin D supplements and those who were, there were no differences in age or in any of the clinical or biological parameters, except for HOMA-IR and diastolic blood pressure, which was higher and lower, respectively, in those consuming vitamin D supplements.

While peak shear rate and NMD did not differ between groups, obese adolescents had a lower FMD than normal weight controls (Table 2). Noting that baseline cutaneous blood perfusion values did not differ between obese adolescents and normal weight controls, the microvascular dilatory response to acetylcholine and sodium nitroprusside iontophoresis, expressed as a percentage increase from baseline, was also lower in obese adolescents. Prior to entering the lifestyle program, there was no difference in any of the parameters of macro- and microvascular reactivity between those randomized to receive vitamin D supplements and those who were not. Concentrations of total 25(OH)D were correlated with acetylcholine-mediated increases in cutaneous blood perfusion in the obese adolescents with a vitamin D deficiency at baseline (n=18, r=0.582, p=0.002).

After the three-month lifestyle program, total and free 25(OH)D increased while body mass index, HOMA-IR and CRP decreased in those who were receiving vitamin D supplements, as well as in those who were not (Table 3). In normal-weight adolescents, after three months, an increase in 25(OH)D was also observed (+20%, data not shown). Vitamin D

deficiency was attenuated (i.e. Vitamin D concentrations raised above 50 nmol/L) in all obese adolescents receiving vitamin D supplements and in 70% (9/13) of those who were not. Moreover, the increase in total 25(OH)D after adjustment for ranked baseline HOMA-IR index, was significantly greater in obese adolescents receiving vitamin D supplements (Table 4).

The vasodilatory response to acetylcholine iontophoresis increased in those receiving vitamin D supplements to a point where there was no longer a difference in microvascular reactivity between those obese adolescents and the normal weight controls. Interestingly, the same improvement in vascular reactivity, was not observed in the obese adolescents who completed the lifestyle program but did not receive vitamin D supplements (Figure 1). Indeed, the increase in endothelium-dependent and endothelium-independent microvascular reactivity after adjustment for ranked baseline HOMA-IR index, was significantly greater in obese adolescents receiving vitamin D supplements. The post-lifestyle program increases in the microvascular responses to acetylcholine tended to be correlated with the increase in 25(OH)D ($r=0.38$, $p=0.09$). Neither FMD nor NMD changed in either of the subgroups of obese adolescents (Figure 2). The results remained the same even when performing a subgroup analysis that included only the 16 obese adolescents with a vitamin D deficiency at baseline.

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DISCUSSION

The main finding of this exploratory, double-blind, randomized controlled trial is that vitamin D supplementation during a lifestyle program in obese adolescents improved endothelium-dependent microvascular reactivity without any effect on macrovascular function. This result

suggests that vitamin D is beneficial to microvascular health, furthering its importance from a paediatric health standpoint.

In accordance with previous research, this study confirmed that vitamin D deficiency and obesity are interrelated. Indeed, similarly to Ganji *et al*. [44], more than 70% of the obese adolescents included in this study had a vitamin D deficiency prior to entering the lifestyle program. The findings from the assessments of macrovascular reactivity in this study also supported those of previous research [4,45], even when discrepancies were reported [5,46]. In addition to confirming an impairment in endothelium-dependent macrovascular reactivity in obese adolescents, this study also demonstrated an impairment in both endothelium-dependent and endothelium-independent microvascular reactivity; supporting data from previous studies in obese children [9].

Whether vitamin D deficiency and vascular dysfunction are associated in obese children has remained hitherto inconclusive. In this present study, there was no relationship between total or free 25(OH)D and FMD in all obese adolescents, supporting findings of previous studies [22,25,26] . In contrast, the significant correlation between total 25(OH)D and acetylcholine-mediated vasodilation in vitamin D-deficient obese adolescents at baseline was a major and novel finding. Vitamin D may have a specific mechanism of action on microcirculation [19], altering the structure and arrangement of the microvasculature by binding to the endothelium's vitamin D receptor and stimulating the synthesis of nitric oxide, a powerful vasodilator; and by reducing the production of reactive oxygen species that decrease nitric oxide bioavailability [47].

The effects of vitamin D supplementation in obese children are still poorly understood [48], especially when considering its impact on vascular function. The lifestyle program in this study, consisting of diet control, exercise and vitamin D supplementation, coincided with an improvement in total and free 25(OH)D concentrations in all obese adolescents,

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presumably due to both a possible release of VD during weight loss and to sun exposure. Interestingly, the sole weight-loss program improved the VD status in obese adolescents not receiving VD supplements, as previously reported [49,50]; noting that this study was carried in the south of France during the spring and that the increased sun exposure would also have contributed to endogenous VD biosynthesis in the obese adolescents (i.e +20% in normal-weight). The weight-loss program also improved HOMA-IR with the greatest enhancement obtained in the obese adolescent receiving VD supplements. The role of vitamin D in the regulation of insulin sensitivity has been highlighted [51]. However, Nader *et al.* [52] found that vitamin D supplementation has no effect on HOMA-IR in obese adolescents. There were also distinctive changes in vascular reactivity, dependent on the artery type. Indeed, there was no change in macrovascular reactivity. A recent meta-analysis in adults reported that vitamin D supplementation alone had no effect on endothelial function [28,29]. The only two studies in obese children that have investigated the effect of vitamin D supplementation on macrovascular function confirmed this lack of effect [12,25]. The null effect of the combined lifestyle program (diet and exercise with vitamin D supplementation) on macrovascular function was a novel finding in this present study.

In contrast, this clinical trial demonstrated an improvement in endothelium-dependent microvascular reactivity after the three-month lifestyle program in obese adolescents receiving vitamin D supplements. These vascular data suggest that physiological differences in the control of vasomotion between small and large blood vessels may underpin the difference in the response to vitamin D supplementation [29]. The lifestyle program combined with vitamin D supplementation resulted in a significant increase in endothelium-dependent microvascular reactivity. In a recent meta-analysis in adults that included three studies that assessed the microcirculation [29], it was reported that vitamin D supplementation alone had a small, but significant benefit, for microvascular reactivity (SMD, 0.43; 95% CI, 0.09–0.76;

P=0.01) [19,30,31]. The clinical significance of this result is not clear. The increase in microvascular function was not associated with decreased inflammation state, body mass index nor HOMA-IR after the weight-loss program. Nevertheless, even if there were no associations between those parameters, it cannot be excluded that the lifestyle intervention could have a mediating role in the effect of VD on microvascular reactivity. Indeed, it is not well understood how increases in the concentration of 25(OH)D could improve vascular function and it is beyond the scope of this study's data to expand on the current body of mechanistic knowledge.

This study is strengthened by its double blind, randomized controlled design, by administering vitamin D supplements in accordance with the international guidelines reported in Saggese *et al.* [53], by the 100% compliance rate in obese adolescents and by the assessment of both microvascular and macrovascular reactivity. However, the present study has several limitations. The relatively small sample size included in this study limits extrapolation of any findings to a wider population. Unfortunately, 1,25(OH)2D, and the synthesis of vitamin D–binding proteins were not monitored in this study. Furthermore, no parathyroid hormone concentrations were available. Additionally, vascular comparisons with normal weight adolescents were only completed at baseline and not after the three-month lifestyle program. Finally, it remains unclear what proportion of influence each interventional factor, diet, exercise or vitamin D supplementation, had on the positive outcomes in 25(OH)D concentration and vascular profiles reported in this study.

In conclusion, our data indicate for the first time that daily vitamin D supplementation in conjunction with a lifestyle program, which controls calory intake and exercise habits, can significantly improve microvascular function in obese adolescents. Considering the small sample of obese adolescents enrolled in this trial, future research needs to confirm the findings of this study in a larger population before vitamin D supplementation can be

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recommended in a lifestyle program to counteract microvascular impairment in childhood obesity.

Conflict of interest: Authors have no conflicts of interest to disclose.

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FIGURE LEGENDS

Figure 1: The percentage increase in cutaneous blood perfusion from baseline measurements following iontophoresis with A) acetylcholine (ACh) and B) sodium nitroprusside (SNP), before and after the intervention in both subgroups of obese adolescents, those who were receiving vitamin D supplements (OS) and those who were not (ONS).

Figure 2: A) flow-mediated dilation (FMD) and B) nitrate-mediated dilation (NMD) before and after the intervention in both subgroups of obese adolescents, those who were receiving vitamin D supplements (OS) and those who were not (ONS).

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Table 1: Baseline characteristics of the participants

Characteristics	All obese adolesents (n=26)	Obese adolescent subgroups		P value	Normal-weight	P value
		OS (n=13)	ONS (n=13)	OS vs ONS	(n=23)	All obese vs NW
Demographics						
Age-yr	14.2 (13.6-14.8)	14.6 (13.6-14.8)	14 (13.1-16.7)	.959	14.9 (14.2-15.3)	0.193
Sex (M-F)	10-16	5-8	5-8	1.0	10-13	.728
Clinical parameters						
BMI (kg/m^2)	33.5 (31.2-36.2)	33.8 (31.3-36.5)	32.7 (31.1-36.3)	0.614	19.2 (18.5-20.2)	**<.0001**
BMI Z-score	4.0 (3.5-4.5)	3.9 (3.4-4.7)	4.0 (3.4-4.6)	0.959	0.1 (-0.3-0.7)	**<.0001**
Fat mass (%)	41.4 (39.8-43.2)	41.9 (39.8-43.6)	41.1 (36.5-44.3)	0.555	23.2 (18.4-27.7)	**<.0001**
SBP (mmHg)	112 (106-120)	112 (106-123)	113 (106-120.5)	0.797	105 (102-109)	**<.001**
DBP (mmHg)	66 (62-68)	59 (57-60)	61 (59-68)	**0.039**	67 (63-69)	**.006**
Biological parameters						
Total 25(OH)D (nmol/L)	38 (35.4-47.8)	39 (38-55.8)	34.5 (32-58.6)	0.191	62 (55-68.3)	**<.0001**
n deficiency in 25(OH)D < 37.5 nmol/L	8	1	7	-	0	-
n insuficiency in 25(OH)D < 50 nmol/L	18	9	9	-	5	-
Free 25(OH)D (pg/mL)	4.1 (1.9-5.3)	4.1 (1.9-5.3)	4.1 (2.4-4.8)	0.918	4.8 (2.6-6.2)	**.006**
HOMA-IR	4.0 (3.3-5.6)	5.6 (3.5-6.7)	3.4 (2.3-4.0)	**0.01**	2.23 (1.4-2.8)	**<.0001**
CRP (mg/mL)	6.1 (3.3-10.1)	7.9 (3.6-13.7)	4.4 (2.3-10.1)	0.331	0.3 (0.2-0.9)	**<.0001**

BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment-insulin resistance; OS, obese participants receiving vitamin D supplements; ONS, obese participants not receiving vitamin D supplements; SBP, systolic blood pressure; 25(OH)D, 25 hydroxyvitamin D.

Data are expressed as median and 95%CI for the median.

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Table 2: Baseline vascular characteristics of the participants

Vascular Parameters	Normal-weight (n=23)	All obese (n=26)	P value All obese vs NW	Obese adolescents subgroups OS (n=13)	ONS (n=13)	P value OS vs ONS
Macrocirculation						
FMD (%)	9.60 (7.69-12.93)	6.66 (5.13-8.09)	**0.015**	6.06 (3.22-10.52)	6.89 (5.19-8.77)	0.442
Peak shear rate (s^{-1})	637.8 (569.0-753.5)	587.1 (507.6-660.2)	0.206	598.7 (490.7-737.8)	578.2 (406.7-763.1)	0.699
FMD/deltaSR (%.s^{-1})	0.019 (0.015-0.036)	0.014 (0.011-0.023)	0.141	0.012 (0.007-0.024)	0.019 (0.009-0.039)	0.349
NMD (%)	22.25 (18.18-29.21)	19.35 (15.41-22.38)	0.107	16.61 (12.24-20.90)	20.34 (15.80-26.24)	0.061
Microcirculation						
Resting CBF (PU)	34.0 (28.0-38.0)	36.2 (34.2-41.7)	0.119	36.2 (32.9-41.4)	38.1 (31.2-45.5)	0.852
Peak ACh CBF (PU)	95.8 (69.7-100.2)	69.3 (64.7-81.5)	0.075	68.7 (55.8-76.0)	74.2 (62.4-106.0)	0.198
ACh CBF increase (%)	150.0 (121.3-187.2)	113.3 (69.6-136.5)	**0.008**	80.7 (40.5-134.3)	124.8 (77.6-148.7)	0.198
Peak SNP CBF (PU)	78.4 (69.7-106-4)	71.7 (60.4-76.1)	0.064	62.4 (45.8-73.8)	75.5 (59.7-87.2)	0.119
SNP CBF increase (%)	136.0 (86.7-170.9)	94.6 (42.5-123.8)	**0.033**	53.3 (36.2-117.9)	120.4 (72.5-153.1)	0.080

Ach, acetylcholine; CBF, cutaneous blood flow; FMD, flow-mediated dilation; NMD, nitrate-mediated dilation; OS, obese participants receiving vitamin D supplements; ONS, obese participants not receiving vitamin D supplements; SNP CVC, sodium nitroprusside cutaneous vascular conductance; SR, shear rate.

Data are expressed as median and 95%CI for the median.

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Table 3: Changes in characteristics in the obese adolescents after completing the 3-month lifestyle intervention

	OS (n=11)		ONS (n=13)	
Outcome variables	Baseline	3-months	Baseline	3-months
Total 25(OH)D (nmol/L)	39 (38-55.8)	70 (59.8-91.7)***	34.5 (32-58.6)	60 (40.2-70.5)***
Free 25(OH)D (pg/mL)	4.1 (1.9-5.3)	6.6 (4.4-8.3)**	4.1 (2.4-4.8)	5.5 (4.1-8.1)**
BMI (kg/m^2)	33.8 (31.3-36.5)	31.6 (28.7-33.1)***	32.7 (31.1-36.3)	30.1 (29.2-32.7)***
HOMA-IR	5.6 (3.5-6.7)	3.3 (2.9-4.2)**	3.4 (2.3-4.0)	2.3 (1.4-3.1)*
CRP (mg/mL)	7.9 (3.6-13.7)	6.5 (1-7.9)*	4.4 (2.3-10.1)	1.8 (0.7-2.5)**

BMI, body mass index; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment-insulin resistance; OS, obese participants receiving vitamin D supplements; ONS, obese participants not receiving vitamin D supplements; 25(OH)D, 25 hydroxyvitamin D. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ between baseline and 3-months.

Data are expressed as median and 95%CI for the median.

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Table 4 : Changes in biological characteristics within and between the two subgroups of obese adolescents, those who were receiving vitamin D supplements and those who were not

Parameters	OS		ONS		p value for the difference between groups#
	median (IQ)*	p value for the difference within group†	median (IQ)*	p value for the difference within group†	
Total 25(OH)D (nmol/L)	22.0 (18.6, 37.2)	**p=0.003**	16.0 (7.2 , 26.3)	**p=0.002**	**p=0.030**
HOMA-IR	-2.7 (-4.5, -0.5)	**p=0.008**	-0.8 (-1.3, 0.07)	**p=0.019**	**p=0.005**
CRP (mg/mL)	-3.5 (-6.8, -0.5)	**p=0.038**	-2.5 (-7.5, 0.9)	**p=0.004**	p=0.884
FMD (%)	0.0 (-3.2, 3.5)	p=1	0.24 (-3.5, 4.8)	p=0.463	p=0.904
NMD (%)	0.15 (-3.6. 3.6)	p=0.953	3.7 (-0.8. 7.0)	p=0.099	p=0.269
ACh CBF increase (%)	49.3 (34.7-133.2)	**p=0.039**	7.9 (-13.9-70.2)	p=0.469	**p=0.002**
SNP CBF increase (%)	49.9 (-24.2-141.9)	p=0.105	-10.6 (-37.0-49.3)	p=0.831	**p=0.035**

CRP, C-reactive protein; FMD, flow-mediated dilation; HOMA-IR, homeostasis model assessment-insulin resistance; IQ, interquartile range; OS, obese participants receiving vitamin D supplements; ONS, obese participants not receiving vitamin D supplements; 25(OH)D, 25 hydroxyvitamin D. * Difference, 3 month follow-up minus baseline; † p values are based on the Wilcoxon signed-rank test to compare the baseline and 3 months values within each group; # p values are based on fitting an analysis of non-parametric ANCOVA using ranked change from baseline variability and ranked baseline HOMA-IR values to compare the changes between the two groups.

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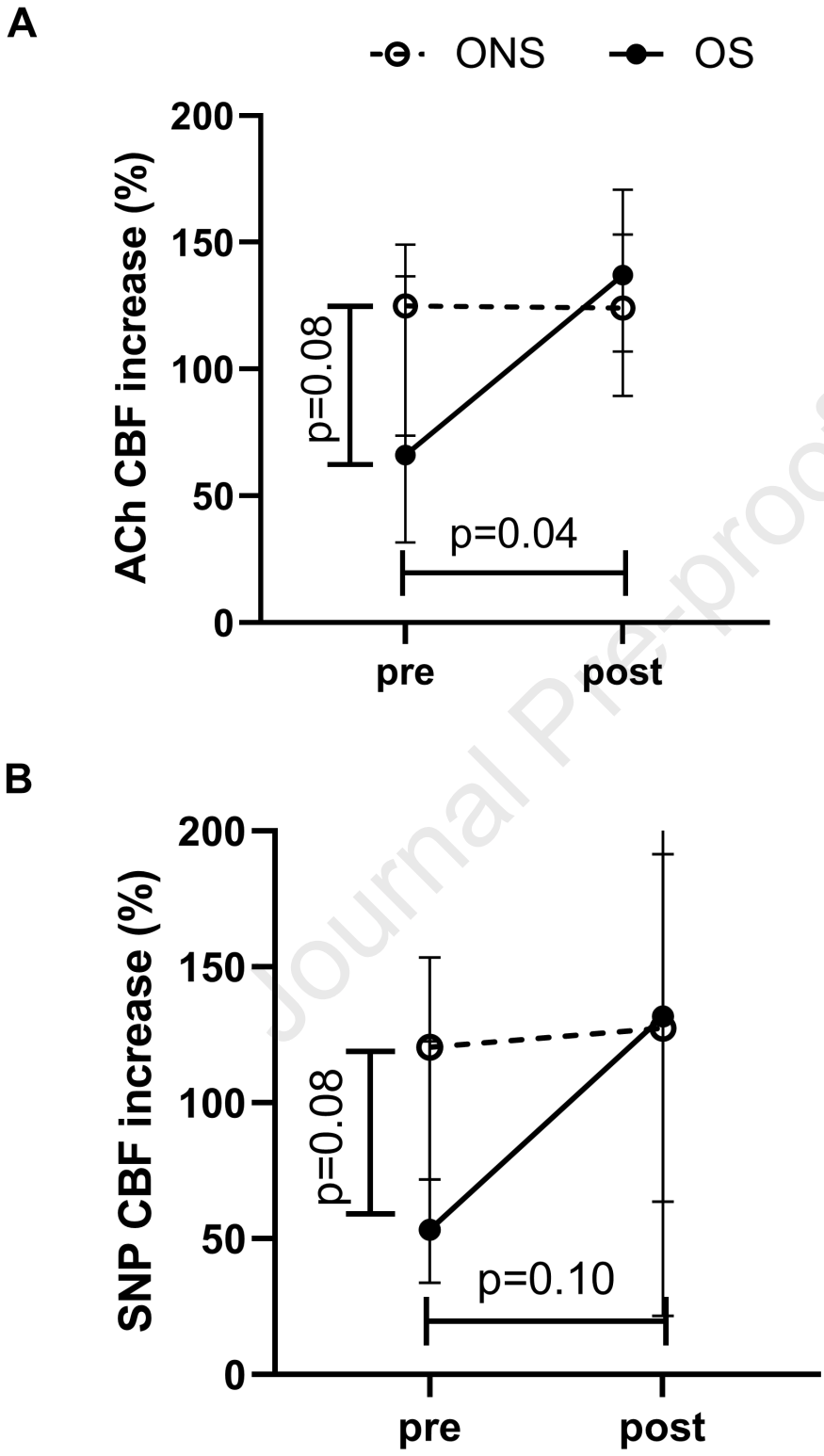

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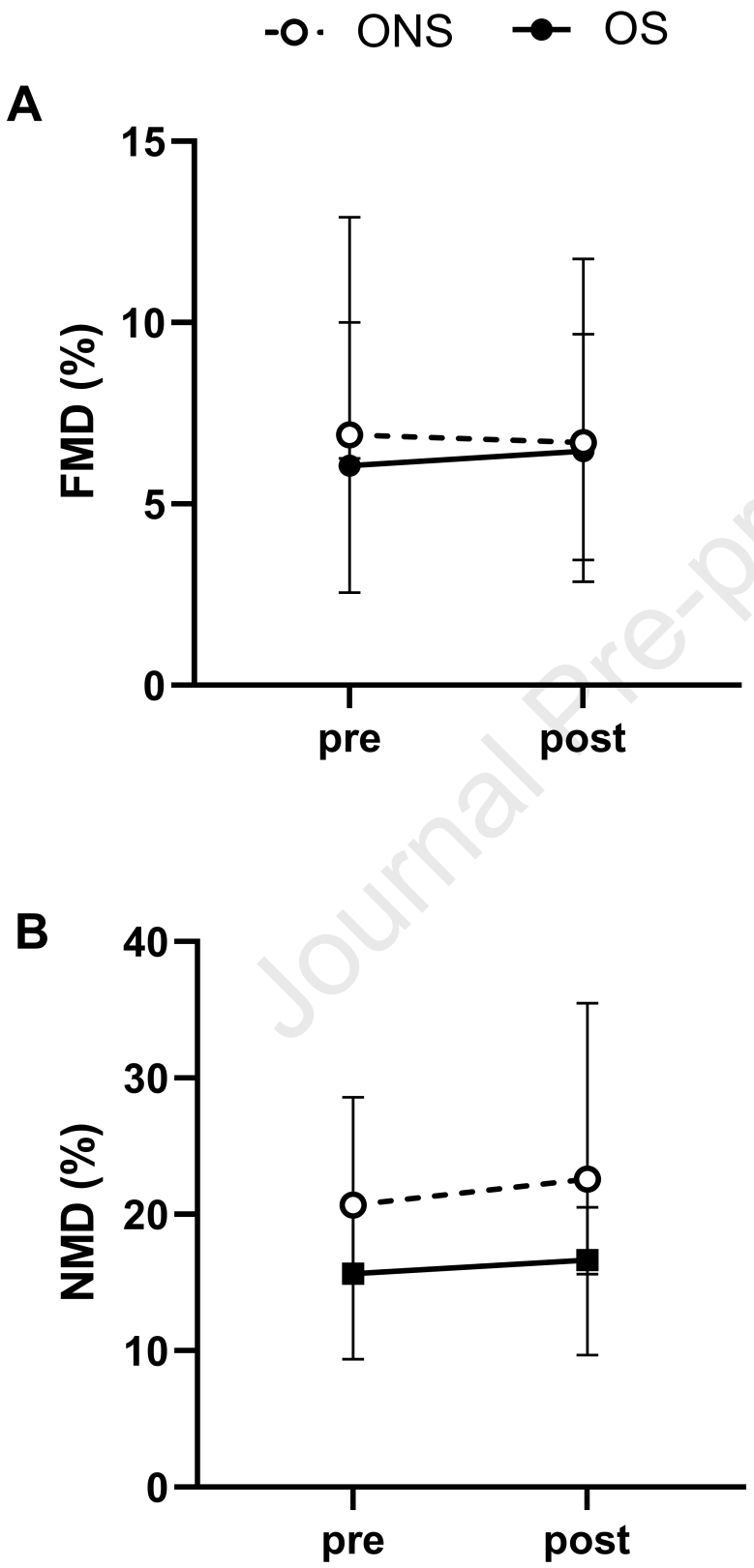

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Highlights

- VD deficiency is associated with endothelial dysfunction in obese adolescents.
- VD supplementation improved microvascular function in obese adolescents.
- VD supplementation had no effect on macrovascular function in obese adolescents.

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