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ORIGINAL RESEARCH

Impact of vitamin D insufficiency on insulin homeostasis and beta cell function in nondiabetic male HIV-infected patients

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Objectives

Vitamin D is thought to play a role in glucose homeostasis and beta cell function. Our aim was to examine the impact of plasma 25-hydroxyvitamin D [25(OH)D] upon *in vivo* insulin sensitivity and beta cell function in HIV-infected male patients without diabetes.

Methods

A cross-sectional study was carried out involving a cohort of HIV-infected patients undergoing regular assessment in a tertiary hospital. Eighty-nine patients [mean (± standard deviation) age 42 ± 8 years] were included in the study: 14 patients were antiretroviral therapy (ART)-naïve, while 75 were on ART. Vitamin D insufficiency (VDI) was defined as 25(OH)D < 75 nmol/L; insulin sensitivity was determined using a 2-h continuous infusion of glucose model assessment with homeostasis (CIGMA-HOMA), using the trapezoidal model to calculate the incremental insulin and glucose areas under the curve (AUCins and AUGglu, respectively). Beta cell function was assessed using the disposition index (DI). Abdominal visceral adipose tissue (VAT) and hepatic triglyceride content (HTGC) were measured by magnetic resonance imaging (MRI) and 1-H magnetic resonance spectroscopy. Multivariate linear regression analysis was performed.

Results

VDI was associated with insulin resistance (IR), as indicated by a higher CIGMA-HOMA index (odds ratio 1.1) [1.01–1.2]. This association was independent of the main confounders, such as age, Centers for Disease Control and Prevention (CDC) stage, ART, lipodystrophy, body mass index, VAT:subcutaneous adipose tissue ratio and HTGC, as confirmed by multivariate analysis ($B = 12.3$; $P = 0.01$; $r^2 = 0.7$). IR in patients with VDI was compensated by an increase in insulin response. However, beta cell function was lower in the VDI subpopulation (33% decrease in DI).

Conclusions

VDI in nondiabetic HIV-positive male patients is associated with impaired insulin sensitivity and a decrease in pancreatic beta cell function.

Keywords: abdominal visceral fat, beta cell, HIV, insulin homeostasis, liver steatosis, vitamin D deficiency

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Introduction

The relationship between vitamin D status and metabolism is increasingly recognized as being of major importance in terms of health outcomes [1,2]. Available data now suggest that 25-hydroxyvitamin D [25(OH)D] levels of

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approximately 75–100 nmol/L (30–40 ng/mL) are associated with the best health outcomes in the general population [3,4]. Epidemiological studies have suggested that a 25(OH)D blood level above 75 nmol/L may have health benefits in terms of reducing the risk of common cancers, autoimmune diseases, type 2 diabetes, cardiovascular disease and infectious diseases [2,5–7]. However, the clinical consequences of vitamin D insufficiency (VDI) in HIV infection remain unclear [1].

Recent European studies have found the prevalence of 25(OH)D < 75 nmol/L in the HIV-infected population to range from 53 to 89%. This is likely to be the result of a combination of traditional risk factors and HIV-associated and antiretroviral therapy (ART)-specific contributors [8–10]. The Endocrine Society has recently published guidelines that recommend screening for vitamin D deficiency in all HIV-infected patients on ART, as ART drugs enhance the catabolism of 25(OH)D and 1.25(OH)2D [5].

VDI and pre-diabetes are prevalent in the general population [11]. Both conditions are associated with an increased risk of diabetes and of its major complication, cardiovascular disease (CVD) [11–13]. Vitamin D is thought to play a role in glucose homeostasis and beta cell function [14]. It has been suggested that the actions of vitamin D in glucose homeostasis are mediated by its autocrine and paracrine functions in the regulation of gene transcription in pancreatic beta cells, skeletal myocytes and immune cells through the enhancement of insulin secretion and sensitivity and the reduction of inflammation [15].

Adiposity is a determinant of 25(OH)D status and influences insulin secretion and sensitivity [14]. However, most studies assessing 25(OH)D-glucose homeostasis relationships have used the body mass index (BMI) as an indirect measure of adiposity for covariate adjustment, and have lacked direct measures of body fat or body fat topography.

In non-HIV-infected patients, consensus remains lacking on the relationship between 25(OH)D concentrations and insulin secretion and insulin sensitivity, as a consequence of conflicting results from cross-sectional and prospective studies. These results discrepancy were probably attributable to differences in the characteristics of the subjects (including age, ethnicity, obesity, physical activity and diet) and in the methods used to assess insulin secretion (oral glucose tolerance test, meal challenge or surrogate indices derived from fasting glucose and insulin levels *vs*. the gold-standard hyperglycaemic clamp), and insulin sensitivity (surrogate indices derived from fasting glucose and insulin levels *vs*. functional tests), and measures of adiposity [12,14,15]. However, on the whole, it appears that low vitamin D levels impair glucose metabolism [7]. In HIV-infected patients, the associations among VDI, pre-diabetes and type 2 diabetes remain unclear. Although a recent cross-sectional study has reported an association between vitamin D deficiency and type 2 diabetes in HIV infection, intervention trials in HIV-infected patients have yielded conflicting results, and their conclusions are questionable because of the methodological limitations involved [16–18].

In this study, we examined the relationships between plasma 25(OH)D and *in vivo* insulin sensitivity and secretion, using a 2-h continuous infusion of glucose with model assessment (CIGMA) in HIV-infected male patients without diabetes, to determine whether plasma 25(OH)D is associated with insulin sensitivity and beta cell function, independently of the visceral:subcutaneous fat ratio and hepatic triglyceride content (HTGC).

Methods

Study design

A cross-sectional observational study was carried out in the Infectious Diseases Unit of a tertiary hospital, between May 2009 and July 2010, following approval by the local independent Ethics Committee.

All men ($n = 89$) without diabetes, as confirmed by glycosylated haemoglobin (HbA1c) < 47 mmol/mol (< 6.5%) and fasting glycaemia < 7 mmol/L [19], belonging to a cohort of HIV-infected patients undergoing follow-up in the unit, with regular assessment of endocrine parameters and cardiovascular risk, and who met the following eligibility criteria, were sequentially sampled: age > 18 years; written informed consent obtained; ART-naïve or on effective ART (< 50 HIV-1 RNA copies/mL); and no changes in the previous 6 months. Patients on treatment were included only if they were receiving two or three nucleoside reverse transcriptase inhibitors (NRTIs) plus an enhanced protease inhibitor (PI) (termed 'the PI group' below) or, in the case of those who had never been treated with PIs, if they were receiving two or three NRTIs and a nonnucleoside reverse transcriptase inhibitor (NNRTI) ('the NNRTI group'). Exclusion criteria were chronic hepatitis C (confirmed by RNA PCR); active AIDS-defining disease [any Centers for Disease Control and Prevention (CDC) class C-defining disease that was diagnosed while the patient was on treatment]; active illegal drug use (methadone excluded); and concomitant treatment with drugs other than ART that interfere with insulin homeostasis.

Outcome variables

Primary

Insulin sensitivity was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR), as well as the 2-h continuous infusion of glucose with model

assessment (CIGMA). High HOMA and CIGMA-HOMA scores denote low insulin sensitivity [20].

CIGMA-HOMA: the 2-h CIGMA consists of a 180 mg/min/m^2 glucose infusion over 120 min. Three blood samples were taken at 4, 8 and 10 min for the measurement of glucose and insulin levels, and again at 120, 125 and 130 min. The means of these three samples were used to estimate the insulin resistance score using the HOMA formula.

HOMA-IR: the HOMA-IR score was calculated as fasting serum insulin (mU/mL) × fasting plasma glucose (mmol/L)/22.5. Three baseline samples, taken at 5-min intervals, were averaged to yield the mean levels of glucose and insulin.

Secondary

For beta cell function, the incremental insulin and glucose areas under the curve (AUCins and AUGglu, respectively) were calculated using a trapezoidal model. $AUC_{0-10\ min}$ and $AUC_{10-120\ min}$ were also determined for the first (0–10 min) and second (10–120 min) phases of insulin secretion, respectively.

The insulinogenic index was calculated as $(insulin_{t\ min} - insulin_{0\ min})/(glucose_{t\ min} - glucose_{0\ min})$, where subscripts '*t* min' and '0 min' indicate time-points *t* and 0, respectively.

The gold standard for beta cell function is the insulin secretion/insulin resistance (disposition) index ($\Delta I/\Delta G \div$ IR, where ΔI is the change in insulin concentration, ΔG is the change in glucose concentration, and IR is insulin resistance) [21]. We defined the disposition index as (AUCins/AUCglu) ÷ HOMA-CIGMA.

Impaired fasting glucose (IFG) was defined as fasting glucose 5.5–7 mmol/L in at least two baseline measurements [19].

Explanatory variables

At enrolment, subjects underwent a full assessment based on their medical history, a physical examination and routine haematological and biochemical tests. Lipodystrophy was determined using a standard questionnaire based on a physical examination and clinical and metabolic data [22]. Sociodemographic and lifestyle variables were examined, as well as variables associated with HIV infection and ART. Adequate physical activity was defined as at least 150 min/week of moderate-intensity aerobic physical exercise [20].

Biochemical measurements

Glucose was measured using the hexokinase method (with the Modular autoanalyser; Roche Diagnostics, Indianapolis, IN, USA). Insulin levels were measured by solid-phase sandwich chemiluminescent immunoassay (using the Immulite 2000 autoanalyser; Siemens, Flanders, NJ, USA). HbA1c levels were determined by high-performance liquid chromatography (HPLC) (using the Adams A1c HA-8160 autoanalyser; Menarini, Florence, Italy), adiponectin levels by an enzyme-linked immunosorbent assay (ELISA) (the Adiponectin ELISA; Mediagnost, Reutlingen, Germany) and apolipoprotein B (ApoB) levels by an immunoturbidimetric assay (using the Modular autoanalyzer).

25(OH) vitamin D

25(OH) vitamin D levels were determined using a Liaison® automatic chemiluminescence immunoassay analyser (DiaSorin, Stillwater, MN, USA). Total 25(OH)D reflects the sum of 25-OH-D2 + 25-OH-D3. Analytical sensitivity was 4 ng/mL. Intra-assay precision was up to 14.7 ng/mL [coefficient of variability (CV) 4.2%] and 62.7 ng/mL (CV 3.1%), and inter-assay precision was up to 14.7 ng/mL (CV 7.7%) and 62.7 ng/mL (CV 6.4%). The following definitions of vitamin D status were used in this study: vitamin D sufficiency (VDS) was defined as 25(OH)D ≥ 75 nmol/L, VDI as 25(OH)D < 75 nmol/L, and vitamin D deficiency as 25(OH)D < 50 nmol/L.

Abdominal fat distribution and intrahepatic lipids

Dual energy X-ray absorptiometry (DEXA) was used to evaluate the percentage body fat (Lunar-DPX-IQ, system number 5808, software 4.7e, GE Healthcare, Chalfont St Giles, UK). Abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were measured by 10-mm single-shot axial magnetic resonance imaging (MRI), between the L4 and L5 vertebrae. HTGC was measured by 1-H magnetic resonance spectroscopy (1H-MRS) (1.5 T GyroscanIntera; Philips Medical Systems, Best, the Netherlands) [23]. Steatosis was defined as > 5% HTGC.

Statistical methods

Values are given as mean ± standard deviation (SD) and as median (25th–75th percentile) where appropriate. Continuous variables were compared with the Student *t*-test or the Mann–Whitney *U*-test. Frequencies were compared with the χ^2 test or Fisher's exact test. Correlations between glucose homeostasis and explanatory variables were tested using Pearson and Spearman correlations where appropriate. Multivariate unconditional linear regression analyses were performed to determine whether an independent association existed between the degree of insulin resistance (CIGMA-HOMA) and beta cell function (disposition index) and VDI. A two-tailed *P*-value of < 0.05 was considered significant. All statistical analyses were performed with SPSS version 17.0 (SPSS Software, Chicago, IL).

Results

Eighty-nine men meeting the inclusion criteria were included in the study. The mean age was 42 ± 8.2 years, the mean duration of HIV infection was 7.8 ± 5.6 years, and the mean CD4 count was 467 cells/μL (range 364–677 cells/μL); 59.6 and 19.1% of the patients were in the CDC class A and C categories, respectively. Seventy-five patients (84.2%) were receiving ART, with a mean time on ART of 67 ± 42 months, and 43.8% belonged to the PI group. Twenty-seven patients [30.7%; 95% confidence interval (CI) 21–40%] presented with lipodystrophy. Mean 25(OH)D was 52.2 ± 27.5 nmol/L. Liver steatosis was present in 24 patients (33.3%; 95% CI 23–42%; available data for HTGC in 72 patients).

Circulating 25(OH)D levels were negatively correlated with HTCG in the right liver lobe (Spearman's rho –0.26; $p = 0.02$), while no correlation was observed between 25(OH)D concentration and parameters related to carbohydrate metabolism, insulin homeostasis, ApoB or adiponectin concentration or body fat distribution.

Factors associated with 25(OH)D < 75 nmol/L are shown in Table 1. VDI was associated with ART use and a sedentary lifestyle. Thirty-seven per cent of patients

Table 1 Factors associated with vitamin D insufficiency in nondiabetic men with HIV infection

	25(OH)D < 75 nmol/L ($n = 72$)	25(OH)D > 75 nmol/L ($n = 19$)	*P*
Age (years) (mean ± SD)	42.8 ± 8.4	38.7 ± 7.1	0.06
Duration of HIV infection (years) (mean ± SD)	7.7 ± 5.7	7.9 ± 5.4	0.8
CD4 count (cells/μL) [median (P25–75)]	470 (368–689)	442 (359–578)	0.3
HIV VL in patients on ART (copies/mL) [median (P25-75)]	39 (39–39)	39 (39–39)	–
HIV VL in naïve patients (copies/mL) [median (P25-75)]	20 100 (2910–44 684)	21 900 (10 000–79 617)	0.4
CDC stage [% (*n*)]			
A	55.6 (40)	76.5 (13)	0.2
B	25 (18)	5.9 (1)	
C	19.4 (14)	17.6 (3)	
Treatment group [% (*n*)]			
Naïve	11.1 (8)	35.3 (6)	0.02*†
NNRTI	44.4 (32)	23.5 (4)	
PI	44.4 (32)	41.2 (17)	
Duration of ART (years) (mean ± SD)	66.8 ± 43.2	69.6 ± 37.4	0.8
Physical exercise < 2.5 h/week [% (*n*)]	84.8 (56)	46.7 (7)	0.03†
Baseline glycaemia (mean ± SD)	96.7 ± 10.2	92 ± 6.7	0.2
IFG [% (*n*)]	37.5 (27)	11.8 (2)	0.057
Insulin resistance (HOMA-baseline ≥ 3.8) [% (*n*)]	12.5 (9)	0 (0)	0.19
HbA1c (%) (mean ± SD)	4.5 ± 0.46	4.3 ± 0.29	0.1
HOMA-baseline (mean ± SD)	1.9 (1.3-2.8)	1.4 (1.1-1.9)	0.02†
CIGMA-HOMA (mean ± SD)	11.7 (5.4-2.9)	7.2 (4.8–11.8)	0.02†
AUC glucose (mg/h/dL) (mean ± SD)	261.8 ± 45	235 ± 28	0.02†
AUC insulin (μU/h/mL) [median (P25-75)]	50.1 (35–77)	34.7 (25–48)	0.02†
AUC ratio (insulin/glucose) (μU/mg) [median (P25-75)]	19 (14–30)	15.5 (9.3–22)	0.06
Disposition index (mean ± SD)	1.9 ± 1.1	2.6 ± 1.6	0.05†
Apolipoprotein B (g/L) (mean ± SD)	0.88 ± 0.22	0.77 ± 0.2	0.06
Adiponectin (μg/mL) (mean ± SD)	7.5 ± 5.1	7.6 ± 3.6	0.9
BMI (kg/m^2) (mean ± SD)	24.9 ± 3.4	23.9 ± 3.3	0.2
WHR (mean ± SD)	0.95 ± 0.07	0.93 ± 0.07	0.3
Lipodystrophy [% (*n*)]	31 (22/71)	29.4 (5/17)	0.89
VAT:SAT ratio [median (P25-75)]	0.56 (0.35–1)	0.35 (0.15–0.61)	0.04†
SAT (mm^3) [median (P25-75)]	3469 (2536–5414)	3569 (1661–5527)	0.9
VAT (mm^3) [median (P25-75)]	2074 (1271–4037)	900 (678–2283)	0.02†
Fat mass DEXA (%) (mean ± SD)	16.8 ± 6.6	14.3 ± 6.6	0.2
HTGC in right hepatic lobe (%) [median (P25-75)]	3.3 (1.8–8.4)	1.6 (0.8–2.7)	0.04†
HTGC in left hepatic lobe (%) [median (P25-75)]	3.59 (2.1–6.2)	2.45 (0.6–3.7)	0.08
Steatosis (yes) [% (*n*)]	38.6 (22/57)	13.3 (2/15)	0.08

To convert to International System of Units: glucose (mg/dL × 0.05551) mmol/L; insulin (μU/mL × 6.945) pmol/L.
ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; IFG, impaired fasting glucose; HOMA, homeostasis model assessment of insulin resistance; CIGMA, 2-h continuous infusion of glucose with model assessment; AUC, area under curve; BMI, body mass index; WHR, waist-to-hip ratio; VAT, visceral adipose tissue; VL, viral load; SAT, subcutaneous adipose tissue; HTCG, hepatic triglyceride content; DEXA, dual energy X-ray absorptiometry; P25–75, 25th–75th percentile; SD, standard deviation.
*Naïve *vs.* ART-experienced.
†$P < 0.05$.

Table 2 Association between explanatory variables and insulin resistance and beta cell function

	CIGMA-HOMA		Disposition index	
	r	*P*	*r*	*P*
Age (years)	0.6	**< 0.001**	–0.51*	**< 0.001**
Duration of HIV infection (years)	0.27	**0.01**	–0.25*	**0.01**
CD4 count (cells/μL)	–0.04	0.3	–0.001	0.9
HIV VL (copies/mL)	–0.11	0.3	0.17	0.1
Duration of ART (years)	0.47	**< 0.001**	–0.37*	**< 0.001**
Non-NRTI exposure (years)	0.3	**0.004**	–0.26*	**0.01**
NRTI exposure (years)	0.43	**< 0.001**	–0.33*	**0.004**
PI exposure (years)	0.29	**0.005**	–0.21*	**0.04**
BMI (kg/m^2)	0.35	**0.001**	–0.1*	0.3
WHR	0.47	**< 0.001**	–0.3*	< 0.001
VAT:SAT ratio	0.36	**0.002**	–0.26	**0.03**
SAT (mm^3)	0.05	0.6	0.01	0.9
VAT (mm^3)	0.39	**0.001**	–0.25	**0.04**
Fat mass DEXA (%)	0.09	0.4	0.1*	0.3
HTGC in right hepatic lobe (%)	0.34	**0.004**	–0.26	**0.02**
HTGC in left hepatic lobe (%)	0.38	**0.006**	–0.27	0.05
	CIGMA-HOMA		**Disposition index**	
	Median (P25–75)	***P***	**Mean ± SD**	***P***
CDC stage				
A	9.9 (5.1–16.2)		2.07 ± 1.1	0.6†
B	11.8 (5.3–21.4)	0.6†	1.88 ± 1	
C	8.6 (4–19.4)		2.3 ± 1.7	
Treatment group				
Naïve	5.4 (4.7–9.6)		2.67 ± 0.9	0.14†
NNRTI	11 (5.3–20.6)	0.14†	2 ± 1.29	
PI	14.3 (7.2–21.4)		1.9 ± 1.28	
Physical exercise				
< 2.5 h/week	11.6 (5.5–21.4)		1.88 ± 1.1	**0.02**
> 2.5 h/week	7.1 (4.7–14.9)	**0.03**	2.66 ± 1.6	
Lipodystrophy				
Yes	15.9 (8.6–29.8)		1.55 ± 0.8	**0.002**
No	9.2 (4.8–16)	**0.006**	2.3 ± 1.3	
Steatosis				
Yes	17.5 (7.7–29.4)		1.9 ± 1.8	0.6
No	9.4 (4.9–15.2)	**0.03**	2.1 ± 1	

BMI, body mass index; CDC, Centers for Disease Control and Prevention; CIGMA-HOMA, 2-h continuous infusion of glucose model assessment with homeostasis model assessment; DEXA, dual energy X-ray absorptiometry; WHR, waist-to-hip ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; HTCG, hepatic triglyceride content; ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; P25–75, 25th–75th percentile; PI, protease inhibitor; SD, standard deviation; VL, viral load.
In the first part of the table, Spearman's rho and *Pearson's correlation were used, as appropriate; in the second part of the table, Student's *t*-test and †analysis of variance (ANOVA) were used, as appropriate. Bold indicates $P < 0.05$.

with VDI had IFG, *vs.* 11.8% in the subgroup with VDS ($P = 0.05$).

Factors associated with CIGMA-HOMA and disposition index scores are presented in Table 2.

Insulin sensitivity and VDI

VDI was associated with a high CIGMA-HOMA index after near-steady-state 5% glucose infusion (Fig. 1). Multivariate analysis confirmed an independent association between a high CIGMA-HOMA score and 25(OH)D < 75 nmol/L [$B = 12.3$; $P = 0.01$; coefficient of determination (r^2) = 0.7] (Table 3). This association persisted on replacing VDI with serum 25(OH)D levels in the same regression model ($B = –0.66$; $P = 0.01$; $r^2 = 0.7$).

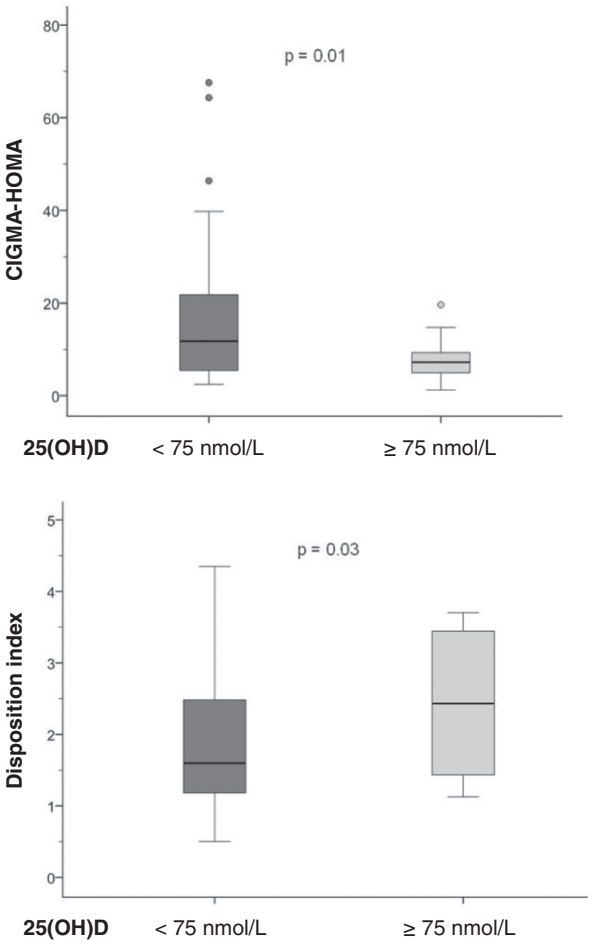

Fig. 1 Insulin sensitivity and beta cell function according to vitamin D status. Box plots represent the median (25th–75th percentile) of each variable. *P*-values were obtained using the Mann–Whitney *U*-test. CIGMA-HOMA, 2-h continuous infusion of glucose model assessment with homeostasis model assessment.

Beta cell function and VDI

While there were no differences in insulinogenic index during intravenous glucose loading at different time-points, there was an increase in insulin secretion, as reflected by a higher AUCins:AUCglu ratio, in the VDI subpopulation (Table 1), compensating insulin resistance. This higher insulin response was also demonstrable in the first and second phases of insulin secretion in CIGMA (Fig. 2).

Table 3 Variables predicting insulin resistance and beta cell function in nondiabetic men with HIV infection

	Multiple stepwise regression					
	CIGMA-HOMA			Disposition index		
	B	r^2	*P*	*B*	r^2	*P*
		0.7*	**0.001***		**0.36†**	**0.005†**
BMI (kg/m^2)	0.36		0.74			
Fat mass (%)	0.97		**0.04**			
Visceral fat (%)	−0.13		0.7			
VAT:SAT ratio	1.7		0.58			
HTGC in right hepatic lobe (%)	0.41		0.17			
Duration of ART (years)	‡		‡			
PI/r exposure (years)	2.22		0.08			
Vitamin D insufficiency (yes)	12.3		**0.01**	0.03		0.9
Age (per 10 years)	−3.37		0.45	−0.62		**0.007**
WHR	120.9		0.05	0.94		0.71
Lipodystrophy (yes)	4.9		0.37	−0.26		0.48
Duration of ART (years)	‡		‡	‡		‡
NRTI exposure (years)	−0.14		0.92	−0.12		0.22
Physical exercise (< 3 h/week)				−0.79		0.06
Duration of HIV infection (years)				0.37		0.33
NNRTI exposure (years)				−0.01		0.99
PI exposure (years)				0.009		0.91

CIGMA-HOMA and the disposition index are the dependent variables. Explanatory variables with statistically significant results in multiple regression analysis, and their significance, are shown in bold.
B, standardized coefficient; r^2, coefficient of determination; BMI, body mass index; CDC, Centers of Disease Control and Prevention; CIGMA-HOMA, 2-h continuous infusion of glucose model assessment with homeostasis model assessment; WHR, waist-to-hip ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; HTCG, hepatic triglyceride content; ART, antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor.
*For the multiple stepwise regression analysis of CIGMA-HOMA, the following were included as explanatory variables: variables with a statistical significance of $P < 0.05$ in simple analysis (shown in the table), and those with a statistical significance of $P < 0.1$ in simple analysis, namely, steatosis, VAT, duration of HIV infection, nadir CD4 count, duration of exposure to NNRTIs, and other clinically relevant variables such as CDC stage (C *vs.* A/B) and ART group (not represented).
†For the multiple stepwise regression analysis of the disposition index, the following were included as explanatory variables: variables with a statistical significance of $P < 0.05$ in simple analysis (shown in the table), and those clinically relevant such as CDC stage (C *vs.* A/B) and ART group (not represented).
‡Variable automatically excluded from the model by the processor.

However, VDI was associated with impairment of pancreatic beta cell function; the disposition index in HIV-infected male patients with VDI was 33% lower than in those with VDS (Fig. 1). Nevertheless, multivariate analysis did not confirm an independent association between beta cell function and 25(OH)D (Table 3). Only age was shown to be an independent factor associated with beta cell function. The disposition index was also lower in patients with IFG than in those with normal glucose tolerance (NGT) (13 *vs.* 19, respectively; $P = 0.003$).

Abdominal fat distribution, HTGC and VDI

No difference in total fat percentage was observed between the two subpopulations of vitamin D status. However, the subgroup of patients with VDI had a greater amount of VAT, an increased VAT:SAT ratio and an increased amount of liver fat (Table 1).

Discussion

The present study showed the parameters of insulin resistance to be higher in subjects with VDI [25(OH)D < 75 nmol/L], even after adjusting for the main confounders such as body composition, visceral/subcutaneous fat and intrahepatic lipids [12]. This was confirmed by the multivariate linear regression model, which was able to explain 70% of the variability in HOMA-CIGMA in our population. This increase in insulin resistance in patients with VDI was accompanied by a compensatory increase in AUC insulin secretion, despite the impairment of pancreatic beta cell function as assessed using the disposition index.

The strengths of the present study include the use of validated functional measures of both insulin resistance and beta cell function, as well as the use of direct measures of adiposity and intrahepatic lipids. Because adiposity is a strong determinant of insulin sensitivity and secretion, in our study we used three methods to evaluate the different components that may modulate the influence of VDI on insulin homeostasis. In addition, we used a functional test based on a near-steady-state 5% glucose infusion, which has been validated against independent measures of insulin sensitivity and beta cell function, including clamp-derived measures [24]. Moreover, CIGMA offers better beta cell function discrimination across subjects with NGT, IGT

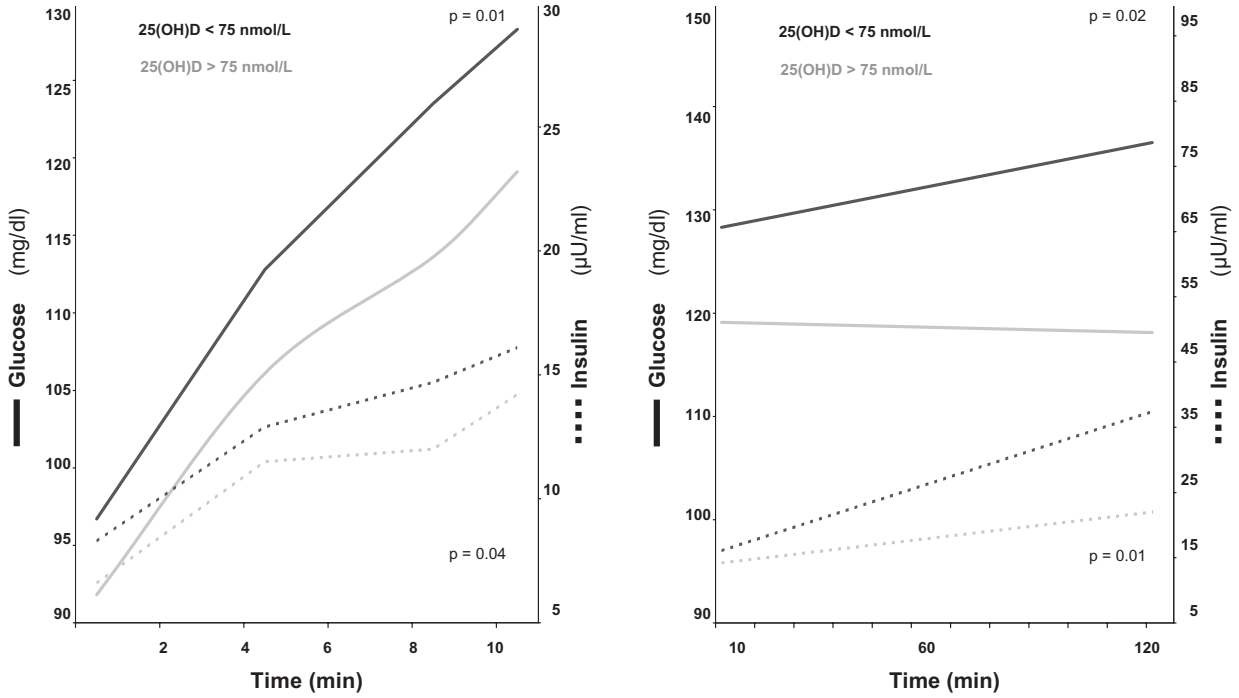

Fig. 2 Area under the curve (AUC) for glucose and insulin in response to 2-h continuous infusion of glucose model assessment (CIGMA) according to vitamin D status. The first (0–10 min) (right) and second (10–120 min) (left) phases of glucose response and insulin secretion are shown. *P* represents the significance of the difference in glucose or insulin AUC based on the presence or absence of vitamin D insufficiency, calculated using Student's *t*-test or the Mann–Whitney *U*-test, respectively. Differences in the AUC values in patients with vitamin D insufficiency *vs.* vitamin D sufficiency, in the first and second phases: AUC for glucose, 0–10 min: 19 ± 2 *vs.* 17.8 ± 1.4 mg/h/dL; AUC for glucose, 10–120 min: 242.8 ± 44 *vs.* 217.5 ± 27.6 mg/h/dL; AUC for insulin, 0–10 min: 2.1 (1.5-3.2) *vs.* 1.8 (1.3-2.2) μU/h/mL; AUC for insulin, 10–120 min: 48.1 (33–75) *vs.* 33.2 (24–46) μU/h/mL. Data are expressed in mg/dL × 0.05551 mmol/L (glucose) and μU/mL × 6.945 pmol/L (insulin).

and type 2 diabetes than measurements derived from the frequently sampled intravenous glucose tolerance test (FSIVGTT) first-phase insulin response, used in other studies in non-HIV-infected populations [25]. The external validity of our results is supported by the methodological robustness of the primary objectives. Furthermore, the fact that our multivariate linear regression model was capable of explaining 70% of the variability in CIGMA-HOMA suggests that it included the major confounding factors. To our knowledge, this is the first study to use this meticulous methodological approach in patients with HIV infection.

The limitations of this study are its small sample size, conditioned by the stringent inclusion criteria, the need for complex functional tests, and the use of expensive imaging procedures. In addition to cross-sectional studies such as this, prospective studies may be useful for examining the impact of 25(OH)D on subsequent changes in insulin homeostasis and the development of type 2 diabetes, and for identifying other temporal risk factors. Although we did not measure sunlight exposure (the study was conducted in Alicante, a Mediterranean city on the southeast coast of Spain; latitude 38°/23° north, with more than 320 days of sun per year), and blood sampling was performed throughout the year, this does not affect the primary endpoint.

Vitamin D regulates the production and secretion of several hormones in addition to maintaining calcium homeostasis, reflecting the ubiquitous distribution of the vitamin D receptor (VDR) and the enzyme CYP27B1 (cytochrome P450, family 27, subfamily B, polypeptide 1) which produces the preferred ligand for VDR, 1.25(OH)2D [2,7].

There is mechanistic support for the hypothesis that vitamin D may influence both insulin secretion and insulin sensitivity, and subsequently the incidence of type 2 diabetes. Previous studies have reported the presence of a specific VDR on pancreatic beta cells and skeletal muscle; the expression of the 1-α-hydroxylase enzyme by pancreatic beta cells; the presence of a vitamin D response element in the human insulin gene promoter; peroxisome proliferator-activated receptor-δ activation; an increase in beta cell replication and resistance to apoptosis; and down-regulation of inflammatory cytokines [14,15,26].

The inverse relationship between 25(OH)D and insulin resistance detected in our study is consistent with associations seen in previous studies in the general and HIV-infected

populations [6,27]. However, when evaluating the relationship between 25(OH)D and glucose homeostasis parameters, it is imperative to use sensitive assessments of body composition and body fat topography in order to differentiate between obesity-modulated and independent relationships, as lower concentrations of 25(OH)D are associated with higher total and abdominal adiposity, which is a strong determinant of insulin sensitivity [12,14].

We used MRI to evaluate VAT:SAT, and DEXA to measure the percentage of body fat. Although DEXA showed no difference in total fat percentage between the VDI and VDS subpopulations, MRI showed abdominal fat distribution to be the factor most strongly associated with the presence of VDI. These results support the need to quantify VAT and the VAT:SAT ratio when the main objective is to study the impact of 25(OH)D on insulin homeostasis in men with HIV infection.

On examining the results for liver fat measured by 1H-MRS, we observed that VDI patients had a greater lobe HTGC and a 3-fold higher prevalence of liver steatosis, which would explain the increase in hepatic insulin resistance. As found by Hammond *et al.* [27], the presence of lipodystrophy was not associated with VDI. However, as the linear regression model indicated lipodystrophy as a determining factor for increased insulin resistance, we recommend its evaluation.

Both genetic and acquired factors (glucotoxicity, lipotoxicity and incretin deficiency/resistance) play a major role in the decline in beta cell function. Our data demonstrate significantly impaired pancreatic beta cell function in HIV-infected male patients with VDI. This seems to be in contradiction to the relatively high insulin levels detected in the VDI subpopulation using the AUCins:AUCglu ratio, but is in fact usual in patients with insulin resistance who show compensatory insulin hypersecretion before type 2 diabetes appears. Correct determination of insulin secretion in an individual therefore requires validation of results in relation to insulin sensitivity. It is thus important to distinguish between the plasma insulin response and beta cell health. Even within the NGT range, beta cell function is the best predictor of progression to IGT and subsequently to type 2 diabetes [21].

In agreement with these data, two prospective 2.7- and 3-year follow-up studies in the general population at risk of type 2 diabetes have confirmed higher baseline 25(OH)D to independently predict a lower risk of incident diabetes and better beta cell function at follow-up [28,29].

With regard to the HIV-infected population, a recent cross-sectional study in 1811 HIV-infected Italian patients showed that, after controlling for other factors, vitamin D deficiency (< 50 nmol/L) was associated with 1.85-fold higher odds of type 2 diabetes [16]. Also, in a retrospective subset analysis of nondiabetic Modena cohort participants with HIV infection ($n = 1574$), 30 000 IU of D3 weekly was associated with an 83% decrease in the incidence of diabetes (hazard ratio 0.17; 95% CI 0.04–0.72) [30].

In HIV infection, only two intervention studies have evaluated the effect of cholecalciferol supplementation on insulin sensitivity, both with negative results [17,18]. The limitations of the studies, such as small sample size, short duration of follow-up, the use of surrogate indices of insulin sensitivity derived from fasting glucose and insulin levels, and the inability to achieve concentrations of 25(OH)D $\geq$ 75 nmol/L (i.e. VDS), reduced the validity of their conclusions.

It is plausible that vitamin D plays a role in improving insulin secretion and sensitivity but may not be effective in insulinopenic situations, or that beyond a certain concentration of vitamin D further supplementation of VDS subjects might be of no value in improving glucose homeostasis. Prospective studies are needed to determine the clinical impact of lower 25(OH)D levels in HIV-infected male patients, and to establish whether VDI in these patients should be regarded as a pre-diabetes equivalent.

The main contribution of our research is the demonstration that 25(OH)D < 75 nmol/L in HIV-infected male patients is associated with impairment of insulin sensitivity and a decrease in pancreatic beta cell function. In fact, to date, no studies in the HIV-infected population have evaluated this association after adjusting for main confounders and validated dynamic tests of insulin resistance and beta cell function. On the basis of our results, with a reduction of one-third in the disposition index in patients with 25(OH)D < 75 nmol/L, and considering the data available in the literature, VDI should be regarded as a factor conferring an increased risk of diabetes (pre-diabetes), at least in the HIV-infected population.

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