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Vitamin D status is not related to insulin resistance in different phenotypes of moderate obesity

Manal Al Masri, Ahmed Jerome Romain, Catherine Boegner, Laurent Maimoun, Denis Mariano-Goulart, Vincent Attalin, Elodie Leprieur, Marion Picandet, Antoine Avignon, and Ariane Sultan

Abstract: Low plasma 25-hydroxy-vitamin D (25OHD) and high levels of parathyroid hormone (PTH) are associated with obesity and could play a role in the occurrence of complications such as insulin resistance. The objective of the study was to evaluate whether the relationship between 25OHD status and phosphocalcic parameters differs between metabolically healthy obese (MHO) and insulin-resistant obese (IRO). This cross-sectional study included 158 consecutive adults (121 females) with obesity (body mass index (BMI) 35.15 ± 2.8 kg/m²), aged 43.21 ± 13.6 years. Serum 25OHD, calcemia, phosphatemia, PTH, plasma lipids, fasting plasma glucose, insulin levels, and body composition were measured. Participants were classified as MHO ($n = 65$) or IRO ($n = 93$) based on homeostatic model assessment insulin-resistance value. IRO patients had a higher BMI ($p = 0.001$), waist circumference ($p = 0.03$), and trunk fat mass ($p = 0.007$) than MHO patients. Mean HbA1c ($p = 0.03$), triglycerides ($p = 0.02$), and hsCRP ($p = 0.04$) plasmatic levels were increased in the IRO group. No between-group difference was found on 25OHD, PTH, calcium, or phosphorus plasmatic levels. Only age-predicted 25OHD levels were identified among IRO participants, whereas no factors were identified in MHO. No predictive factors of PTH plasmatic level were identified in the IRO and MHO groups. Although MHO and IRO patients have different metabolic profiles, we did not detect any difference regarding either 25OHD or PTH. Insulin resistance was not a predictive factor of vitamin D status. Our results confirm the absence of link between vitamin D status and insulin resistance in moderate obesity.

Key words: obesity, insulin resistance, metabolism, body composition.

Résumé : Une faible concentration plasmatique de 25-hydroxy-vitamine D (25OHD) et une forte concentration de parathormone (PTH) sont associées à l'obésité et pourraient jouer un rôle au sujet de l'occurrence de ses complications telles l'insulinorésistance. Cette étude a pour objectif de vérifier si l'association entre le statut de 25OHD et les variables phosphocalciques diffère entre les obèses métaboliquement sains (MHO) et les obèses insulinorésistants (IRO). L'étude transversale comprend 158 adultes consécutifs (121 femmes) obèses (IMC : $35,15 \pm 2,8$ kg/m²) âgés de $43,21 \pm 13,6$ ans. On mesure les variables suivantes : 25OHD sérique, calcémie, phosphatémie, PTH, lipides plasmatiques, glucose sanguin à jeun, taux d'insuline et composition corporelle. Les participants sont classés MHO ($n = 65$) ou IRO ($n = 93$) d'après la valeur d'HOMA-IR. Comparativement aux patients MHO, les patients IRO présentent un IMC plus élevé ($p = 0,001$), un plus grand tour de taille ($p = 0,03$) et une plus grande masse adipeuse au tronc ($p = 0,007$). Les concentrations plasmatiques moyennes de HbA1c ($p = 0,03$), de triglycérides ($p = 0,02$) et d'hsCRP ($p = 0,04$) sont plus élevées dans le groupe IRO. On ne note pas de différences entre les groupes à propos des concentrations plasmatiques des variables suivantes : 25OHD, PTH, calcium et phosphore. Au sujet des facteurs de prédiction de 25OHD, seul l'âge prédit le taux de 25OHD chez les patients IRO; on n'observe aucun facteur dans le groupe MHO. Aussi, il n'y a pas de facteurs de prédiction du taux plasmatique de PTH dans les deux groupes, IRO et MHO. Même si les patients MHO et IRO présentent des profils métaboliques différents, on n'observe aucune différence de 25OHD ou de PTH. L'insulinorésistance n'est pas un facteur de prédiction du statut de la vitamine D. D'après nos résultats, il n'y a pas d'association entre le statut de la vitamine D et l'insulinorésistance en présence d'obésité modérée. [Traduit par la Rédaction]

Mots-clés : obésité, insulinorésistance, métabolisme, composition corporelle.

Introduction

Obesity is well recognized as a global epidemic (Popkin 2010) and is associated with various comorbidities, including hypertension, insulin resistance (IR), and other components of the metabolic syndrome (Botella-Carretero et al. 2007; Kayaniyil et al. 2011). Obesity and its complications significantly contribute to modern health care costs,

morbidity, and mortality (Wang et al. 2008; Yach et al. 2006). Further, mechanisms involved in IR are not fully understood.

Over the past years, several abnormalities in the regulation of mineral metabolism, including vitamin D deficiency (Bell et al. 1985; Buffington et al. 1993) and increased levels of parathyroid hormone (PTH) (Adam et al. 2010; Bolland et al. 2005; Kamycheva et al. 2004), have been reported in obese individuals. They have

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been associated with IR (Chiu et al. 2004; Deleskog et al. 2012; Reis et al. 2007), systemic inflammation (Sukumar et al. 2011), arterial calcifications (Gilardini et al. 2011), and presence of metabolic syndrome components (Hjelmsaeth et al. 2009). However, it is not clear whether the observed low vitamin D and high circulating PTH levels in obese individuals can further and independently contribute to the risk of developing metabolic diseases or whether they should be considered as a disturbance of mineral metabolism per se. Indeed, the question of the relationships between anthropometric, metabolic factors, and vitamin D status remains partly unanswered. Cross-sectional studies showed correlations between the aforementioned factors (Bellia et al. 2012; Hjelmsaeth et al. 2009). Indeed, one study recently showed that among class III obesity, metabolically healthy obese patients (MHO) had higher levels of 25OHD than insulin-resistant obese (IRO) patients, with comparable levels of circulating PTH (Bellia et al. 2012). This study further showed an independent inverse association between 25OHD and IR in both MHO and IRO patients. Conversely, interventional studies using vitamin D supplementation have yielded conflicting results, reporting either a decreased IR (Nagpal et al. 2009; Von Hurst et al. 2010) or no effect (Jorde and Figenschau 2009; Wamberg et al. 2013).

Therefore, our objective was to evaluate whether the relationship between vitamin D status and phosphocalcic parameters differs between MHO and IRO patients, suffering from moderate class I obesity, to determine if vitamin D status could be associated with IR.

Materials and methods

Inclusion and exclusion criteria

Obese patients referred to our department for metabolic and physical assessment of their obesity were assessed for eligibility. Inclusion criteria were aged <65 years, body mass index (BMI) >30 kg/m², no calcium or vitamin D supplementation, and no past bariatric surgery.

Patients with known diabetes mellitus were excluded (HbA1c > 6.5%; fasting plasmatic glycemia > 7 mmol/L and (or) presence of any antidiabetic medications). All participants had normal cardiopulmonary and kidney functions as determined by medical history, physical examination, creatinine clearance calculation, and electrocardiogram realized by one investigator of the study. Height and weight were measured wearing light clothing and no shoes; BMI was calculated as weight in kilograms divided by the square of height in metres (kg/m²). Waist circumference was recorded to the nearest 0.1 cm midway between the last rib and the crest of the ileum using a nonstretch tape measure on lightly clad participants.

Patient consent

All participants gave written informed consent. The study was performed according to the principles of the Declaration of Helsinki and was approved by the local ethics committee. All patients were entered into a registry with data collected from their hospitalization including anthropometric, clinical, and biological information (N°DC-2009-1052).

Laboratory procedures

Blood was drawn in the morning after an 8-h overnight fast. Fasting plasma glucose was measured by routine biochemistry analysis. Total and high-density lipoprotein cholesterol were measured by routine enzymatic methods (KonePro; Konelab, Epoo, Finland). Low-density lipoprotein cholesterol was calculated according to the Friedwald formula. Hemoglobin A1c (HbA1c) was measured with routine high-performance liquid chromatography based ion-exchange procedure (HA-8140; Menarini, Rungis CEDEX, France). Hormones (insulin and PTH) were measured using ECLIA Electroluminescence, and 25OHD was measured using the CLIA method (Elecsys; Roche, France).

Vitamin D deficiency was defined as plasma levels <50 nmol/L (20 ng/mL) in agreement with current guidelines (Holick et al. 2011). Hyperparathyroidism was defined as plasmatic PTH concentration >62 pg/mL, regarding laboratory standards.

All patients underwent an oral glucose tolerance test using a load of 75 g of glucose. The results were evaluated according to American Diabetes Association criteria as diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance (American Diabetes Association 2010). The homeostatic model assessment insulin resistance (HOMA-IR) value was calculated as follows: fasting insulin × fasting glucose/22.5. Using a stratification based on HOMA-IR, 2 subgroups were defined: IRO (HOMA-IR values were ≥3) and MHO sub-groups (HOMA-IR values were ≤3) (Matthews et al. 1985).

Each participant underwent a total body scan using dual-energy X-ray absorptiometry (Dual Energy X-ray absorptiometry, Hologic QDR 4500 A.).

Statistical analyses

Statistical analysis was performed between MHO and IRO groups. To analyze the differences between groups, one-way ANOVA was performed on the different selected outcomes. Then, to obtain the effect size of the between-group difference, Cohen's *d* was computed and by convention, 0.2, 0.5, and 0.8 represent small, medium, and large effects, respectively. To detect predictive factors of 25OHD and PTH plasmatic levels, multiple regression analysis was carried out. The multiple regression analyses were conducted among the entire sample and separately in the MHO and IRO sample. The independent variables were high-sensitivity C-reactive protein (hsCRP), fasting glucose, 2-h glucose, and fasting insulin and were selected on the basis of a previous study (Bellia et al. 2012). Only age was added to control for its effect. Significance was set at 0.05. Statistical analyses were performed with Statistica 7.1 (Statsoft, France).

Results

A total of 158 consecutive patients were included, of which 65 were IRO and 93 were MHO patients. Seventy-six percent of the patients enrolled were female. Nonetheless, the proportion of females did not differ between groups ($p = 0.49$).

Anthropometric and metabolic parameters of MHO and IRO patients

Main clinical and metabolic characteristics of MHO and IRO patients are reported in Table 1. The MHO group had lower trunk fat (19.97 ± 4.24 kg vs 22.23 ± 6.04 kg; $p = 0.007$), along with lower BMI values (34.57 ± 2.69 kg/m² vs 35.98 ± 2.78 kg/m²; $p = 0.001$) and lower waist circumference (106.25 ± 10.18 cm vs 109.93 ± 10.30 cm; $p = 0.03$) compared with IRO patients. Mean age tended to be higher in MHO patients ($p = 0.08$).

In addition, MHO individuals exhibited lower levels of fasting glucose ($p = 0.0001$), 2-h postload glucose ($p = 0.001$), and triglycerides ($p = 0.02$), whereas plasmatic level of total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol did not differ between groups. As markers of systemic inflammation, circulating levels of hsCRP were significantly reduced in MHO compared with IRO patients ($p = 0.04$). Furthermore, 19% of MHO patients were taking antihypertensive agents compared with 25% of IRO patients.

Mineral metabolism-related parameters in MHO and IRO patients

Mean 25OHD plasmatic levels were not different between the 2 subgroups (19.50 ± 8.11 ng/mL in MHO vs 21.94 ± 9.69 ng/mL in IRO; $p = 0.11$) (Table 1). Similarly, PTH plasmatic concentrations did not differ between the 2 subgroups (37.45 ± 24.35 ng/L in MHO vs 31.97 ± 13.69 ng/L in IRO; $p = 0.13$). In the whole cohort, the resulting proportion of patients with vitamin D deficiency was about

Table 1. Anthropometric and metabolic parameters of the study participants.

	MHO	IRO	<i>p</i>	Cohen's <i>d</i>
Gender (male/female)	20/73	17/48	0.49	—
Age (y)	44.79±13.27	40.96±13.86	0.08	0.28
Weight (kg)	94.56±10.94	100.71±12.81	0.001	0.52
BMI (kg/m ²)	34.57±2.69	35.98±2.78	0.001	0.52
Waist circumference (cm)	106.26±10.81	109.94±10.31	0.033	0.35
Total fat mass (%)	41.29±5.87	40.54±6.94	0.48	0.12
Fat mass trunk (kg)	19.97±4.24	22.23±6.04	0.007	0.45
HbA1c (%)	5.57±0.38	5.85±1.12	0.033	0.36
Total cholesterol (mmol/L)	5.31±1.18	5.54±1.56	0.296	0.17
TG (mmol/L)	1.62±0.71	1.85±0.71	0.047	0.33
LDL (mmol/L)	3.28±1.04	3.43±1.56	0.466	0.12
HDL (mmol/L)	1.30±0.33	1.27±0.47	0.597	0.07
hsCRP (mg/L)	4.87±4.75	6.94±7.65	0.041	0.34
25OHD deficiency (%)	59.26	40.74	0.16	0.22
25OHD (nmol/L)	19.50±8.11	21.94±9.69	0.111	0.28
PTH (ng/L)	37.45±24.35	31.97±13.69	0.130	0.27
Calcium (nmol/L)	2.36±0.15	2.35±0.25	0.650	0.05
Phosphorus (nmol/L)	1.33±1.89	1.14±0.56	0.440	0.13
HOMA-IR	1.72±0.77	4.86±2.15	—	2.11
2-h glucose (mmol/L)	6.59±1.82	7.56±1.71	0.001	0.55
T score spine	0.06±1.21	-0.037±1.14	0.637	0.08
T score hips	0.32±1	0.23±0.98	0.608	0.09

Note: Variables are expressed as means ± SD unless otherwise stated. Bold values indicate significant *p* value. MHO, metabolically healthy obese; IRO, insulin-resistant obese; BMI, body mass index; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; 25OHD, 25-hydroxy-vitamin D; PTH, parathyroid hormone; HOMA-IR, homeostatic model assessment–insulin resistance.

Table 2. Multivariate linear regression among MHO participants.

Outcome variable	Value	<i>p</i>
25OHD		0.87
Age	0.034 (0.12)	0.77
hsCRP (mg/L)	0.097 (0.11)	0.39
Fasting glucose (mmol/L)	-0.138 (0.13)	0.28
2-h glucose (mmol/L)	-0.012 (0.12)	0.91
Fasting insulin (μ/mL)	-0.39 (0.11)	0.72
PTH		0.55
Age	0.13 (0.12)	0.28
hsCRP (mg/L)	0.14 (0.11)	0.21
Fasting glucose (mmol/L)	0.030 (0.13)	0.81
2-h glucose (mmol/L)	-0.08 (0.12)	0.47
Fasting insulin (μ/mL)	0.12 (0.11)	0.25

Note: Data are expressed as standardized coefficients (standard errors). Age was forced in the model. MHO, metabolically healthy obese; 25OHD, 25-hydroxy-vitamin D; hsCRP, high-sensitivity C-reactive protein; PTH, parathyroid hormone.

Table 3. Multivariate linear regression among IRO patients.

Outcome variable	Value	<i>p</i>
25OHD		0.051
Age	0.40 (0.14)	0.005
hsCRP (mg/L)	0.01 (0.12)	0.92
Fasting glucose (mmol/L)	-0.039 (0.12)	0.76
2-h glucose (mmol/L)	0.01 (0.13)	0.93
Fasting insulin (μ/mL)	-0.078 (0.12)	0.51
PTH		0.18
Age	0.15 (0.14)	0.27
hsCRP (mg/L)	-0.26 (0.12)	0.041
Fasting glucose (mmol/L)	-0.057 (0.13)	0.66
2-h glucose (mmol/L)	0.08 (0.14)	0.57
Fasting insulin (μ/mL)	-0.024 (0.12)	0.84

Note: Data are expressed as standardized coefficients (standard errors). Age was forced in the model. Bold values indicate significant *p* values. IRO, insulin-resistant obese; 25OHD, 25-hydroxy-vitamin D; hsCRP, high-sensitivity C-reactive protein; PTH, parathyroid hormone.

47.36% (*n* = 81) and was substantially smaller in IRO patients (40.74%; *n* = 33) than in MHO patients (59.26%; *n* = 48) (*p* = 0.16). Moreover, we found that 86% (*n* = 41) of the MHO subgroup had a vitamin D deficiency without hyperparathyroidism compared with 96% (*n* = 31) in the IRO group (*p* = 0.34). Circulating levels of calcium, phosphorus, and bone mineral density were similar between the two subgroups, as displayed in Table 1.

Predictive factors of 25OHD and PTH plasmatic levels

All coefficients of regression with their standard errors and their *p* value are provided in Table 2 (MHO patients), Table 3 (IRO patients), and Supplementary Table S1¹ (entire population).

Regarding the 25OHD plasmatic level, the regression model was not significant for the entire sample size ($F_{[5,152]} = 0.93$; *p* = 0.46) or

the MHO group ($F_{[5,87]} = 0.35$; *p* = 0.87). Concerning the IRO patients, the model of regression was very close to the significance threshold ($F_{[5,59]} = 1.95$; *p* = 0.051) with a *R*² of 0.16. Through the dependent variable, only age was positively and significantly associated with the 25OHD plasmatic level (*p* = 0.005).

The same analysis was performed for the PTH plasmatic level. Among IRO participants, although hsCRP was negatively associated with level of PTH (*p* = 0.04), the final regression model was not significant ($F_{[5,59]} = 1.55$; *p* = 0.18). The final model of regression was not significant in the MHO group ($F_{[5,87]} = 0.79$; *p* = 0.55) and in the entire population ($F_{[5,152]} = 0.72$; *p* = 0.60).

Discussion

We demonstrated in this cross-sectional study of grade 1 obese patients with 2 different phenotypes of obesity that (i) MHO pa-

tients and IRO patients have comparable plasmatic levels of 25OHD, calcium, phosphorus, and PTH; (ii) in IRO patients, only age was associated with 25OHD plasmatic levels; and (iii) metabolic parameters did not predict PTH plasmatic levels in both groups.

It is largely known that individuals with obesity have lower levels of 25OHD than people from the general population (Chapuy et al. 1997; Hyppönen and Power 2007). It has already been reported that 25OHD deficiency concerns 85% of obese subjects (Hyppönen and Power 2007) compared with 30%–50% of the general population (Holick et al. 2011). In our cohort, this prevalence was 47%, which is below the aforementioned prevalence among people with obesity. This difference could be explained by the geographical location and moderate obesity of our patients. The increased prevalence of vitamin D deficiency during obesity may be explained by several mechanisms. A higher amount of abdominal fat has been reported to contribute mostly to the adipose tissue sequestration of 25OHD (Arunabh et al. 2003; Vilarrasa et al. 2007). This mechanism could partly explain the lower prevalence we found compared with more severe obesity. Other mechanisms such as nutritional deficiency due to dietary restriction or lower sun exposure can also be evocated (Hyppönen and Power 2007).

Regarding the association between levels of Vitamin D and IR, we showed that MHO and IRO patients had comparable circulating 25OHD. On the contrary, a recent study from Bellia et al. (2012) showed that 25OHD plasmatic levels were higher in MHO than in IRO with 174 subjects. This discrepancy could be explained by some methodological considerations. First, the 2 samples had different BMI: the mean BMI of included participants was 45 kg/m² (class III obesity) in Bellia's study, whereas it was 35.15 ± 2.8 kg/m² (class II obesity) in ours. As previously explained, the adipose tissue is highly associated with vitamin D regulation and higher BMI is associated with higher adipose tissue. Second, their patients were <50 years old, whereas our exclusion criterion regarding age was set at 65 years old. Thus, this inclusion criterion may explain that age was positively and significantly associated with 25OHD in the IRO group. In fact, although reasons are unclear, age is known to be associated with 25OHD levels (Lagunova et al. 2009) even though it remains a negligible factor compared with BMI or PTH (Bischof et al. 2006). Nonetheless, another study seems to corroborate the absence of differences in 25OHD levels in more severe form of obesity associated with metabolic syndrome (Hjelmessaeth et al. 2009). A clarification could be obtained from a review highlighting that vitamin D supplementation decreases IR only when the vitamin D bioavailability is considered as normal (>50 nmol/L) (Poolsup et al. 2016). Therefore, this may partly explain the lack of association in our sample given that almost half of our participants had a vitamin D deficiency (Poolsup et al. 2016).

Furthermore, our results are in agreement with most studies evaluating effects of vitamin D supplementation on IR, which mainly gave negative results in adults, arguing for an absence of link concerning the role of 25OHD in IR. Indeed, Mason et al. (2014) did not find these benefits with a 12-month supplementation among postmenopausal women, aged between 50 and 75, involved in a weight loss program. Similarly, another study carried out among African-Americans reported no effects of a 3-month supplementation on postload glucose (Harris et al. 2012). In the same vein, supplementation did not benefit IR (measured by the HOMA-IR and by the 2-h postload glucose) in healthy overweight women (Salehpour et al. 2013). In fact, these studies are corroborated by a meta-analysis showing no overall effects of the supplementation on IR (George et al. 2012). Nevertheless, this meta-analysis showed that the subgroup of patients with an abnormal glucose tolerance could benefit from this supplementation (George et al. 2012). This is further supported by Kelishadi et al. (2014) who reported a reduction in IR (measured by the HOMA-IR) in obese (pre-)adolescents after a 3-month vitamin D supplementation.

Furthermore, mean plasmatic PTH levels or bone mineral density did not differ between groups. Further, we found that 88% of

MHO had a vitamin D deficiency without elevated PTH, compared with 96% in the IRO group. Nevertheless, blunted PTH response in the presence of vitamin D deficiency is not readily apparent (Kamycheva et al. 2003). Indeed, dysfunction of the parathyroid glands such as abnormalities of the parathyroid calcium sensing receptor, or abnormalities of the 1,25(OH)₂D receptor, or even less common causes such as abnormal expression of growth repressing genes within the gland could be involved (Kamycheva et al. 2003). Another hypothesis is that obese people have elevated levels of circulating estrogens (Schindler et al. 1972), which is known to suppress RANK ligand-induced osteoclast differentiation (Shevde et al. 2000) and to increase 1 α -hydroxylase activity, which stimulates 25OHD conversion into 1,25(OH)₂D, leading to the inhibition of PTH and thus to decreased bone turnover (Tanaka et al. 1976).

Finally, some study limitations should be acknowledged, mainly related to the cross-sectional design that did not allow us to assess any causal relationships and to the small sample size that precludes stratifying the sample by subgroups to evaluate a hypothetical threshold effect. Information is lacking regarding dietary vitamin D intake, weight history, and duration of sun exposure. In addition we did not assess urinary calcium excretion or bone turnover markers, which would have been useful to better characterize the regulation of mineral metabolism in our patients. Last, we assessed IR with HOMA-IR, easily measured in routine, and not with the euglycemic hyperinsulinemic clamp.

Conclusion

Even though MHO and IRO patients have different metabolic profiles, we did not detect any difference regarding either plasmatic vitamin D concentration or the PTH level. Furthermore, in IRO the only dependent variable associated with 25OHD level was age. Thus, in view of the negative results of vitamin D supplementation on IR (Gagnon et al. 2014; Poolsup et al. 2016), our study reinforces the absence of a link between vitamin D status and IR in moderate obesity. Future studies should use a cluster randomized controlled design to analyze whether a more aggressive 25OHD supplementation among overweight individuals with normal levels of 25OHD would have effects on IR.

Conflict of interest statement

The authors have no relevant conflict of interest to disclose.

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