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ORIGINAL ARTICLE

Impact of circulating bacterial DNA in long-term glucose homeostasis in non-diabetic patients with HIV infection: cohort study

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Abstract In HIV-infected patients, the damage in the gut mucosal immune system is not completely restored after antiretroviral therapy (ART). It results in microbial translocation, which could influence the immune and inflammatory response. We aimed at investigating the long-term impact of bacterial-DNA translocation (bactDNA) on glucose homeostasis in an HIV population. This was a cohort study in HIV-infected patients whereby inclusion criteria were: patients with age >18 years, ART-naïve or on effective ART (<50 HIV-1 RNA copies/mL) and without diabetes or chronic hepatitis C. Primary outcome was the change in HbA1c (%). Explanatory variables at baseline were: bactDNA (qualitatively detected in blood samples by PCR [broad-range PCR] and gene 16SrRNA - prokaryote), ART exposure, HOMA-R and a dynamic test HOMA-CIGMA [continuous infusion of glucose with model assessment], hepatic steatosis (hepatic triglyceride content - 1H-MRS), visceral fat / subcutaneous ratio and inflammatory markers. Fifty-four men (age 43.2 ± 8.3 years, BMI 24.9 ± 3 kg/m^2, mean duration of HIV infection of 8.1 ± 5.3 years) were included. Baseline HbA1c was 4.4 ± 0.4% and baseline presence of BactDNA in six patients. After 8.5 ± 0.5 years of follow-up, change in HbA1c was 1.5 ± 0.47% in patients with BactDNA vs 0.87 ± 0.3% in the rest of the sample $p < 0.001$. The change in Hba1c was also influenced by protease inhibitors exposure, but not by baseline indices of insulin resistance, body composition, hepatic steatosis, inflammatory markers or anthropometric changes. In non-diabetic patients with HIV infection, baseline bacterial translocation and PI exposure time were the only factors associated with long-term impaired glucose homeostasis.

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Introduction

HIV infection and antiretroviral therapy (ART) are associated with perturbations in glucose metabolism and increasing incidence of diabetes [1, 2]. Current ART regimens are less toxic for cellular function and metabolism but have failed to completely eliminate metabolic dysfunction associated with HIV infection [3]. Several causes of this residual inflammation have been proposed including ongoing low-level HIV replication and microbial translocation associated with a dysbiotic intestinal community and an impaired gut barrier [4–6].

Type 2 diabetes and insulin resistance are associated with changes in gut bacterial composition or function (dysbiosis) [7]. Bacterial components and metabolites, such as short chain fatty acids (SCFAs) produced by gut microbes, may be related to the development of insulin resistance and type 2 diabetes. An increase in plasma lipopolysaccharides (LPS) and bacterial

fragments (bacterial translocation), defined as metabolic endotoxaemia, are associated with a low-grade systemic inflammation and are involved in host metabolism-microbiome interactions.

Studies in HIV infected untreated patients have suggested that determination of microbial translocation may be useful in predicting clinical events, while reports in ART-experienced individuals are less consistent. In a cross-sectional study among HIV-infected patients, high LPS was associated with a lower insulin sensitivity [8]. However, there is no data about the effect of metabolic endotoxemia on long-term glucose homeostasis.

We aimed at investigating the impact of bacterial-DNA translocation on glucose homeostasis evolution in an HIV population.

Material and methods

Study population

A cohort study was carried out in the Infectious Diseases and Endocrinology Units of a tertiary hospital in Alicante (Spain). The local ethics committee approved the study. All HIV-infected men from a cohort of 600 HIV-infected patients, with regular assessment of endocrine parameters and cardiovascular risk, were invited to participate in this study if they were ≥18 years of age, ART-naïve or on effective ART (< 50 copies RNA/mL), with no changes in the previous 6 months. Only patients receiving two nucleoside-reverse transcriptase inhibitors (NRTIs) and a boosted protease inhibitor (PI/r) or a non-NRTI (NNRTI) (efavirenz or nevirapine) that had never been treated with PIs were included. Those with chronic hepatitis C, diabetes mellitus, active AIDS disease, active illegal drug use, or psychiatric illness were excluded. All patients gave written informed consent. The baseline study was conducted from March 2009 to October 2010, and glucose homeostasis status updated data from January 2016 to September 2016.

Participants were required to fast for 12 h prior to the blood sample, which was performed between 8:00 and 9:00 AM. The samples were centrifuged and serum and plasma were stored at −70 °C until determination.

Outcome variable

Change in HbA1c (%) was the outcome variable and levels were determined at baseline and at the end of the follow-up by high-performance liquid chromatography (HPLC) (using the Adams A1c HA-8160 autoanalyzer; Menarini, Florence, Italy).

Explanatory variables

Clinical evaluation

At enrolment, subjects underwent a full assessment based on their medical history, a physical examination and routine hematological and biochemical tests. Sociodemographic and lifestyle variables were examined, as well as variables associated with HIV infection and ART.

Bacterial translocation

Bacterial DNA was studied in blood. Total DNA extraction was carried out by using the QIAamp DNA blood Mini kit (QIAgen), according to the manufacturer's instructions. A broad-range polymerase chain reaction (PCR) followed by nucleotide sequencing of the 16SrRNA gene was performed according to the methodology described elsewhere [9].

Insulin homeostasis

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), according to the methodology previously described [10]. High HOMA and CIGMA-HOMA scores denote low insulin sensitivity.

Abdominal fat distribution and intrahepatic lipids

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. Hepatic triglyceride content (HTGC) was measured by 1-H magnetic resonance spectroscopy (1H–MRS) (1.5 T GyroscanIntera; Philips Medical Systems, Best, the Netherlands) [11]. Hepatic steatosis was defined as HTGC > 5%.

Systemic inflammatory markers

Systemic inflammatory markers were measured: high sensitivity C-reactive protein (hsCRP) (turbidimetry kinetics; IMMAGE, Beckmann Coulter, Inc., Chasca, MN, USA), plasminogen activator inhibitor-1 (PAI-I), tumor necrosis factor-alpha (TNF-a), soluble forms of TNF-1 and -2 receptors (sTNF1R, sTNF2R) and interleukin-6 (IL-6) (enzyme immunoassay; Quantikine, R&D Systems, Abingdon, UK); adiponectin levels were measured by an enzyme-linked immunosorbent assay (ELISA) (the Adiponectin ELISA; Mediagnost, Reutlingen, Germany).

Statistical analysis

The associations between the different variables and bactDNA were calculated using the chi-square test for qualitative variables and the Student t-test or Mann-Whitney U-test for quantitative variables. Linear multivariate regression analysis was used to identify factors independently associated with HbA1c change, using variables with $p < 0.1$ statistical significance in the bivariate analysis, and those clinically relevant. In all cases, a p-value of <0.05 was considered statistically significant. The SPSS version 19.1 statistical package (SPSS Inc., Chicago, IL, USA) was used throughout.

Results

Baseline characteristics

Eighty-nine patients were included in baseline evaluation and monitored for a median of 8.5 years; 35 patients were lost in the follow-up, so finally 54 subjects were included in the study. Mean age was 43.2 ± 8.3 years, BMI 24.9 ± 3 kg/m^2, and mean duration of HIV infection was 8.1 ± 5.3 years, while 51.9% were in CDC class A, 94.4% on ART, with a mean time on ART of 64 ± 41 months. Thirty-four and 32.6% presented lipodystrophy and hepatic steatosis, respectively. Baseline characteristics did not differ in patients with missed follow-up. Bacterial translocation was present in six patients (bactDNA+) at baseline. After 8.5 ± 0.5 years of follow-up mean change in Hba1c was 0.94 ± 0.4%.

Hba1c change, bacterial translocation, insulin homeostasis, hepatic steatosis and abdominal fat distribution

It should be noted that at baseline, bactDNA+ patients were thinner and had a lower HbA1c (Table 1). There was no difference in the other baseline clinical, HIV-related and metabolic parameters evaluated between bactDNA+ and bactDNA-. After follow-up, bactDNA+ was associated with a greater change in HbA1c (1.5 ± 0.47 vs 0.87 ± 0.3%;

Table 1 Baseline clinical–metabolic parameters and bacterial translocation

Parameter	Bact DNA negative	Bact DNA positive	p-value
Age, years Mean ± SD	43.3 ± 8.2	41.5 ± 11.4	0.62
HIV evolution, years Mean ± SD	7.9 ± 5.2	9.5 ± 5.8	0.51
Hba1c (%) Mean ± SD	4.5 ± 0.41	3.9 ± 0.63	**0.01**
Glucose (mg/dl) Mean ± SD	97.1 ± 9.9	92.8 ± 5.63	0.31
HOMA-IR Median (P_{25}-P_{75})	2.01 (1.35–2.73)	1.52 (1.06–2.38)	0.36
CIGMA-HOMA Mean ± SD	15 ± 12.6	16.8 ± 11.7	0.73
Total cholesterol (mg/dl) Mean ± SD	196 ± 41	195.5 ± 39	0.92
LDL cholesterol (mg/dl) Mean ± SD	132.9 ± 39.9	134.5 ± 30.6	0.97
HDL cholesterol (mg/dl) Mean ± SD	48.3 ± 11.5	54.6 ± 10.5	0.21
BMI (kg/m^2) Mean ± SD	25.2 ± 3	22.3 ± 2.7	**0.03**
VAT/SAT ratio Mean ± SD	2.06 (1.13–3.71)	1.2 (0.98–2.09)	0.19
HTGC right lobe (%) Median (P_{25}-P_{75})	2.84 (1.69–9.68)	2.18 (1.38–4.13)	0.36
Median (P_{25}-P_{75})			

SD standard deviation, *HDL* high-density lipoprotein, *BMI* body mass index, *HOMA-IR* homeostatic model assessment-insulin resistance, *CIGMA* continuous infusion of glucose with model assessment, *VAT/SAT* ratio abdominal visceral adipose tissue / subcutaneous adipose tissue, *HTGC* hepatic triglyceride content

Qualitative variables were expressed as relative and absolute frequencies. Parametric variables were expressed as means ± SD, nonparametric variables as medians and percentiles 25–75. The differences between populations were evaluated using Student t-test or Mann-Whitney U-test. Statistically significant values are in bold text

$p < 0.001$ [Fig. 1]). Linear bivariate analysis confirmed bactDNA+ impact in HbA1c change (B, standardized coefficient 0.67 [0.28–1.04] ($p < 0.001$)) and discharged baseline indices of insulin resistance, VAT/SAT ratio, lipodystrophy, hepatic steatosis, and anthropometric changes as predictor factors of impaired glucose homeostasis evolution.

Hba1c change, ART and systemic inflammatory markers

Linear bivariate analysis identified PI exposure time (years) as the only ART variable influencing HbA1c change (B, standardized coefficient 0.064 [0.02–0.1] (p 0.007)). Regarding inflammation, baseline sTNF1R level was associated positively with HbA1c change (p = 0.02) and hsCRP was close to statistically significant association (p = 0.052).

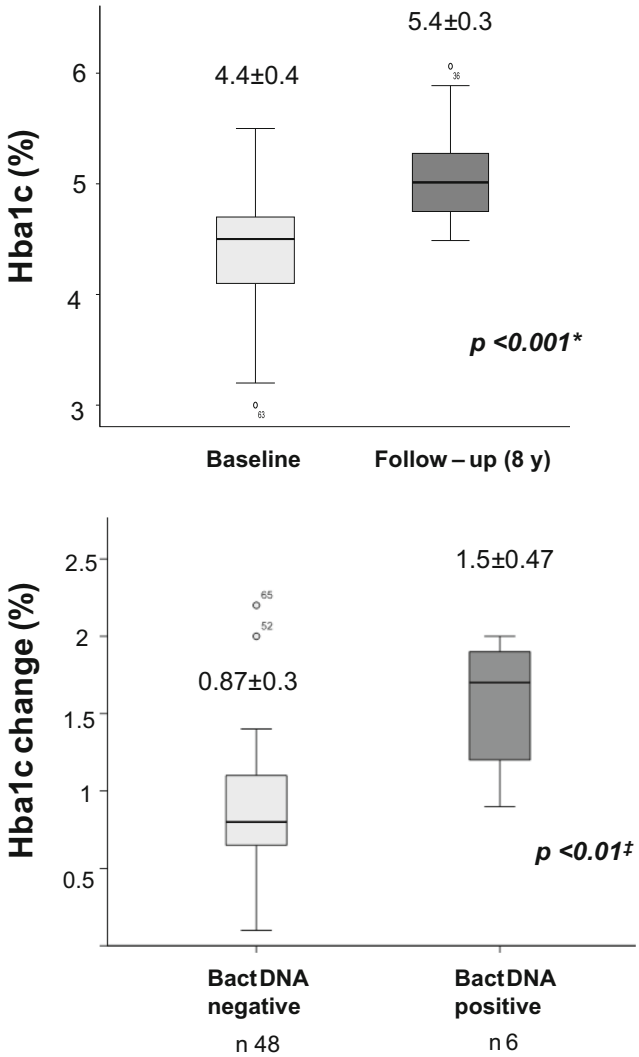

Fig. 1 HbA1c change in global cohort and according to bacterial traslocation at baseline. *Box plots* represent the median (25th–75th percentile) of each variable. *p*-values were obtained using Student t-test* and the Mann–Whitney U-test‡

Multivariate analysis

The multivariate linear regression model analysis, adjusted by confounding factors, confirmed an independent association between bactDNA, PI exposure time and HbA1c change (Table 2).

Discussion

The present study shows that, in non-diabetic HIV-infected males, bacterial translocation and PI exposure time are the only baseline factors associated with long-term impaired glucose homeostasis, even after adjusting for the main confounders such as BMI, age, duration of HIV infection and time on ART [3]. In addition, we found that a higher sTNF1R level was positively associated with HbA1c change; however, after multivariate analysis this association did not remain significant. To our knowledge, this is the first study that has evaluated the effect of bacterial translocation on impaired glucose homeostasis evolution in an HIV population.

The strengths of the present study are: the exhaustive baseline evaluation, use of validated functional measures of insulin resistance (CIGMA-HOMA), as well as the use of "gold standard" direct measures of adiposity and intrahepatic lipids (1H–MRS). The fact that the multivariate linear regression model was able to explain 60% of the variability in HbA1c change suggests that it included the major confounding factors. Some limitations should be acknowledged in the interpretation of our results, mainly the small sample size, conditioned by the stringent inclusion criteria and the high number of losses due to the long follow-up.

Gut microbiome has been reported to influence almost all organs and systems that contribute to metabolic control: it modulates appetite and food intake, absorption of nutrients from the gut, hepatic steatosis, inflammation, triglyceride accumulation in adipose tissue and fatty acid oxidation in skeletal muscle and liver [7]. A fine characterization of the functional gene content of the gut microbiota and its metabolic pathways in ART-treated subjects showed enrichment of genes involved in different pathogenic processes, LPS biosynthesis, bacterial translocation and other inflammatory pathways [12]. Probiotic supplementation has been studied as an approach to restore gut microbial health, potentially improving glucose and fat metabolism [3]. Initial findings of the Probio-HIV study suggest that 48 weeks of probiotic supplementation reduces inflammation and microbial translocation in HIV infected patients [13], and prospective, double-blinded, placebo-controlled pilots trials are underway [14].

HIV infected patients are challenged with metabolism disruption related with the disease or its treatment and with an increased risk for other non-communicable diseases. Despite that current ART regimens are significantly less metabolically toxic than prior medications, HIV and ART use remain independently

Table 2 Variables predicting long-term change in HbA1c in nondiabetic men with HIV infection

Variable	HbA1c change (%)		
	B (IC95%)	r^2 0.6*	p-value 0.04*
Age (10 years)	0.07 (−0.09–0.2)		0.37
Duration of HIV (years)	−0.05 (−0.05–0.04)		0.8
ART exposure (years)	−0.04 (−0.14–0.04)		0.26
PI/r exposure (years)	0.084 (0.004–0.16)		**0.04**
Bacterial translocation	0.62 (0.17–1.07)		**0.01**
BMI (kg/m^2)	−0.015 (−0.07–0.04)		0.16
sRTNF1 (pg/L)	0.0 (−0.01–0.0)		0.26
hsRCP (mg/dL)	−0.28 (−1–0.46)		0.4
Constant	1.25 (−1.1–3.6)		0.28

B standardized coefficient, *r2* coefficient of determination, *BMI* body mass index, *ART* antiretroviral therapy, *PI/r* protease inhibitor, *hsCRP* high sensitivity C-reactive protein, *sRTNF1* soluble form of tumor necrosis factor–alpha receptor type 1

HbA1c change (%) is the dependent variable. Explanatory variables with statistically significant results in multiple regression analysis, and their significance, are shown in bold

* For the multiple stepwise regression analysis of HbA1c change, the following were included as explanatory variables: variables with a statistical significance of $p < 0.05$ in simple analysis, and those with a statistical significance of $p < 0.1$ in simple analysis, and other clinically relevant variables such as age, duration of HIV infection, BMI at the end of the follow-up, and global ART exposure time (shown in the table)

associated with mitochondrial dysfunction, fatty acid metabolism, insulin resistance an altered glucose. These metabolic perturbations contribute to the elevated incidence of cardiovascular disease, type 2 diabetes or non-alcoholic fatty liver disease [1]. The current DHHS preferred nucleoside analogs, tenofovir and abacavir, do not induce mitochondrial toxicity [15], darunavir and atazanavir, the preferred PIs, have a favorable effect on lipids and a limited or modest impact on insulin sensitivity [16, 17]. Finally, the integrase inhibitors have demonstrated a favourable profile in terms of lipids and glucose metabolism [18].

In the setting of HIV infection, impaired glucose metabolism has been associated with altered levels of adipokines, including increased adiponectin and soluble-tumor necrosis factor-receptor 1 (sTNFR1) and decreased leptin [19]. Our data support a partial role of systemic inflammatory markers, however, loss of association in multivariate analysis along with the lack of association with most of the evaluated markers, suggest that other not measured or unknown factors must also be implicated.

In summary, we observed bacterial translocation in about 10% of a population of men with stable HIV infection. Bacterial translocation at baseline and longer exposure to PIs were independent factors associated with impaired glucose homeostasis evolution determined by HbA1c change, after 8 years of follow-up. Conversely, functional indices of insulin resistance, visceral fat distribution and hepatic steatosis were not useful as long-term predictors of changes in glucose homeostasis.

As patients with HIV live longer on ART, the effect of atherogenic dyslipidemia, diabetes and cardiovascular disease on morbidity and mortality will probably worsen unless effective management strategies are developed. A focus on improving underlying metabolic perturbations in HIV should be definitive to avoid these derangements, and could significant impact on quality of life and treatment outcomes. A comprehensive preventive management strategy focused on traditional cardiovascular risk factors has been claimed [20], but our results prompt to pay attention to non-traditional, HIV-specific risk factors, such as bacterial translocation, that could play a crucial role.

Strategies aiming at the correction of dysbiosis, mucosal immune imbalances and metabolic endotaoxemia may represent promising approaches for the treatment of HIV infection-related metabolic complications.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The local Ethics Committee approved the study.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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