Title page

Effect of vitamin D supplementation on cardiometabolic factors and inflammatory status in adolescents with obesity enrolled in a weight-loss program.

Authors: Morrissey Christopher[1,2]; Perez-Martin Antonia[3]; Defoort Catherine[2]; Raverdy Cecile[4]; Masson Delphine[4]; Tardivel Catherine[2]; Sandrine Gayrard[1], Aurélie Goncalvez[5]; Myriam Carrere[6], Landrier Jean-François[2], Vinet Agnes[1*], Amiot Marie Josèphe[6*]

Authors' affiliations

[1] LaPEC, University of Avignon, France

[2] C2VN, AMU, INRA, INSERM, Marseille, France

[3]Vascular Medicine and Laboratory, Nimes University Hospital, France

[4] SSR Institut Saint Pierre, Palavas-les-Flots, France

[5] Chrome Unit, EA 7352, University of Nîmes, France

[6] MOISA, INRA, CIRAD, CIHEAM, SupAgro, Univ Montpellier,

Corresponding author:

* Agnès VINET, Avignon University, LAPEC EA4278, 74 rue Louis Pasteur, 84000 AVIGNON, France

Tel: +334 90 16 29 30; Fax: + 334 90 16 29 01 agnes.vinet@univ-avignon.fr.

* Marie Josèphe Amiot, PhD, MOISA, Univ Montpellier, INRA, CIRAD, CIHEAM-IAAM, Montpellier SupAgro, 2 place Viala, 34060 Montpellier, France +334 99 61 22 46; +334 67 54 58 05 (fax) marie-josephe.amiot-carlin@inra.fr.

Sources of support: This research was supported by grants from Nimes University Hospital (France).

Short running head*:* Vitamin D supplementation during weight loss in children

 doi:10.20944/preprints201908.0105.v1

List of abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; hs-CRP, ultrasensitive C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance index; IL-6, interleukin 6; LDL-C, high-density lipoprotein cholesterol; MBP, mean blood pressure; OS, obesity group supplemented with vitamin D; ONS, obesity group non-supplemented with vitamin D; PTH, parathyroid hormone; SBP, systolic blood pressure; SD, standard deviation; TG, triglycerides; TNF, tumor necrosis factor; VD, vitamin D.

Clinical Trial Registration: NCT02400151, https://clinicaltrials.gov/.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

Abstract

Obesity in children is associated with vitamin D (VD) deficiency and cardiometabolic abnormalities. To analyze the effects of VD supplementation in adolescents with obesity enrolled in a weight-loss program. Adolescents with obesity (n=26) and with normal weight (n=23; controls) were matched for age, sex, and puberty stage. The obesity group followed a 3-month weight-loss program that combined a reduced caloric intake with interval training physical activity and during which they received or not VD supplementation (4000 IU/d) (n=13/group; random assignation). The anthropometric parameters (BMI z-score, fat mass); serum levels of 25(OH)D, calcium and parathyroid hormone (PTH), cardiometabolic factors (triglycerides, HDL, and LDL cholesterol), fasting glucose and insulin, and homeostasis model assessment of insulin resistance index; diastolic, systolic and mean blood pressure, and inflammatory status (C-reactive protein, CRP) were measured at baseline and at the end of the 3-month program. At baseline, 25(OH)D concentration was lower and VD insufficiency (25(OH)D levels <50 nmol/L) rate was higher (73% vs 22%) in the obesity than in the normal-weight group. All cardiometabolic factors were altered in the obesity compared with the normal-weight group. After the 3-month weight-loss program, 25(OH)D levels was >50 nmol in all adolescents with obesity, but only in 46% of normal-weight adolescents. Moreover, the weight-loss program improved the cardiometabolic factors, inflammatory status (CRP) and physical performance, but VD supplementation did not have any additional effect. Analysis only of the adolescents with obesity and VD deficiency (25(OH)D <50 nmol/L) at baseline showed a significant correlation between the change in PTH and CRP (p=0.02) in the supplemented obesity group, while the increase in 25(OH)D only tended to be correlated with CRP decrease. Vitamin D supplementation could reduce VD insufficiency in adolescent with obesity, but does not have any additional effect on cardiometabolic factors when combined with a weight-loss program.

 doi:10.20944/preprints201908.0105.v1

Key words: lifestyle program, 25-hydroxyvitamin D, parathyroid-hormone, anthropometry, cardiometabolic factors

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

Introduction

Vitamin D (VD) deficiency and obesity are two major health concerns worldwide [1].The increasing prevalence of early childhood overweight and obesity raises major public health issues, and lifestyle measures need to be implemented to avoid comorbidities. Childhood obesity is strongly linked to cardiovascular risk factors, dyslipidemia, insulin sensitivity, inflammatory, and oxidative stress profiles [2][3]. To date, few studies have investigated the potential positive effects of VD on the cardiometabolic risk factors in children and adolescents with obesity, with varying conclusions. Javed and al. [4] showed no effect of VD supplementation (2000 IU maximal dose) on insulin activity in non-diabetic adolescents with obesity without VD insufficiency. On the other hand, Belenchia and al. [5] found that 4000 IU VD3 corrected VD insufficiency and improved insulin sensitivity in adolescents with obesity.

The classical approach for weight management in childhood obesity includes diet and physical activity [6][7][8]. In children with obesity, weight-loss programs (diet and/or exercise training) reduce body fat mass and inflammation [9] improve insulin sensitivity, and also the lipid profile [10][11][12]. Moreover, two literature reviews concluded that weight management through diet alone or with physical activity leads to normalization of 25-hydroxyvitamin D (25(OH)D) level in adults with obesity [13][14]. Data on childhood obesity are still limited [15][16]. We hypothesized that a weight loss intervention combining also VD supplementation could result in additional health benefits in adolescents with obesity.

The present double-blind, randomized, placebo-controlled clinical study evaluated the possible added effects of VD supplementation on body composition, aerobic fitness, and cardiometabolic risk factors (e.g., blood pressure, insulin resistance, lipid and inflammation profiles) in adolescents with obesity enrolled in a weight-loss program that included diet and physical exercise.

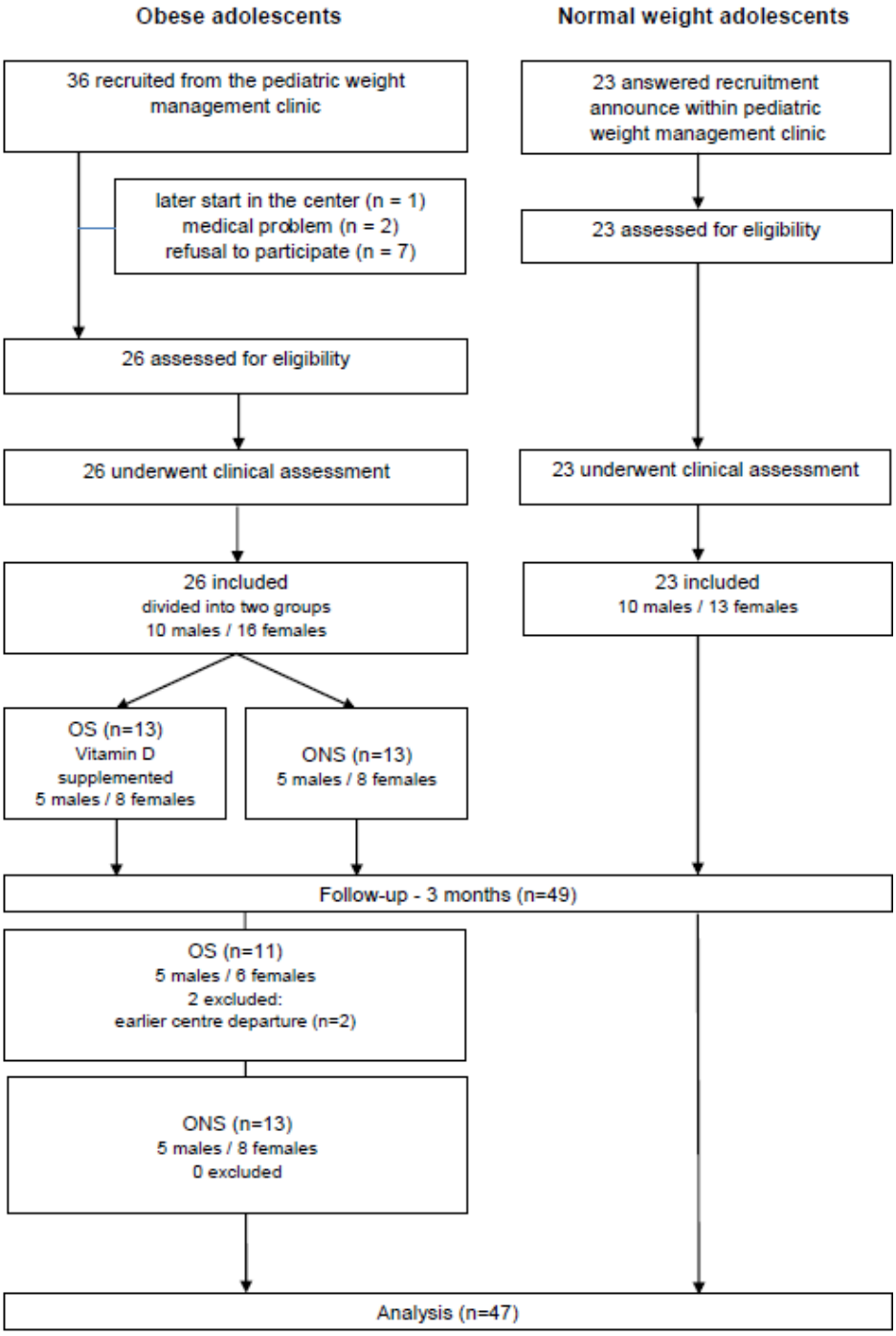
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

Methods

Study population

Adolescents (12 to 17 years of age) with obesity were enrolled at a pediatric weight management clinic, whereas the age-, sex-, puberty stage- and season-matched controls with normal weight were volunteers (Fig. 1).

Figure 1: Study flowchart



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

As reported by Gutierrez-Medina et al [17] , the pubertal status should be taken into account when assessing obesity-related VD deficiency. Obesity was defined according to the age and sex-specific cut-off points of childhood obesity, as recommended by the International Obesity Task Force [18]. General exclusion criteria were: history or presence of premature cardiovascular or metabolic disease in a first-degree family member, active smoking, regular intake of any medication, pubertal status lower than Tanner stage 2, and not willing to take VD supplementation. Specific exclusion criteria were: weight loss higher than 5% of their total weight during the previous 3 months for adolescents with obesity, and body mass index (BMI) above the 90th percentile for their sex and age for adolescents with normal weight. To minimize the potential moderating effect of physical activity, adolescents who participated in extra-school sport activities more than three hours per week were also excluded.

Study Design

Adolescents with obesity (n=36) who voluntarily accepted to follow a 3-month weight loss program (diet and exercise training) at a pediatric weight-management center were asked to participate in a double-blind, randomized, placebo-controlled clinical study, from April 2015 to July 2015 (1st group) and from April 2016 to July 2016 (2nd group). Seven adolescents refused to participate, two were considered ineligible for medical reasons, and one arrived after the program start date. Finally, 26 adolescents were recruited and 24 (10 males and 14 females) completed the study. Adolescents with obesity were randomized by blocks of random size (2 or 4), with a 1:1 ratio in two arms: (1) supplementation with 4000 IU/day of VD3 (Uvedose 100000 UI/2 mL, Crinex laboratory, France) given in a fruit juice (n=13; OS group); and (2) no VD3 supplementation in the fruit juice (n=13; ONS group). It has been shown that VD3 is equally bioavailable in orange juice and capsules [19]. The randomization list was generated with the SAS software, version 9.4. The inclusion of adolescents with

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

obesity was managed with the INCLUSIO software, developed by Nimes University Hospital (France), and using a secure individual connection (login and password).

All adolescents with obesity followed the same moderately hypocaloric diet (reduction of 300 to 500 calories per day) based on a balanced intake of carbohydrates (55%), proteins (15%), and lipids (30% in total, with less than 10% of saturated fats)[16]. During the 3-month program, exercise training consisted in supervised moderate-to-vigorous intensity exercise for 180 min/week. Each session mostly involved aerobic exercise, including training circuit, boxing, basket-ball and Nordic walk, with alternating short periods of high-intensity exercise interspersed among periods of lower intensity, as previously described [20].

The 23 healthy adolescents with normal weight were recruited from the community to serve as controls at baseline and after the 3-month program.

The study protocol was approved by the local Ethics Committee and was performed in accordance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all adolescents and their parents.

Dietary assessment

All participants were asked to complete a three-day dietary record to assess the total energy dietary intake, and a food-frequency questionnaire containing calcium- and VD-related nutrients to determine their usual dietary intake pattern.

Outcome measures

Anthropometric data and body composition

Anthropometry and body composition were assessed at baseline and after the 3-month intervention. BMI was calculated as weight in kilograms divided by height in squared meters ($kg.m^{-2}$). The BMI z-scores were also calculated. Adolescents with obesity underwent dual-energy X-ray absorptiometry (QDR 2000 – Discovery, Hologic, Bedford, MA) before and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

after the 3-month program, and controls only at baseline. Body fat mass and abdominal fat mass were reported [18]. Skin tone was categorized in light, medium and dark [3].

Aerobic fitness test

Only adolescents with obesity performed the 20-meter shuttle run test [21]. The initial running speed was set at 8.0 $km.h^{-1}$ and was increased by 0.5 $km.h^{-1}$ each minute. Adolescents were instructed and encouraged to complete as many stages as possible until volitional exhaustion. The maximal heart rate (Polar) at the end of the test and the number of shuttles (20 m) were recorded.

VD, PTH and calcium status, cardiometabolic risk factors, and inflammatory status

25(OH)D, parathyroid hormone (PTH), and calcium, glycemia, insulin, lipid profile (triglycerides, TG; total cholesterol; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C), inflammatory (ultrasensitive C-reactive protein, hs-CRP; tumor necrosis factor, TNF; interleukin-6, IL-6; leptin), and anti-inflammatory (adiponectin) markers were measured in venous blood samples collected after overnight fasting at baseline and after the 3-month program (24h after the last training session for adolescents with obesity). Total 25(OH)D concentrations were determined by the electrochemiluminescence immunoassay (ECLIA) method, using Roche Diagnostics kits (Roche Elecsys vitamin D total). Free form of 25(OH)D was quantified by ELISA (DIAsource ImmunoAssays, Louvain-La-Neuve, Belgium). According the Committee on Nutrition of the French Society of Paediatrics, VD insufficiency corresponds to 25(OH)D levels <50 nmol/L [22]. Hs-CRP, TNF and IL-6 were measured using commercially available ELISA kits purchased from R&D systems (Minneapolis, USA) according to the manufacturer's specifications. LDL-C was calculated using Friedwald formula (LDL-C (mmol/l) = total cholesterol (mmol/L) – HDL-C (mmol/L) – TG (nmol/L) x 0.5). Insulin resistance was expressed by using the homeostasis model assessment of insulin resistance index (HOMA-IR = glycemia (mmol/L) x insulinemia

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

(mU/L)/22.5) [23]. Hs-CRP, TNFα, IL-6, adiponectin and leptin were measured in serum samples using commercially available Elisa kits from R&D Systems (Minneapolis, USA), according to the manufacturer's specifications. For analysis of hs-CRP, adiponectin and leptin, serum samples were diluted 1:100 with sample diluent prior to analysis.

Statistical analysis

All statistical analyses were performed using the free R software (*R Core Team 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/*). All variables were expressed as medians and their associated dispersion index, such as 1st and 3rd quartiles. As our sample included 26 adolescents with obesity (and 23 control teenagers) divided in two balanced groups (OS and ONS), and due to the fact that variables were not normally distributed (even after a log-transformation to approximate normality), non-parametric tests were used. Differences in baseline characteristics between the normal-weight and obesity groups, and between the OS and ONS groups were compared using the Wilcoxon exact test implemented in the {coin}-R package [24][25].

The delta change rates (Δ) in all adolescents with obesity and in the OS and ONS groups were analyzed using Spearman's correlations. Bivariate associations between Δ25(OH)D, ΔPTH ΔPTH/25(OH)D and Δ variables were determined by calculating the Spearman's correlation coefficients. A p-value <0.05 was considered statistically significant.

Results

Baseline characteristics

At baseline, normal-weight controls and adolescents with obesity were matched for age, sex, and Tanner stage (Tables 1 and 2). 25(OH)D levels was lower (44.2±13.8 vs 61.1±12.9 nmol/L, p<0.05), and the rate of VD insufficiency (25(OH)D levels <50 nmol/L) was higher

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

(73% vs 22%) in adolescents with obesity than in normal-weight controls. Although systolic and diastolic blood pressure (SBP and DBP) were higher and lower, respectively, in adolescents with obesity than in controls, no case of hypertension was reported (Table 1). Insulin, HOMA-IR and TG also were higher (+116%, +113% and +43%, respectively), HDL-C levels were lower (-33%), and the TG/HDL-C ratio was higher in adolescents with obesity than in controls. The mean of TG/HDL-C ratio in adolescents with obesity was higher than the cut-off previously defined as a metabolic syndrome indicator in children [26]. The insulin values in the obesity group were above the threshold (>100 pmol/L), defined as ‘‘at risk’’ by Delvin et al [27]. No significant difference was observed for calcium, resting heart rate, and LDL-C between groups. However, hs-CRP, IL-6 and leptin levels were higher and adiponectin levels were lower in adolescents with obesity than in controls.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

Table 1: Baseline characteristics of participants

Characteristics	All adolescents with obesity (n=26)	Controls with normal weight (n=23)	P value Obesity v normal weight
Age (years)	14.4 ± 1.6	14.8 ± 1.5	0.324
Sex (M-F)	10-16	10-13	0.728
Tanner stage	3.6 ± 0.8	3.4 ± 1.0	0.358
BMI ($kg.m^2$)	33.8 ± 3.2	19.6 ± 2.4	**<0.0001**
BMI z-score	4.0 ± 0.6	0.2 ± 0.8	**<0.0001**
Fat mass (%)	41.3 ± 4.2	22.7 ± 6.8	**<0.0001**
Abdominal fat mass (kg)	43.1 ± 3.6	22.7 ± 8.1	**<0.0001**
Lean mass (kg)	53.0 ± 7.5 log	39.2 ± 7.6	**<0.0001**
25(OH)D (nmol/L)	44.2 ± 13.8	61.1 ± 12.9	**<0.001**
Free 25(OH)D	3.923± 0.843*	4.685± 0.866*	0.06
25(OH)D deficiency (<37.5 nmol/L)	8	0	-
25(OH)D insufficiency (<50 nmol/L)	18	5	-
Calcium (mmol/L)	2.28 ± 0.06	2.28 ± 0.08	0.735
PTH (pmol/L)	4.10 ± 1.38	4.59 ± 1.48	0.310
SBP (mmHg)	114.0 ± 9.8	103.8 ± 7.0	**<0.001**
DBP (mmHg)	61.5 ± 5.1	65.6 ± 6.1	**0.007**

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

MBP (mmHg)	79.4 ± 5.7	79.0 ± 5.5	0.833
Resting heart rate (bpm)	66.5 ± 11.1	63.4 ± 7.0	0.640
Number of 20 m	36.1 ± 13.0 log	-	-
Maximal heart rate (bpm)	196.3 ± 9.8	-	-
Insulin (µUI/L)	133.11 ± 58.92	61.44 ± 30.80	**<0.0001**
HOMA-IR	4.7 ± 2.3	2.20 ± 1.12	**<0.0001**
HbA1c (%)	5.37 ± 0.37	5.25 ± 0.25	0.099
Triglycerides (g/L)	0.87 ± 0.34	0.61 ± 0.23	**<.0001**
HDL-C (g/L)	0.43 ± 0.11	0.65 ± 0.16	**<0.0001**
LDL-C (g/L)	1.00 ± 0.31	0.93 ± 0.18	0.754
TG/HDL	2.21 ± 1.27	0.97 ± 0.50	**<0.0001**
Hs-CRP (mg/mL)	7.28 ± 5.08	1.61 ± 2.63	**<0.0001**
IL-6 (pg/mL)	2.33 ± 0.65	0.98 ± 0.66	**<0.0001**
Adiponectin (µg/mL)	2.36 ± 0.48	2.58 ± 0.41	**0.025**
Leptin (ng/mL)	29.00 ± 12.94	6.28. ± 4.77	**<0.0001**

BMI, body mass index based; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; 25(OH)D, 25-hydroxyvitamin D; HOMA-IR, homeostasis model assessment-insulin resistance index; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, ultrasensitive C-reactive protein; IL-6, interleukin 6.

* free vitamin D n=21 for obese adolescents and 22 for normal weight adolescents. Significant p-values are written in bold.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

Comparison of the baseline characteristics in the OS and ONS groups (Table 2) showed that the clinical and metabolic variables were comparable between groups, but for PTH, DBP, insulin, and particularly the HOMA-IR index that was higher than 3.16 in the OS groups, suggesting insulin resistance [28].

Table 2: Changes in clinical and metabolic variables after the 3-month intervention in the OS and ONS groups

Characteristics	Time	OS (n=13)				ONS (n=13)				P value			
		Mean	Median	P25	P75	Mean	Median	P25	P75	T0 (Os vs ONS)	T3 vs T0 OS	T3vs T0 ONS	T3 Os vs ONS)
Age (years)	T0	14.11	14.21	13.21	14.70	14.49	14.00	13.21	16.69	0.820			
Sex (M-F)	T0	5-8				5-8							
Tanner stage	T0	3.7				3.5							
Anthropometry													
BMI (kg.m2)	T0	34.01	33.80	31.30	36.50	33.47	32.70	31.20	35.00	0.765	**<0.001**	**<0.001**	0.722
	T3	31.06	31.60	29.35	32.90	30.69	30.10	29.30	32.40				
BMI z-score	T0	4.10	3.98	3.36	4.69	4.00	4.01	3.45	4.55	0.876	**<0.001**	**<0.001**	0.619
	T3	3.69	3.68	3.04	4.27	3.55	3.52	3.26	3.84				
Fat mass (%)	T0	42.00	41.90	40.35	43.60	40.72	41.10	36.80	43.50	0.522	**<0.001**	**<0.001**	0.638

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

	T3	37.5	38.3	35.3	39.4	36.67	36.00	33.50	40.80				
Abdominal fat mass (kg)	T0	8.33	8.41	8.04	8.90	7.96	7.86	6.26	9.09	0.531	**<0.001**	**<0.001**	0.765
	T3	6.97	6.82	6.63	7.45	6.53	7.05	5.28	7.65				
Lean mass (kg)	T0	54.61	52.68	51.29	59.62	51.90	50.40	44.03	57.73	0.303	0.218	0.399	0.331
	T3	54.22	52.15	50.10	59.30	51.49	50.62	45.50	58.02				
VD and associated factors													
25(OH)D (nmol/L)	T0	48.27	44.00	38.00	56.00	41.33	34.50	32.00	50.50	0.183	**<0.001**	**<0.001**	0.023
	T3	74.73	70.00	63.50	83.50	56.54	56.00	46.00	68.00				
PTH (pmol/L)	T0	4.48	4.40	3.45	5.55	3.34	2.95	2.68	3.63	**0.021**	**<0.001**	**<0.001**	0.145
	T3	3.95	3.70	3.40	4.15	3.29	2.80	2.70	3.80				
Calcium (mmol/L)	T0	2.30	2.29	2.27	2.34	2.28	2.26	2.22	2.35	0.502	0.990	0.990	0.853
	T3	2.32	2.32	2.27	2.38	2.31	2.33	2.28	2.33				
Cardiometabolic factors													
SBP (mmHg)	T0	115.50	119.00	106.50	123.00	113.80	113.00	106.00	120.00	0.540	0.104	0.099	0.161
	T3	116.80	117.00	109.00	122.50	109.80	111.00	105.00	114.00				
DBP (mmHg)	T0	59.30	59.00	57.00	60.00	63.38	61.00	59.00	67.00	**0.050**	**0.040**	**0.030**	**0.062**
	T3	57.56	57.00	56.00	58.00	60.31	61.00	58.00	63.00				
MBP (mmHg)	T0	74.43	76.70	72.65	81.15	80.17	78.70	76.00	84.30	0.246	**0.030**	**0.030**	0.366
	T3	76.14	74.70	73.70	78.00	76.82	77.70	75.00	79.00				

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

Resting HR (bpm)	T0	67.00	68.00	64.00	73.00	66.08	63.00	57.00	72.00	0.484	**<0.001**	**<0.001**	0.167
	T3	59.14	56.00	54.50	64.00	52.67	50.00	45.75	55.75				
Maximal HR (bpm)	T0	196.10	194.50	191.50	203.00	196.50	198.00	190.00	206.00	0.939	0.674	0.467	**0.098**
	T3	192.70	198.00	182.00	202.00	201.40	202.00	198.00	206.50				
Glycemia (mmol/L)	T0	4.77	4.80	4.40	5.20	4.47	4.50	4.00	4.90	0.121	0.175	0.210	0.396
	T3	4.67	4.80	4.60	4.90	4.56	4.60	4.40	4.90				
Insulinemia (mUI/L)	T0	174.70	172.60	141.10	190.10	99.09	92.55	83.60	116.80	**0.003**	**<0.001**	**<0.001**	**0.010**
	T3	103.60	108.50	82.45	119.60	73.85	66.30	50.40	89.40				
HOMA-IR	T0	6.26	6.20	4.90	7.10	3.35	3.35	2.80	3.95	**0.002**	**<0.001**	**<0.001**	**0.006**
	T3	3.57	3.40	3.05	4.20	2.55	2.20	1.80	3.10				
Triglycerides (g/L)	T0	1.02	0.91	0.66	1.14	0.85	0.80	0.59	0.94	0.521	**<0.001**	**<0.001**	0.966
	T3	0.68	0.54	0.44	0.90	0.62	0.62	0.46	0.76				
Cholesterol total	T0	1.65	1.62	1.56	1.84	1.64	1.56	1.40	1.89	0.679	**<0.001**	**<0.001**	0.467
	T3	1.36	1.34	1.28	1.46	1.35	1.25	1.21	1.46				
HDL-C (g/L)	T0	0.40	0.40	0.34	0.42	0.47	0.46	0.39	0.51	0.102	0.201	0.115	0.432
	T3	0.41	0.43	0.37	0.46	0.44	0.47	0.33	0.51				
LDL- C (g/L)	T0	1.05	1.09	0.85	1.23	1.00	0.99	0.78	1.20	0.541	**<0.001**	**<0.001**	0.484
	T3	0.81	0.79	0.74	0.91	0.78	0.73	0.64	0.83				
TG/HDL-C	T0	2.82	2.28	1.56	3.22	2.00	1.83	1.16	2.54	0.331	**<0.001**	**<0.001**	0.910

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

	T3	1.77	1.34	0.97	2.24	1.55	1.42	0.96	1.81				
Inflammatory status													
Hs-CRP (mg/mL)	T0	9.40	7.91	5.26	13.13	5.86	4.37	2.74	9.61	0.173	**<0.001**	**<0.001**	**0.046**
	T3	5.35	6.52	1.86	7.75	1.81	1.77	0.82	2.26				
TNF (pg/mL)	T0	1.43	1.37	1.19	1.69	1.21	1.24	1.00	1.32	0.264	**<0.001**	**<0.001**	0.173
	T3	1.19	1.18	1.16	1.35	1.03	1.02	0.91	1.14				
Adiponectin (μg/mL)	T0	2.09	2.18	1.76	2.85	2.59	2.34	1.66	3.40	0.511	0.312	0.937	0.558
	T3	2.19	2.41	1.66	3.13	2.60	2.48	1.93	3.42				
IL-6 (pg/mL)	T0	3.03	2.69	2.51	2.89	2.20	2.33	1.67	2.72	0.219	**<0.001**	**<0.001**	**0.086**
	T3	1.94	1.78	1.64	2.26	1.39	1.23	0.92	1.67				
Leptin (ng/mL)	T0	34.61	31.98	21.32	44.02	27.32	23.08	17.64	30.78	0.260	**<0.001**	**<0.001**	0.487
	T3	18.77	15.16	8.75	32.13	14.24	12.78	8.11	22.20				
Physical performance													
Number of 20 m	T0	34.30	30.00	25.25	43.50	37.69	36.00	27.00	46.00	0.820	**<0.001**	**<0.001**	0.616
	T3	49.50	49.00	36.50	56.00	53.36	57.00	37.50	59.50				

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

Changes in clinical and metabolic variables after the 3-month intervention in the OS and ONS groups

As expected for lifestyle interventions, anthropometric variables (BMI, BMI z-score, fat mass and abdominal fat mass) were significantly and similarly reduced in both groups (OS and ONS) after the 3-month weight-loss program compared with baseline (Table 2). Serum 25(OH)D was significantly increased without any significant difference between groups (59% vs 43%), possibly due to increased sun exposure and outdoor physical activity. Indeed, serum 25(OH)D also increased (+20%, data not shown) in the group with normal weight. However, adequate 25(OH)D levels (>50 nmol) at the 3-month follow-up were detected in all adolescent in the OS group, but only in 46% of adolescent in the ONS group. Moreover, PTH was significantly decreased in the OS group, but not in the ONS group. After the 3-month program, all cardiometabolic variables were improved in both groups. Although the HOMA-IR was strongly reduced in both groups (-45% in OS, $p<0.001$ and -34%, in ONS, $p<0.001$), it was still significantly different between groups. Indeed, it was below 3.16, the cut-off for insulin resistance [28], in all adolescents of the ONS group, but only in the first quartile of the OS group. The lipid profiles were similarly improved in both groups with reduced TG (-41% and -22%, respectively), total cholesterol (-17% and -20%), and LDL-C (-27.5% and -26%) levels compared with baseline (p values <0.001 for all lipid variables), but for HDL-C. The intervention also reduced the inflammatory marker levels in both groups: hs-CRP (-17% and -59%, respectively; $p = 0.046$ between groups), TNF-α (-14% and -18%), IL-6 (-34% and -47%; $p=0.086$ between groups) and leptin (-53% and -44%; p values <0.001).

Correlations between changes in 25(OH)D, PTH or PTH/25(OH)D ratio and cardiometabolic variables in adolescents with obesity and VD deficiency.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

When the analysis was restricted to all the adolescents with obesity and baseline VD insufficiency (25(OH)D <50 nmol/L; n=18, 9 OS and 9 ONS), the change (□□□in 25(OH)D between baseline and the end of the intervention was strongly and negatively correlated with the □TG/HDL-C ratio (r =-0.7, p<0.001). Similarly, the □PTH/25(OH)D ratio was negatively correlated with the □BMI z-score (r =-0.51, p<0.04), and □SBP (r=-0.53, p 0.03). These correlations were maintained after adjusting for sex and skin tone (data not shown).

In the nine adolescents of the OS group, the strongest correlations were obtained between □PTH and □PTH/25(OH)D ratio and □DBP (r= -0.86 p = 0.01) and □MBP (r = -0.89 p <0.001). Moreover, □PTH was correlated with □CRP (p 0.02).

In the nine adolescents of the ONS group, □PTH and □PTH/25(OH)D ratio were not correlated with □DBP or □MBP. Conversely, □PTH was significantly correlated with changes in glycemia and HOMA-IR, and □PTH/25(OH)D ratio was correlated with the change in glycemia. These correlations were not found in the OS group.

Discussion

The present study confirms that pediatric obesity impairs many clinical and cardiometabolic variables. Notably, we found in adolescents with obesity enrolled in a lifestyle program a higher prevalence of insulin resistance, higher TG/HDL-C ratio and inflammatory status compared with adolescents with normal weight. An increase in the TG/HDL-C ratio can be used to predict increased arterial stiffness in young subjects who have developed obesity, as previously suggested [26]. In our study, the insulin and HOMA-IR values of adolescents with obesity were above the unfavorable thresholds established by Delvin et al [27] and Keskin et al [28] (i.e. >100 pmol/L and >3.16, respectively). As other previous cross-sectional studies [29][30][31], low serum 25(OH)D concentrations were associated with obesity in adolescents. In our study, 70% of adolescents with obesity had baseline 25(OH)-D concentrations lower

than 50 nmol/L (equal to 20 ng/mL), which defines deficiency according to the French guidelines [32]. Obesity-related VD insufficiency could be explained by many factors: sun underexposure, inadequate intake of VD-rich foods and beverages, genetic factors [33], sequestration of this fat-soluble vitamin, and its volumetric dilution [34][35]. Cutaneous VD production varies according to the latitude, season, skin pigmentation, frequency and the time of sun exposure, type of clothing… Generally, children with obesity are not sufficiently exposed to sunlight. Moreover, foods rich in VD, such as fatty fish, are rarely eaten by children, as confirmed in our study (data not shown). The sequestration hypothesis postulates a decreased VD bioavailability from cutaneous and dietary sources because of its storage in body fat compartments. The volumetric dilution is more related to the greater distribution volume of 25(OH)D in the tissue mass of individuals with obesity [13]. Both VD storage and dilution in the body fat mass make VD physiologically unavailable for circulation in the blood and for transformation into its biologically active form in the liver and kidneys. Suboptimal VD status has been associated with abnormalities in metabolic regulation (insulin resistance, hyperlipidemia, and hypertension) in children [36][37]. Although many cross-sectional studies indicate that VD might have a beneficial role in obesity and associated diseases, the causality has not been demonstrated yet.

Our finding that 25(OH)D levels as total and free were increased also in the ONS group after the 3-month lifestyle program suggests a possible release of VD during weight loss in the absence of VD supplementation. Previous studies also reported increased circulating 25(OH)D levels in adults with obesity after adiposity loss through lifestyle interventions without VD supplementation [14]. Note that our study was carried in the South of France from April to July, and the increased sun intensity/exposure during the spring/summer months could have contributed to endogenous VD biosynthesis. This hypothesis was validated by finding that the mean 25(OH)D level in the control group also was increased by 20% (data not shown) at the end of the same 3-month period. Nevertheless, satisfactory 25(OH)D plasma

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

levels (close to 75 nmol/L) were observed only in the OS group at the end of the intervention, in accordance with studies reporting the efficacy and safety of 4000 IU VD [5]. Moreover, the significant decrease in PTH observed in our study with a dose of 4000 IU VD is in agreement with the results reported by Bhagatwala et al [38] in African Americans with overweight/obesity and suboptimal vitamin D status. Lower doses do not have any significant effect on PTH and 25(OH)D levels.

In the adolescents with baseline vitamin D deficiency (25(OH)D <50 nmol/L), the 3-month lifestyle program significantly improved the PTH/25(OH)D ratio and this change was negatively correlated with the BMI z-score. The increase in 25(OH)D was not correlated with fat mass loss (p value close to 0.1), while previous studies reported a significant linear increase in circulating 25(OH)D levels as a function of the adiposity volume loss in adults with obesity [39][40]. Although blood pressure was within the normal values in both OS and ONS groups, the 25(OH)D increase was significantly correlated with the decrease of DBP and MBP in the OS group. Conversely, a previous interventional trial did not find any change or difference in SBP and DBP [41]. However, in other studies including adolescents, 25(OH)D deficiency was associated with elevated DBP [42][43]. Petersen et al. [44] showed that each 10 mmol/L serum 25(OH)D increase is associated with a decrease of DBP (−0.3 mmHg, 95% confidence interval −0.6, −0.0) (P = 0.02), independently of the fat mass index. The meta-analysis by Withman et al. [45] on 11 randomized trials in adults showed a small reduction in DBP in adults with hypertension and receiving VD supplementation. VD level increase has been associated with PTH decrease, and higher serum PTH concentration was linked to impaired endothelial function. This suggests that the circulating PTH level could be associated with higher hypertension risk through different potential molecular mechanisms [46], and a reduction of the PTH/25(OH)D ratio could be taken as an indicator of reduced cardiovascular risk.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

Our study did not find any additional effect of VD supplementation on glycemia and estimated insulin resistance expressed by HOMA-IR, while insulin sensitivity is improved by this intervention [47]. Our result is in agreement with an intervention trial (VD supplementation and resistance exercise training) in adults with overweight and obesity [48] . The presence of a significant effect on glycemia and HOMA-IR also in the ONS group indicates that a lifestyle program can on its own improve glucose metabolism. A recent meta-analysis on different populations (adults, people with obesity, women…) showed that there is a non-linear association between VD status and fat mass percentage in a dose-effect analysis, without any beneficial effect with VD doses higher than 2000 UI [49]. This suggests that the high dose of VD used in our study could explain the absence of additional effects related to this supplementation. Future studies should evaluate the dose-effect relationship.

VD supplementation did not have any additional effect on lipid metabolism, in contrast with results previously reported in children with severe obesity, where 25(OH)D concentrations have been positively associated with HDL-C levels [50].

Finally, although hs-CRP reduction was more important in the ONS group, we found a positive association between changes in PTH concentrations and in the PTH/25(OH)D ratio and changes in hs-CRP levels in the OS group (p=0.02 and 0.07 respectively). It has been reported that VD has anti-inflammatory actions [51].

Our study has several limitations: the relatively small size of our cohort that included both girls and boys; only the OS group showed signs of insulin resistance at baseline; the use of a single-dose supplementation; and the assessment of serum parameters only at two time points. Unfortunately, we did not measure the active metabolite 1,25(OH)2D and 25-hydroxylase activity, and also we did not assess the synthesis of vitamin D–binding proteins.

 doi:10.20944/preprints201908.0105.v1

Our study protocol has also some strengths because dietary intake (VD, calcium), seasonality, and sun exposure time were the same in both OS and ONS groups. We controlled for the pubertal status because it may play a role in changes in BMI, lipid and glucose levels.

In conclusion, the combination of exercise, including recreational intermittent physical activities, and healthy diet has strong positive effects on different cardiometabolic parameters. VD supplementation (4000 IU) allowed improving 25(OH)D concentration, but did not have any additional beneficial effects on cardiometabolic factors and associated factors. It could contribute to improving the inflammation status and blood pressure by reducing PTH levels. More studies are needed to specify the dose-effect relationship and with longer VD supplementation in larger samples of adolescents with obesity.

Acknowledgments

This study was sponsored by Nîmes University Hospital.

Disclosure Summary

The authors have nothing to disclose.

References

1. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; Murad, M.H.; Weaver, C.M. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 1911–1930.

2. Codoñer-Franch, P.; Tavárez-Alonso, S.; Simó-Jordá, R.; Laporta-Martín, P.; Carratalá-Calvo, A.; Alonso-Iglesias, E. Vitamin D status is linked to biomarkers of oxidative stress, inflammation, and endothelial activation in obese children. *J. Pediatr.* **2012**, *161*, 848–854.

3. Reyman, M.; Verrijn Stuart, A.A.; van Summeren, M.; Rakhshandehroo, M.; Nuboer, R.; de Boer, F.K.; van den Ham, H.J.; Kalkhoven, E.; Prakken, B.; Schipper, H.S. Vitamin D deficiency in childhood obesity is associated with high levels of circulating

inflammatory mediators, and low insulin sensitivity. *Int. J. Obes.* **2013**, *38*, 46.

4. Javed, A.; Vella, A.; Balagopal, P.B.; Fischer, P.R.; Weaver, A.L.; Piccinini, F.; Dalla Man, C.; Cobelli, C.; Giesler, P.D.; Laugen, J.M. Cholecalciferol supplementation does not influence β-cell function and insulin action in obese adolescents: a prospective double-blind randomized trial. *J. Nutr.* **2014**, *145*, 284–290.

5. Belenchia, A.M.; Tosh, A.K.; Hillman, L.S.; Peterson, C.A. Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: a randomized controlled trial. *Am. J. Clin. Nutr.* **2013**, *97*, 774–781.

6. Kelishadi, R.; Mirmoghtadaee, P.; Najafi, H.; Keikha, M. Systematic review on the association of abdominal obesity in children and adolescents with cardio-metabolic risk factors. *J. Res. Med. Sci. Off. J. Isfahan Univ. Med. Sci.* **2015**, *20*, 294.

7. Montero, D.; Walther, G.; Perez-Martin, A.; Mercier, C.S.; Gayrard, S.; Vicente-Salar, N.; Miguel Sempere-Ortells, J.; Martinez-Peinado, P.; Roche, E.; Vinet, A. Effects of a Lifestyle Program on Vascular Reactivity in Macro- and Microcirculation in Severely Obese Adolescents. *J. Clin. Endocrinol. Metab.* **2014**, *99*, 1019–1026.

8. Ribeiro, M.M.; Silva, A.G.; Santos, N.S.; Guazzelle, I.; Matos, L.N.; Trombetta, I.C.; Halpern, A.; Negrão, C.E.; Villares, S.M. Diet and exercise training restore blood pressure and vasodilatory responses during physiological maneuvers in obese children. *Circulation* **2005**, *111*, 1915–1923.

9. Slusher, A.L.; McAllister, M.J.; Huang, C.-J. A therapeutic role for vitamin D on obesity-associated inflammation and weight-loss intervention. *Inflamm. Res.* **2015**, *64*, 565–575.

10. Pourshahidi, L.K. Vitamin D and obesity: current perspectives and future directions. *Proc. Nutr. Soc.* **2015**, *74*, 115–124.

11. Strasser, B. Physical activity in obesity and metabolic syndrome. *Ann. N. Y. Acad. Sci.* **2013**, *1281*, 141.

12. Bruyndonckx, L.; Hoymans, V.Y.; De Guchtenaere, A.; Van Helvoirt, M.; Van Craenenbroeck, E.M.; Frederix, G.; Lemmens, K.; Vissers, D.K.; Vrints, C.J.; Ramet, J. Diet, exercise, and endothelial function in obese adolescents. *Pediatrics* **2015**, *135*, e653–e661.

13. Gangloff, A.; Bergeron, J.; Pelletier-Beaumont, E.; Nazare, J.-A.; Smith, J.; Borel, A.-L.; Lemieux, I.; Tremblay, A.; Poirier, P.; Almeras, N.; et al. Effect of adipose tissue volume loss on circulating 25-hydroxyvitamin D levels: results from a 1-year lifestyle intervention in viscerally obese men. *Int. J. Obes.* **2015**, *39*, 1638–1643.

14. Pannu, P.K.; Zhao, Y.; Soares, M.J. Reductions in body weight and percent fat mass increase the vitamin D status of obese subjects: a systematic review and metaregression analysis. *Nutr. Res.* **2016**, *36*, 201–213.

15. Apovian, C.; Bigornia, S.; Cullum-Dugan, D.; Schoonmaker, C.; Radziejowska, J.; Phipps, J.; Gokce, N.; Istfan, N.; Meyers, A.; Lenders, C. Milk-based nutritional supplements in conjunction with lifestyle intervention in overweight adolescents. *ICAN Infant Child Adolesc. Nutr.* **2009**, *1*, 37–44.

16. Reinehr, T.; de Sousa, G.; Alexy, U.; Kersting, M.; Andler, W. Vitamin D status and parathyroid hormone in obese children before and after weight loss. *Eur. J. Endocrinol.* **2007**, *157*, 225–232.

17. Medina, S.G.; Gavela-Pérez, T.; Domínguez-Garrido, M.N.; Gutiérrez-Moreno, E.; Rovira, A.; Garcés, C.; Soriano-Guillén, L. The influence of puberty on vitamin D status in obese children and the possible relation between vitamin D deficiency and insulin resistance. *J. Pediatr. Endocrinol. Metab.* **2015**, *28*, 105–110.

18. Cole, T.J.; Bellizzi, M.C.; Flegal, K.M.; Dietz, W.H. Establishing a standard definition for child overweight and obesity worldwide: international survey. *Bmj* **2000**, *320*, 1240.

19. Biancuzzo, R.M.; Young, A.; Bibuld, D.; Cai, M.H.; Winter, M.R.; Klein, E.K.; Ameri, A.; Reitz, R.; Salameh, W.; Chen, T.C. Fortification of orange juice with vitamin D2 or vitamin D3 is as effective as an oral supplement in maintaining vitamin D status in adults. *Am. J. Clin. Nutr.* **2010**, *91*, 1621–1626.

20. Morrissey, C.; Montero, D.; Raverdy, C.; Masson, D.; Amiot, M.-J.; Vinet, A. Effects of Exercise Intensity on Microvascular Function in Obese Adolescents. *Int. J. Sports Med.* **2018**, *39*, 450–455.

21. Leger, L.; Lambert, J. A maximal multistage 20-m shuttle run test to predict VO_2 max. *Eur. J. Appl. Physiol.* **1982**, *49*, 1–12.

22. Vidailhet, M.; Mallet, E.; Bocquet, A.; Bresson, J.-L.; Briend, A.; Chouraqui, J.-P.; Darmaun, D.; Dupont, C.; Frelut, M.-L.; Ghisolfi, J.; et al. Vitamin D: Still a topical matter in children and adolescents. A position paper by the Committee on Nutrition of the French Society of Paediatrics. *Arch. Pediatr.* **2012**, *19*, 316–328.

23. Bonora, E.; Formentini, G.; Calcaterra, F.; Lombardi, S.; Marini, F.; Zenari, L.; Saggiani, F.; Poli, M.; Perbellini, S.; Raffaelli, A. HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study. *Diabetes Care* **2002**, *25*, 1135–1141.

24. Hothorn, T.; Hornik, K.; Van De Wiel, M.A.; Zeileis, A. A Lego System for Conditional Inference. *The American Statistician* **2006**, *60*, 257-263.

25. Hothorn, T.; Hornik, K.; Van De Wiel, M.A.; Zeileis, A. Implementing a class of permutation pests: the coin package. *Journal of Statistical Software*, **2008**, *28*, 1-23

26. Urbina, E.M.; Khoury, P.R.; McCoy, C.E.; Dolan, L.M.; Daniels, S.R.; Kimball, T.R. Triglyceride to HDL-C ratio and increased arterial stiffness in children, adolescents, and young adults. *Pediatrics* **2013**, *131*, e1082–e1090.

27. Delvin, E.E.; Lambert, M.; Levy, E.; O'Loughlin, J.; Mark, S.; Gray-Donald, K.; Paradis, G. Vitamin D status is modestly associated with glycemia and indicators of lipid metabolism in French-Canadian children and adolescents. *J. Nutr.* **2010**, *140*, 987–991.

28. Keskin, M.; Kurtoglu, S.; Kendirci, M.; Atabek, M.E.; Yazici, C. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. *Pediatrics* **2005**, *115*, e500–e503.

29. Gul, A.; Ozer, S.; Yilmaz, R.; Sonmezgoz, E.; Kasap, T.; Takci, S.; Demir, O. Association between vitamin D levels and cardiovascular risk factors in obese children and adolescents. *Nutr. Hosp.* **2017**, *34*, 323–329.

30. Holmlund-Suila, E.; Pekkinen, M.; Ivaska, K.K.; Andersson, S.; Makitie, O.; Viljakainen, H. Obese young adults exhibit lower total and lower free serum 25-hydroxycholecalciferol in a randomized vitamin D intervention. *Clin. Endocrinol. (Oxf.)* **2016**, *85*, 378–385.

31. Rajakumar, K.; Fernstrom, J.D.; Holick, M.E.; Janosky, J.E.; Greenspan, S.L. Vitamin D status and response to vitamin D-3 in obese vs. non-obese African American children. *Obesity* **2008**, *16*, 90–95.

32. Anses. Actualisation des repères du PNNS: révision des repères de consommations alimentaires. **2016**, https://www.anses.fr/fr/content/l'anses-actualise-les-repères-de-consommations-alimentaires-pour-la-population-française

33. Javier Ruiz-Ojeda, F.; Anguita-Ruiz, A.; Leis, R.; Aguilera, C.M. Genetic Factors and Molecular Mechanisms of Vitamin D and Obesity Relationship. *Ann. Nutr. Metab.* **2018**, *73*, 89–99.

34. Drincic, A.T.; Armas, L.A.G.; Van Diest, E.E.; Heaney, R.P. Volumetric Dilution, Rather Than Sequestration Best Explains the Low Vitamin D Status of Obesity. *Obesity* **2012**, *20*, 1444–1448.

35. Wortsman, J.; Matsuoka, L.; Chen, T.; Lu, Z.; Holick, M. Decreased bioavailability of vitamin D in obesity. *Am. J. Clin. Nutr.* **2000**, *72*, 690–693.

36. Challa, A.S.; Makariou, S.E.; Siomou, E.C. The relation of vitamin D status with metabolic syndrome in childhood and adolescence: an update. *J. Pediatr. Endocrinol. Metab.* **2015**, *28*, 1235–1245.

37. Gilbert-Diamond, D.; Baylin, A.; Mora-Plazas, M.; Marin, C.; Arsenault, J.E.; Hughes, M.D.; Willett, W.C.; Villamor, E. Vitamin D deficiency and anthropometric indicators of adiposity in school-age children a prospective study. *Am. J. Clin. Nutr.* **2010**, *92*, 1446–1451.

38. Bhagatwala, J.; Zhu, H.; Parikh, S.J.; Guo, D.-H.; Kotak, I.; Huang, Y.; Havens, R.; Pham, M.; Afari, E.; Kim, S. Dose and time responses of vitamin D biomarkers to

monthly vitamin D 3 supplementation in overweight/obese African Americans with suboptimal vitamin d status: A placebo controlled randomized clinical trial. *BMC Obes.* **2015**, *2*, 27.

39. Rosenblum, J.L.; Castro, V.M.; Moore, C.E.; Kaplan, L.M. Calcium and vitamin D supplementation is associated with decreased abdominal visceral adipose tissue in overweight and obese adults. *Am. J. Clin. Nutr.* **2012**, *95*, 101–108.

40. Hannemann, A.; Thuesen, B.H.; Friedrich, N.; Voelzke, H.; Steveling, A.; Ittermann, T.; Hegenscheid, K.; Nauck, M.; Linneberg, A.; Wallaschofski, H. Adiposity measures and vitamin D concentrations in Northeast Germany and Denmark. *Nutr. Metab.* **2015**, *12*.

41. Dong, Y.; Stallmann-Jorgensen, I.S.; Pollock, N.K.; Harris, R.A.; Keeton, D.; Huang, Y.; Li, K.; Bassali, R.; Guo, D.-H.; Thomas, J.; et al. A 16-Week Randomized Clinical Trial of 2000 International Units Daily Vitamin D-3 Supplementation in Black Youth: 25-Hydroxyvitamin D, Adiposity, and Arterial Stiffness. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 4584–4591.

42. Pacifico, L.; Anania, C.; Osborn, J.F.; Ferraro, F.; Bonci, E.; Olivero, E.; Chiesa, C. Low 25(OH)D-3 levels are associated with total adiposity, metabolic syndrome, and hypertension in Caucasian children and adolescents. *Eur. J. Endocrinol.* **2011**, *165*, 603–611.

43. Tomaino, K.; Romero, K.M.; Robinson, C.L.; Baumann, L.M.; Hansel, N.N.; Pollard, S.L.; Gilman, R.H.; Mougey, E.; Lima, J.J.; Checkley, W.; et al. Association Between Serum 25-Hydroxy Vitamin D Levels and Blood Pressure Among Adolescents in Two Resource-Limited Settings in Peru. *Am. J. Hypertens.* **2015**, *28*, 1017–1023.

44. Petersen, R.A.; Dalskov, S.-M.; Sorensen, L.B.; Hjorth, M.F.; Andersen, R.; Tetens, I.; Krarup, H.; Ritz, C.; Astrup, A.; Michaelsen, K.F.; et al. Vitamin D status is associated with cardiometabolic markers in 8-11-year-old children, independently of body fat and physical activity. *Br. J. Nutr.* **2015**, *114*, 1647–1655.

45. Witham, M.D.; Nadir, M.A.; Struthers, A.D. Effect of vitamin D on blood pressure: a systematic review and meta-analysis. *J. Hypertens.* **2009**, *27*, 1948–1954.

46. Zhang, Y.; Zhang, D. Circulating parathyroid hormone and risk of hypertension: A meta-analysis. *Clin. Chim. ACTA* **2018**, *482*, 40–45.

47. Zuk, A.; Fitzpatrick, T.; Rosella, L.C. Effect of Vitamin D-3 Supplementation on Inflammatory Markers and Glycemic Measures among Overweight or Obese Adults: A Systematic Review of Randomized Controlled Trials. *PLOS ONE* **2016**, *11*.

48. Carrillo, A.E.; Flynn, M.G.; Pinkston, C.; Markofski, M.M.; Jiang, Y.; Donkin, S.S.; Teegarden, D. Impact of vitamin D supplementation during a resistance training intervention on body composition, muscle function, and glucose tolerance in overweight and obese adults. *Clin. Nutr.* **2013**, *32*, 375–381.

 doi:10.20944/preprints201908.0105.v1

49. Golzarand, M.; Hollis, B.W.; Mirmiran, P.; Wagner, C.L.; Shab-Bidar, S. Vitamin D supplementation and body fat mass: a systematic review and meta-analysis. *Eur. J. Clin. Nutr.* **2018**, *72*, 1345–1357.

50. Iqbal, A.M.; Dahl, A.R.; Lteif, A.; Kumar, S. Vitamin D Deficiency: A Potential Modifiable Risk Factor for Cardiovascular Disease in Children with Severe Obesity. *Child.-BASEL* **2017**, *4*.

51. Querfeld, U. Vitamin D and inflammation. *Pediatr. Nephrol.* **2013**, *28*, 605–610.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2019 doi:10.20944/preprints201908.0105.v1

Table 3: Correlations between the changes in 25(OH)D, PTH, PTH/25(OH)D and the changes in anthropometric, cardiometabolic, inflammatory, and physical activity parameters (significant correlations in bold; NS: not significant) in 18 adolescents with obesity and VD deficiency (9 0S and 9 ONS).

	BMI (kg.m2)		BMI z-score		Fat mass (%)		Abdominal fat mass (kg)		Lean mass (kg)		Calcium		SBP (mmHg)		DBP (mmHg)		MBP (m
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r
Δ 25(OH)D																	
All	-0.13	NS	0.35	NS	-0.41	NS	0.21	NS	0.13	NS	-0.39	NS	0.33	NS	0.17	NS	0.21
OS	-0.56	NS	0.5	NS	-0.67	0.1	-0.16	NS	-0.11	NS	-0.52	NS	0.71	0.07	0.75	**0.05**	0.83
ONS	0.15	NS	0.18	NS	-0.10	NS	0.33	NS	0.39	NS	-0.48	NS	0.08	NS	-0.22	NS	0.05
Δ PTH																	
All	0.15	NS	-0.3	NS	0.43	0.1	0.03	NS	-0.11	NS	0.23	NS	-0.22	NS	-0.2	NS	-0.21
OS	0.07	NS	-0.41	NS	0.11	NS	-0.18	NS	0	NS	0.18	NS	-0.43	NS	-0.86	**0.01**	-0.89
ONS	0.26	NS	-0.03	NS	0.6	0.09	0.35	NS	-0.28	NS	0.26	NS	0.1	NS	0.63	0.07	0.26
Δ PTH/25(OH)D																	
All	-0.05	NS	-0.51	**0.04**	0.36	NS	-0.11	NS	-0.33	NS	0.32	NS	-0.53	**0.03**	-0.36	NS	-0.44
OS	0.43	NS	-0.37	NS	0.46	NS	0.14	NS	0.04	NS	0.39	NS	-0.67	0.1	-0.85	**0.02**	-0.89
ONS	-0.29	NS	-0.45	NS	0.23	NS	-0.08	NS	-0.52	NS	0.27	NS	-0.28	NS	0.13	NS	-0.27