Metabolic Response to a ^{13}C-Glucose Load in Human Immunodeficiency Virus Patients Before and After Antiprotease Therapy

Marion Korach, Pascale Leclercq, François Péronnet, and Xavier Leverve

Changes in glucose and fat metabolism associated with human immunodeficiency virus (HIV) infection have received attention because of the development of glucose intolerance, dyslipidemia, and lipodystrophy associated with protease inhibitor (PI) therapy. The response to ingested [^{13}C]glucose (1.4 g/kg) was determined in 9 asymptomatic male HIV patients before and after 4.8 months of PI therapy (nelfinavir, 2,250 mg/d) compared with 9 matched seronegative HIV controls. No significant difference was observed for basal plasma glucose, insulin, and C-peptide concentrations between controls and patients before PI therapy. After 4.8 months of PI therapy, basal plasma glucose concentration was slightly, but significantly, increased (≈ 15%) compared with controls or HIV patients prior to receiving PI therapy. Over the first hour following ingestion of the glucose load, plasma glucose and insulin concentrations were higher in HIV patients than in controls, both before (≈ 15% and ≈ 29%, respectively) and after (≈ 32% and ≈ 43%, respectively) PI therapy. In addition, plasma C-peptide concentration was approximately 61% higher after PI therapy. The oxidation rate of fat, endogenous, and exogenous glucose was computed from the $\dot{V}O_2$ and respiratory exchange ratio corrected for protein oxidation and from $^{13}C/^{12}C$ in expired CO_2. The only difference between controls and patients both before and after PI therapy was observed over the first 120 minutes following ingestion of the glucose load, when HIV patients oxidized approximately 18% more glucose and approximately 19% less fat than controls. This was not due to a larger oxidation rate of exogenous glucose, but to a larger oxidation rate of endogenous glucose (≈ 50%) in patients compared with controls. These data indicate that HIV infection is associated with minor changes in glucose metabolism, and that PI therapy with nelfinavir for 4.8 months only slightly further impairs glucose metabolism as assessed in response to a large oral glucose load. However, the larger stimulation of total and endogenous glucose oxidation and the larger reduction in fat oxidation, observed in the metabolic response to the glucose load in HIV patients, over time, could result in the accumulation of body fat and could contribute to lipodystrophy.

CHANGES IN GLUCOSE AND fat metabolism associated with human immunodeficiency virus (HIV) infection have received attention because of the development of glucose intolerance,[1-4] dyslipidemia,[5] and lipodystrophy,[6] which have been described following therapy with protease inhibitors (PIs). Early data from Hommes et al[7] and Heyligenberg et al[8] suggested an increased insulin sensitivity and glucose tolerance in HIV infection. However, more recent data indicate a lower insulin sensitivity and glucose tolerance in HIV patients,[9-12] which could be worsened by PIs.[5,10,13-15] These changes in glucose metabolism associated with HIV infection and/or PIs might be related to changes in fat metabolism, which include an increase in low-density lipoprotein (LDL)-cholesterol,[16] Lp(a), triglyceride concentrations,[4,17] and fat accumulation on the trunk.[18]

The purpose of the present longitudinal study was, thus, to describe possible changes in glucose tolerance and in the metabolic response to an exogenous glucose load using indirect respiratory calorimetry associated with ^{13}C-labeling in HIV patients following 4.8 months of therapy with a PI. We hypothesized, first, that glucose tolerance will be impaired in patients before PI therapy and will further deteriorate following PI therapy. In addition, when ingested in large amounts, exogenous glucose could be oxidized, deposited in the form of glycogen, or converted into fat. Exogenous glucose also promotes endogenous glucose oxidation and glycogen turnover,[19,20] while inhibiting fat oxidation.[21] Impairment in carbohydrate and fat metabolism, and lipodystrophy are frequent features associated with HIV infection and/or PI therapy.[18,22] We hypothesized that this could be due, in part, to changes in the respective contributions of the various routes in the disposal of a glucose load, which could favor conversion of glucose into fat and fat accumulation.

MATERIALS AND METHODS

Subjects

The study was conducted on 9 male asymptomatic HIV-1–infected patients (patients) and 9 healthy seronegative male control subjects (controls) matched for age, height, body mass, body mass index (BMI), and percent body fat (impedancemetry: BIA 101/S, Akern-RJL Systems, Clinton, MI) (Table 1). The patients and controls gave their informed written consent to volunteer in the study, which was approved by the Institutional Board on the use of human subjects in research. The controls were studied once, while the patients were studied immediately before and 4.8 ± 0.6 months (mean ± SE) following initiation of therapy with a PI (nelfinavir, Viracept, Roche, Neuilly sur Seine, France; 2,250 mg/d), given in association with nucleoside and/or nonnucleoside analog reverse transcriptase inhibitors (Table 2). The drugs were well tolerated except for occasional mild diarrhea, and no episode of acute surinfection was observed. As expected, a dramatic reduction in HIV mRNA copies occurred following 4.8 months of PI therapy (Table 2).

Experiment

The subjects were studied between 12:00 AM and 6:00 PM in a laboratory with controlled temperature (20°C), 5 hours following a

From the Laboratoire de bioénergétique fondamentale et appliquée, Université Joseph Fourier, Grenoble, France; and the Département de kinésiologie, Université de Montréal, Montréal, Canada.

Submitted April 26, 2001; accepted September 5, 2001.

Supported by grants from Roche (France) and from the Natural Sciences and Engineering Research Council of Canada.

Address reprint requests to François Péronnet, PhD, Departement de kinésiologie, Université de Montréal, CP 6128-Centre Ville, Montréal PQ, Canada H3C 3J7.

0026-0495/02/5103-0008$35.00/0

doi:10.1053/meta.2002.30505

Table 1. Characteristics of the Controls and Patients and Fasting Plasma Values (Mean ± SE)

	Controls	Patients	
		Before PI	After PI
Age (yr)	36.2 ± 1.8	37.8 ± 1.4	—
Height (cm)	175.1 ± 2.7	178.7 ± 1.9	—
Body mass (kg)	67.9 ± 2.5	69.0 ± 2.2	68.1 ± 1.8
BMI (kg/m^2)	22.2 ± 0.8	22.0 ± 0.7	21.7 ± 0.8
Body fat (%)	17.5 ± 1.8	15.2 ± 0.8	16.6 ± 1.2
Lean body mass (kg)	56.7 ± 0.4	58.2 ± 0.2	56.4 ± 0.2
Glucose (mmol/L)	4.33 ± 0.12	4.46 ± 0.18	5.20 ± 0.26*†
Insuline (pmol/L)	20.0 ± 5.9	35.8 ± 13.3	28.0 ± 6.5

*Significantly different from controls ($P < .05$).
†Significantly different from patients before PI ($P < .05$).

light breakfast (≈ 360 kcal; proteins, ≈ 15%; carbohydrates, ≈ 65%; fat, ≈ 20%). To keep a low background ^{13}C enrichment of plasma glucose and expired CO_2, ingestion of carbohydrates from plants with the C_4 photosynthetic cycle, which are naturally enriched in ^{13}C, was avoided 1 week before each experiment. The subjects and patients also refrained from exercising for 2 days before each experiment. In addition, the evening meal the day preceding each experiment was standardized (≈ 1,420 kcal; proteins, ≈ 25%; carbohydrates, ≈ 50%; fat, ≈ 25%).

After measurement of body mass and percent body fat, the subjects emptied their bladder and the urine was discarded. They were then placed in a semisupine position for the remainder of the experiment. Following a 30-minute rest period, a catheter (Becton Dickinson, 20-gauge, Grenoble, France) was inserted into an antecubital vein for subsequent blood sampling, and the subject was placed under a canopy for the measurement of oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) (Deltatrac I, MBM 100, Datex; Ohmeda SAS, Helsinki, Finland). Respiratory exchanges were allowed to stabilize for 30 minutes before performing control measurements. The canopy was then briefly lifted, and the subjects ingested in a single bolus 1.4 g/kg of glucose in 7 mL of water/kg at room temperature. The glucose ingested, which was naturally enriched in ^{13}C (Sigma, Lyon, France), was artificially enriched in ^{13}C with U-^{13}C glucose (Isotec, Miamisburg, OH; $^{13}C/^{12}C > 99\%$) to achieve a final isotopic composition close to 20 $^{o}/_{oo}$ δ ^{13}C PDB_1: actual value measured by mass spectrometry = 21.6 $^{o}/_{oo}$ δ ^{13}C PDB_1 (SIRA 10, VG Isogas, Middlewich, UK). Water was provided ad libitum immediately after ingestion of the glucose solution (120 ± 30 mL), as well as during the following observation period (amount ingested: 320 ± 50 mL with no significant difference between the 3 experimental situations).

The observations were made over a 4-hour period following glucose ingestion. Respiratory exchanges were measured and recorded continuously, while 10-mL blood samples were withdrawn at regular intervals for the measurement of plasma glucose and insulin concentrations and of ^{13}C-enrichment of plasma glucose (see figures). Plasma C-peptide and free fatty acid (FFA) concentrations were also measured immediately before glucose ingestion and at 30 and 60 minutes (C-peptide) and 120 and 240 minutes (FFA). In addition, the urine produced over the observation period was collected for measurement of urea excretion.

Measures and Computations

Protein oxidation and the associated amount of energy provided were computed from urea excretion in urine, and $\dot{V}O_2$ and $\dot{V}CO_2$ were corrected for protein oxidation.[23] Glucose and fat oxidation, and the amounts of energy provided, were then computed when the nonprotein respiratory quotient was less than 1.0,[23] while glucose oxidation, the amount of glucose converted into fat, and the amount of fat synthesized were computed when the nonprotein respiratory quotient was larger than 1.0.[24]

The amount of ingested glucose, which was actually oxidized, was computed from $^{13}C/^{12}C$ in expired CO_2. For this purpose, 40-mL samples of expired gases were collected in vacutainers (Becton Dickinson), and the ^{13}C enrichment of expired CO_2 was determined by mass spectrometry (SIRA 10, VG Isogas), as previously described, and expressed by reference to the International Standard Pee Dee Belemnitela (PDB_1) ($^{13}C/^{12}C = 1.1237\%$): $^{o}/_{oo}$ δ ^{13}C $PDB_1 = [(Rspl/Rstd) - 1] \times 1{,}000$, where Rspl and Rstd are the $^{13}C/^{12}C$ in the sample and standard, respectively. The amount of labeled glucose (Exo glucose) oxidized was then computed as follows:

$$\text{Exo. glucose (g/min)} = \dot{V}CO_2\,[(Rexp\text{-}Rref)/(Rexo - Rref)]/(k_1 \times k_2) \quad (1)$$

In this equation, $\dot{V}CO_2$ is in L/min, Rexp is the observed isotopic composition of expired CO_2, Rref is the isotopic composition of expired CO_2 before glucose ingestion, Rexo is the isotopic composition of the glucose ingested, k_1 is the volume of CO_2 provided by the oxidation of glucose (0.7426 L/g), and k_2 is the fractional recovery at the mouth of the CO_2 produced in tissues. The value of Rexo was measured by mass spectrometry (SIRA 10, VG Isogas) in a 5-mL sample of the

Table 2. Clinical Status of the Patients Before and After PI Therapy and Drugs Taken in Addition to Nelfinavir (2,250 mg/d)

Patients	Times of Infection (mo)	Antiretroviral Drugs	CD4 Counts (cells/μL)		HIV mRNA (copies/mL)	
			Before PI	After PI	Before PI	After PI
1	96	AZT ddI	780	940	11,072	BDL*
2	1	ddI Nevirapine	370	560	13,180	BDL
3	48	AZT 3TC	640	630	4,203	2,190
4	120	3TC d4T	450	550	62,210	BDL
5	108	3TC d4T	550	550	12,554	BDL
6	53	AZT 3TC	360	500	5,927	BDL
7	150	d4T ddI	430	940	2,927	BDL
8	17	3TC AZT	760	510	650	BDL
9	108	ddC Nevirapine	730	590	8,351	903
Mean	78		563	641	13,452	499†
SE	16.7		56	58	6,265	225

Abbreviations: AZT, zidovudine; ddI, didanosine; ddC, zalcitabine; 3TC, lamivudine; d4T, stavudine.
*BDL, below detection level (200 copies/mL).
†Taking 200 copies/mL as the default value when BDL.

ingested solution following lyophilization and combustion, as previously described.[25] As for the value of k_2, when uniformly labeled glucose is the tracer, it has been estimated at 54% by Schneiter et al.[26]

The amount of endogenous glucose oxidized was computed by the difference between total glucose oxidation and exogenous glucose oxidation. In addition, based on the assumption that over the 4-hour observation period, all the exogenous glucose was absorbed, glycogen accretion (glucose ingested – exogenous glucose oxidized) and glycogen balance (glycogen accretion – endogenous glucose oxidation) was also computed, taking into account de novo lipogenesis, if present.

The ^{13}C enrichment of plasma glucose was determined as previously described[25] by using a technique combining gas chromatography and isotope-ratio mass spectrometry (SIRA 10, VG Isogas). Briefly, the plasma samples were deproteinized with perchloric acid, neutralized, and partially purified by sequential anion-cation exchange chromatography. The neutral eluate fraction was then lyophilized and derivatized as glucose pentacetate. After removing the excess of derivatization products under nitrogen, the sample was resuspended in 50 mL $CHCl_3$, and the glucose was separated by gas chromatography (Hewlett Packard 5890, Evry, France) on a CP SIL19CB column (Chrompack Inc, Bridgewater, NJ) maintained at 260°C. The glucose peak in the effluent was oxidized at 800°C in the presence of CuO, and the effluent was driven through a water trap to the isotope-ratio mass spectrometer. Based on the isotopic composition of plasma glucose before ($Rglu_0$) and following glucose ingestion (Rglu), and on the value of Rexo, the percentage of plasma glucose derived from ingested glucose was computed as:

$$\%\ \text{exogenous glucose} = [(Rglu - Rglu_0)/(Rexo - Rglu_0)] \times 100$$

Plasma glucose and FFA concentrations were measured using automated spectrophotometric assays (Sigma Diagnostics, Mississauga, Canada and Roche Diagnostics, Laval, Canada), while plasma insulin and C-peptide concentrations were measured using radioimmunoassays (Bi-insulin IRMA ERIA, Pasteur, Paris, France).

Statistics

Data are presented as mean ± SE. The main effects of HIV infection, PI, and time following ingestion of the glucose load, as well as HIV infection-PI interaction, were tested by analysis of variance with repeated-measures when pertinent (time and PI) (Statistica package, Statsoft, Tulsa, OK). Newman-Keuls post hoc test was used to identify the location of significant differences ($P < .05$) when the analysis of variance yielded a significant F ratio.

RESULTS

Before ingestion of the glucose load, when compared with the controls, no significant difference was observed for plasma glucose, insulin, C-peptide, and FFA concentrations in patients infected with HIV before initiation of the therapy (Tables 1 and 3). After 4.8 months of PI therapy, basal plasma glucose concentration was significantly increased, while no change was observed for insulin, C-peptide, and FFA concentrations.

Table 3. Plasma C-Peptide and FFA Concentrations in Controls and Patients (Mean ± SE)

	Time (min)	Controls	Patients	
			Before PI	After PI
C-peptide (ng/mL)	0	1.52 ± 0.20	2.14 ± 0.28	2.14 ± 0.27
	30	4.25 ± 0.59	5.93 ± 0.89	6.72 ± 0.8*
	60	8.08 ± 0.9	9.32 ± 0.8	8.84 ± 0.9
FFA (μmol/L)	0	483 ± 134	423 ± 87	554 ± 162
	120	37.4 ± 10.5	26.7 ± 5.4	23.4 ± 8.3
	240	332.4 ± 82	209 ± 61	240 ± 81

*Significantly different from controls ($P < .05$).

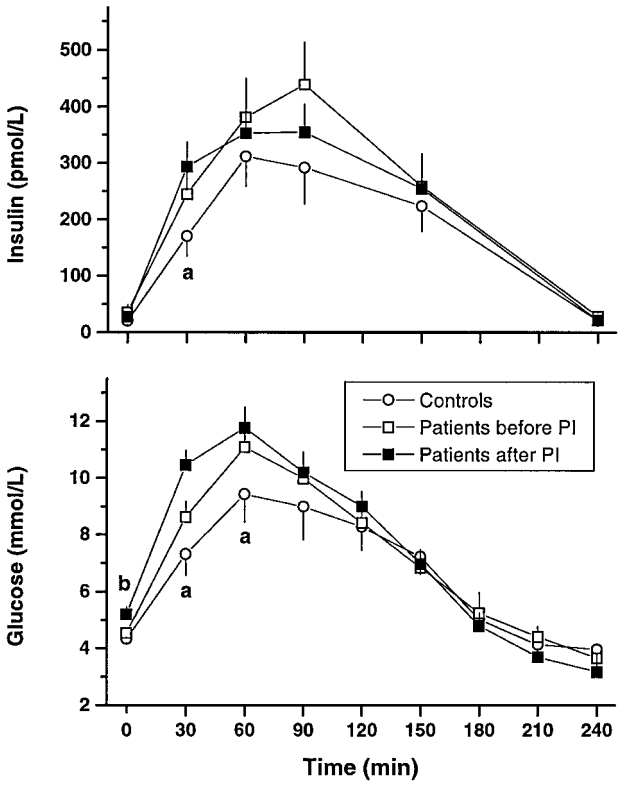

Fig 1. Plasma glucose and insulin concentrations before and following ingestion of the glucose load (mean ± SE). (a) Significantly different from patients; (b) significantly different from patients before PI.

In response to the glucose load in both patients and controls, plasma glucose and insulin concentrations significantly increased over the basal corresponding values (Fig 1). Over the first hour following ingestion of the glucose load, plasma glucose (at both 30 and 60 minutes), and insulin concentrations (at 30 minutes) were higher in patients than in controls, both before and after PI therapy. Plasma C-peptide concentration also increased in response to the glucose load and at 30 minutes was significantly higher in patients after 4.8 months of PI therapy than in controls (Table 3). Plasma FFA concentration was markedly decreased at 120 and 240 minutes following glucose ingestion, with no significant difference between controls and patients, and no significant effect of PI therapy (Table 3).

Over the 4-hour period following glucose ingestion, urea excretion was similar in controls and patients before initiation of the therapy (95.7 ± 9.5 *v* 94.9 ± 8.3 mmol). Accordingly, the amount of proteins oxidized was similar in controls and in patients, providing 20.5% and 18.7% of the energy yield (Table 4). After 4.8 months of PI therapy, an approximately 20% reduction in urea excretion (76.8 ± 4.7 mmol) and protein

Table 4. Substrate Oxidation Over the 4 Hours Following Glucose Ingestion (Mean ± SE)

	Controls		Patients			
			Before PI		After PI	
	Minutes 0-120	Minutes 120-240	Minutes 0-120	Minutes 120-240	Minutes 0-120	Minutes 120-240
Energy (kcal)	154.0 ± 7.4	150.4 ± 7.4	156.4 ± 6.2	151.3 ± 6.5	152.3 ± 5.5	150.7 ± 7.30
Proteins						
(g)	15.3 ± 1.8		14.0 ± 1.3		11.9 ± 0.6	
(% energy)	20.5 ± 1.5		18.7 ± 1.3		16.5 ± 0.9	
Fat						
(g)	7.4 ± 0.5	2.5 ± 0.3	5.4 ± 0.7*	2.5 ± 0.6	5.8 ± 0.7*	2.7 ± 0.4
(% energy)	47.6 ± 2.7	16.7 ± 2.6	33.8 ± 3.8*	15.6 ± 3.6	37.1 ± 4.2*	18.3 ± 2.6
Glucose total						
(g)	12.8 ± 1.1	24.3 ± 1.5	19.3 ± 1.3*	25.5 ± 1.6	18.5 ± 1.9*	25.8 ± 2.4
(% energy)	32.2 ± 2.9	62.6 ± 2.7	47.9 ± 3.8*	65.3 ± 3.7	46.7 ± 4.6*	65.1 ± 3.2
Exogenous glucose						
(g)	4.6 ± 0.4	19.6 ± 0.84	4.8 ± 0.3	18.8 ± 1.2	4.9 ± 0.3	19.4 ± 1.4
(% energy)	5.9 ± 0.5	24.6 ± 0.6	6.0 ± 0.4	23.5 ± 1.6	6.1 ± 0.3	24.2 ± 1.2
Endogenous glucose						
(g)	6.6 ± 1.3	5.0 ± 1.0	12.8 ± 0.9*	6.6 ± 0.8	12.5 ± 1.5*	6.4 ± 1.2
(% energy)	8.2 ± 1.8	6.3 ± 1.3	16.0 ± 1.2*	8.1 ± 0.9	15.6 ± 1.7*	7.8 ± 1.3
Glycogen accretion (g glucose)	69.7 ± 3.8		70.2 ± 3.0		68.1 ± 2.0	
Glycogen balance (g glucose)	51.8 ± 3.9		47.1 ± 2.8		46.2 ± 3.0	

*Significantly different from controls ($P < .05$).

oxidation (Table 4) was observed, which, however, failed to reach statistical significance ($P = .054$).

The oxidation rate of fat, endogenous, and exogenous glucose computed from the $\dot{V}O_2$ and respiratory exchange ratio corrected for protein oxidation and from $^{13}C/^{12}C$ in expired CO_2 (Fig 2), are shown in Fig 3. Ingestion of the glucose load increased glucose oxidation and decreased fat oxidation, but no net de novo lipogenesis was observed at any time during the observation period. The only difference between controls and patients was observed over the first 120 minutes following ingestion of the glucose load (Table 4). Over this period, the patients oxidized approximately 18% more glucose and approximately 19% less fat than controls. This was not due to a larger oxidation rate of exogenous glucose, but to a larger (≈ 50%) oxidation rate of endogenous glucose in patients than controls. These differences between patients and controls were present both before and after PI therapy.

The percentage of plasma glucose derived from exogenous glucose was not significantly different in controls and patients before therapy, but a significant (≈ 20%) increase was observed in patients following PI therapy (Fig 2).

The amount of exogenous glucose converted into glycogen, computed by difference between the amount of glucose ingested and oxidized, was not significantly different in controls and patients (Table 4). Due to the larger amount of endogenous glucose oxidized, glycogen balance was slightly lower in patients, although this did not reach statistical significance.

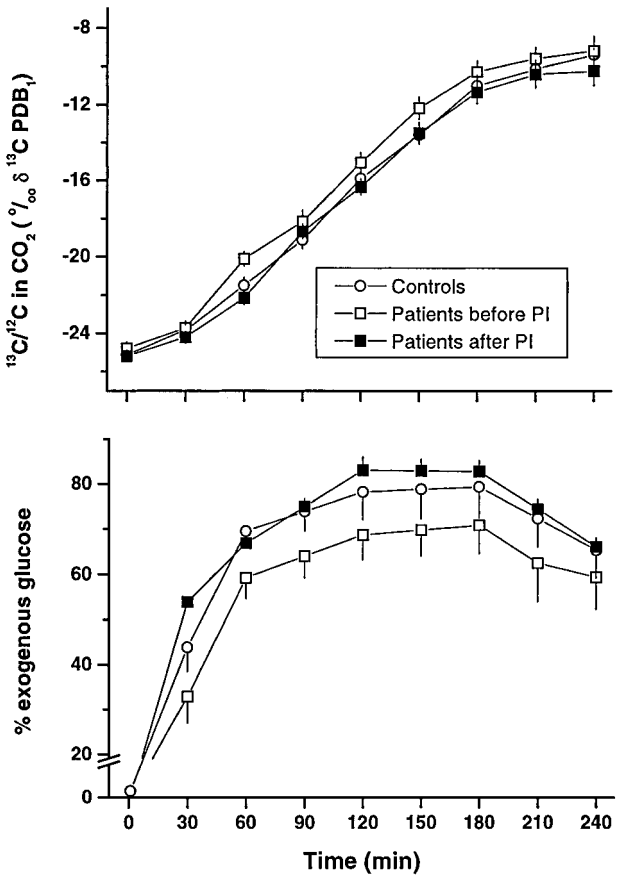

Fig 2. Changes in $^{13}C/^{12}C$ in expired CO_2 (top) and percentage of plasma glucose derived from exogenous glucose (bottom) over the 4 hours following ingestion of the glucose load (mean ± SE); values observed following PI are significantly higher than before.

DISCUSSION

Changes in glucose metabolism in HIV patients were first described by Hommes et al[7] and by Heyligenberg et al.[8] Fasting plasma glucose was similar[8,27] or somewhat lower[7] in patients than in controls, while plasma insulin concentration was similar,[7,8,27] and plasma C-peptide concentration was similar or

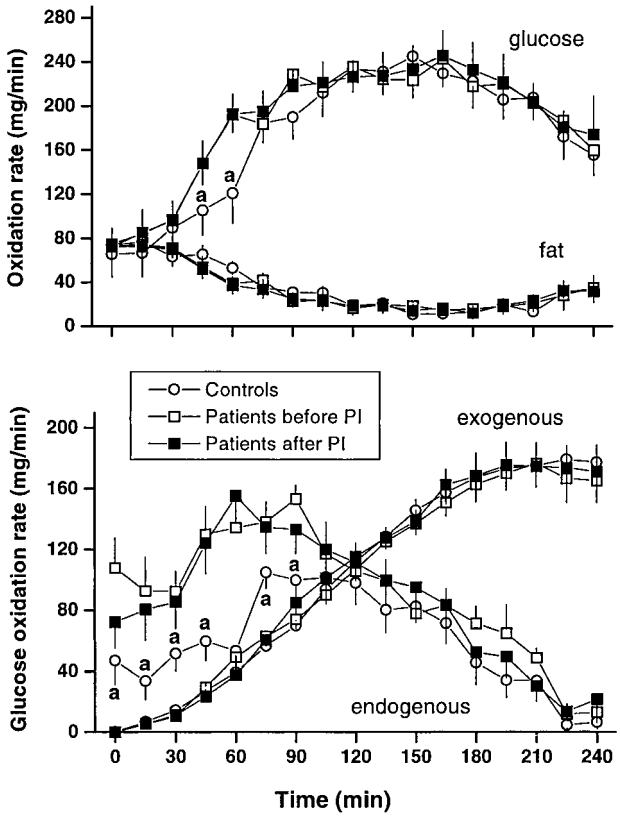

Fig 3. Rate of total glucose and fat oxidation (top) and of endogenous and exogenous glucose oxidation (bottom) over the 4 hours following ingestion of the glucose load (mean ± SE). (a) Significantly different from patients ($P < .05$).

slightly higher in HIV patients.[8] In addition, during an euglycemic clamp, insulin clearance was higher and plasma insulin concentration was lower in HIV patients, but glucose uptake was similar to that observed in HIV seronegative controls. Results from a subsequent study from the same laboratory[8] showed that the increased glucose tolerance in HIV patients was due to a higher peripheral sensitivity to insulin, without any changes in non–insulin-mediated glucose uptake. Taken together, these results suggested that HIV infection, unlike other infections,[28] results in an increased insulin sensitivity and glucose tolerance. However, this phenomenon has not been confirmed in subsequent studies. Consistent results from the 8 studies available in the literature, which have directly compared controls and HIV patients (not treated with PI) indicate similar fasting plasma glucose, but somewhat higher plasma insulin and C-peptide concentrations in patients versus controls (Table 5). There appears to be only 2 studies describing the plasma glucose response to an oral glucose tolerance test in HIV patients not receiving PI, none of them including controls.[5,10] In the study by Saint-Marc et al,[5] plasma glucose concentration both before ingestion of the 75-g glucose load, and 120 minutes after, were within the normal range. In contrast, in the study by Behrens et al,[10] although fasting plasma glucose concentration (3.3 to 5.5 mmol/L) was within the normal range, the average plasma glucose concentration at 120 minutes was 6.8 mmol/L (range, 3.8 to 10.2 mmol/L) with 24% of the patients above the American Diabetes Association criteria for glucose intolerance (> 7.8 mmol/L).[15] Finally, in a recent study by Hardin et al,[11] glucose disappearance rate under maximal insulin stimulation was about 30% lower in acquired immunodeficiency syndrome (AIDS) patients (without PI) than in controls.

Results from the present experiment are consistent with these findings and indicate a small impairment of glucose tolerance and a reduction in peripheral insulin sensitivity in response to a large oral glucose load (95.3 ± 1.2 g) in patients before initiation of PI therapy. Indeed, immediately before ingestion of the glucose load, plasma glucose, insulin, and C-peptide concentrations were similar in patients and controls. However, in response to the glucose load, plasma glucose was 29% and 17% higher at 30 and 60 minutes in patients than in controls, while insulin, as well as C-peptide concentrations, were, respectively, 56% and 49% higher at 30 minutes. Direct comparison between patients without PI and controls following an oral glucose load, thus, confirm the results from the study by Behrens et al[10] and indicate that HIV infection is associated with

Table 5. Fasting Plasma Glucose, Insulin, and C-Peptide Concentrations Reported in Various Studies in Controls and HIV Patients With or Without PIs

Studies	Subjects (n)	Glucose (mmol/L)	Insulin (pmol/L)	C-peptide (ng/mL)
Hommes et al[7]	Controls (10)	5.09	50.2	1.76
	Patients without PI (10)	4.9	50.2	1.6
	Patients with PI	—	—	—
Hommes et al[27]	Controls (7)	4.8	49	2.8
	Patients without PI (8)	4.3	41	4.4
	Patients with PI	—	—	—
Heyligenberg et al[8]	Controls (5)	5.2	54.9	1.9
	Patients without PI (7)	5.3	60.4	2.7
	Patients with PI	—	—	—
Walli et al[13]	Controls (13)	4.0	—	—
	Patients without PI (13)	4.2	57.4	—
	Patients with PI (67)	4.4	100.4	—
Carr et al[3]	Controls (47)	5.1	36.6	1.1
	Patients without PI (32)	4.9	51.6	2.1
	Patients with PI (116)	4.9	65.3	2.6
Hadigan et al[34]	Controls (30)	4.3	54.2	—
	Patients without PI	—	—	—
	Patients with PI (70)	5.07	114.0	—
Christeff et al[6]	Controls (20)	4.1	42.31	—
	Patients without PI	—	—	—
	Patients with PI (37)	3.1	76.2	—
Hadigan et al[35]	Controls (20)	5.2	74.6	—
	Patients without PI (30)	5.6	155.62	—
	Patients with PI (20)	5.1	93.9	—
Mean ± SE	Controls (152)	4.7	50.43	1.43
	Patients without PI (100)	4.9	68.90	2.41
	Patients with PI (310)	4.9	89.61	2.60

NOTE. Number of subjects indicated in parentheses.

small impairments in plasma glucose, insulin, and C-peptide concentrations, which are indicative of a reduced peripheral insulin sensitivity.

PIs given with other drugs could be associated with a further reduction in insulin sensitivity. Indeed, in cross-sectional studies, although fasting plasma glucose is not different, fasting plasma insulin concentration is higher in patients with PIs (Table 5). Several case reports have also shown that in a small percentage of the patients, this phenomenon can lead to the development of diabetes mellitus.[1,4,14,29] For example, in a large cohort of 116 patients receiving PIs, including 27 patients with a 1- to 39-month follow-up, based on fasting plasma glucose values, 2% of the patients were diagnosed with diabetes and a further 7% with impaired glucose tolerance.[9] These figures increased to 7% and 16% in response to an oral glucose tolerance load. The development of glucose intolerance following PI therapy could be more prevalent in patients with a family history of type 2 diabetes.[29] In addition, short-term treatment with nelfinavir does not appear to be associated with an impairment in glucose tolerance,[1,10,14] and recent data suggest that nucleoside analog reverse transcriptase inhibitors, which are given in association with PIs, could also be involved in the development of glucose intolerance.[4]

In the present experiment, 4.8 months following initiation of the PI therapy, only a small further impairement in glucose metabolism was observed in the basal situation, as well as at 30 minutes following ingestion of the glucose load. Indeed, an increase in basal plasma glucose concentration was observed in all patients, but 1 (range, -0.2 to 1.9 mmol/L), with a value as high as 6.7 mmol/L in one of them, and an average plasma glucose 15% higher than before initiation of the therapy. Plasma glucose, as well as plasma insulin concentrations, were also slightly ($\approx$ 20%), but significantly, increased 30 minutes following ingestion of the glucose load. This slight impairment in glucose metabolism following PI therapy is in line with data reported by Behrens et al,[10] although these investigators showed a larger deterioration in the response of plasma glucose and insulin, as well as C-peptide concentrations, which were observed later following ingestion of the glucose load (at 120 minutes and after). These differences could be due to the fact that in this cross-sectional study by Behrens et al,[10] the patients with PIs were taking the drugs for an average of 18 months (*v* 4.8 months in the present study). In addition, only a small percentage of these patients (7/38) were taking nelfinavir, while most of them were taking other PIs, which could have more pronounced effects on glucose metabolism.[14]

In the present experiment, the progressive ^{13}C-enrichment of plasma glucose indicates that 2 hours following ingestion of the ^{13}C-labeled glucose load, the percentage of exogenous glucose in the circulating glucose pool peaked between 70% and 75%, a value well in accordance with data observed in a previous experiment in healthy subjects at rest following ingestion of 100 g of glucose.[30] However, the percentage of exogenous glucose in the circulating pool was slightly, but significantly, increased 4.8 months after PI therapy ($\approx$ 20% higher between 30 and 240 minutes). In the absence of data concerning plasma glucose flux, this increase should be interpreted with caution, because changes in glucose absorption, liver glucose output, and peripheral glucose disposal could all modify the percentage of plasma glucose arising from exogenous glucose. However, the changes observed are consistent with a reduction in peripheral insulin sensitivity and in glucose tolerance, with a small accumulation of exogenous glucose in the extracellular fluid.

No data are available in the literature concerning the metabolic response to ingestion of a glucose load in HIV infected patients. Changes in oxidation versus storage as glycogen and/or fat of the ingested glucose and/or changes in the oxidation of proteins, fat, and endogenous glucose could, in part, explain fat accumulation on the trunk[18] and dyslipidemias[4,16,17] associated with HIV infection and/or PIs. In the present experiment, the metabolic response was observed following a comparatively large oral glucose load (1.4 g/kg). Use of exogenous glucose labeled with ^{13}C, combined with indirect respiratory calorimetry corrected for protein oxidation, provides a detailed picture of the metabolic routes involved in the disposal of the glucose load. Therapy with a PI did not significantly modify these routes. Indeed, observations made in patients before intitiation of PI therapy and 4.8 months after were very similar over the entire observation period following ingestion of the glucose load. In addition, no significant difference was observed between patients and controls over the last 2 hours of the observation period. In contrast, small, but significant, differences were observed bewteen controls and patients over the first 2 hours following ingestion of the glucose load. During this period, total glucose oxidation significantly increased both because exogenous glucose oxidation increased and because endogenous glucose oxidation was stimulated, with a parallel reduction in fat oxidation. However, when compared with controls, changes in glucose versus fat oxidation were more pronounced in patients, both before and 4.8 months after PI therapy. This was due to a larger increase in endogenous glucose oxidation and a larger reduction in fat oxidation. As a consequence, although net de novo lipogenesis was not present, this observation suggests that HIV patients are more prone to preserve their fat stores when the availability of glucose is high, such as following a meal. This difference, which could be due to HIV infection per se, to the nutritional consequences of the disease and the associated changes in the diet, and/or to the medication, is small, and the effect on fat balance is negligible following a single glucose load. However, the resulting effect of this phenomenon on body fat balance, when cumulated following each meal, could, in part, explain the accumulation of fat and the development of lipodystrophy and dyslipidemias observed in HIV patients with PIs and/or nucleoside reverse transcriptase inhibitors.[18,31-33] In contrast, the larger stimulation of glucose oxidation following ingestion of a glucose load resulted in a smaller positive glycogen balance in patients than in controls.

Taken together, these data indicate that HIV infection is associated with only minor deterioration in glucose metabolism, and that PI therapy with nelfinavir for 4.8 months only slightly further impairs glucose metabolism as assessed in response to a large oral glucose load. However, the small difference observed in the metabolic response to the glucose load between patients and controls, namely the larger stimulation of total and endogenous glucose oxidation, and the larger reduction in fat oxidation could be involved in the accumulation of body fat in HIV-infected patients.

REFERENCES

1. Dubé MP, Jonhson DL, Currier JS, et al: Protease inhibitor-associated hyperglycemia. Lancet 350:713-714, 1997
2. Kilby JM, Tabereaux PB: Severe hyperglycemia in an HIV clinic: Preexisting versus drug-associated diabetes mellitus. J Acquir Immune Defic Syndr Hum Retrovirol 17:46-50, 1998
3. Carr A, Samaras K, Burton S, et al: A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS 12:F51-58, 1998
4. Qaqish RB, Fisher E, Rublein J, et al: HIV-associated lipodystrophy syndrome. Pharmacotherapy 20:13-22, 2000
5. Saint-Marc T, Partisani M, Poizot Martin I, et al: Fat distribution evaluated by computed tomography and metabolic abnormalities in patients undergoing antiretroviral therapy: Preliminary results of the LIPOCO study. AIDS 14:37-49, 2000
6. Christeff N, Melchior JC, de Truchis P, et al: Lipodystrophy defined by a clinical score in HIV-infected men on highly active antiretroviral therapy: Correlation between dyslipidaemia and steroid hormone alterations. AIDS 13:2251-2260, 1999
7. Hommes MJ, Romijn JA, Endert E, et al: Insulin sensitivity and insulin clearance in human immunodeficiency virus-infected men. Metabolism 40:651-656, 1991
8. Heyligenberg R, Romijn JA, Hommes MJ, et al: Non-insulin-mediated glucose uptake in human immunodeficiency virus-infected men. Clin Sci (Colch) 84:209-216, 1993
9. Carr A, Samaras K, Thorisdottir A, et al: Diagnosis, prediction, and natural course of HIV protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: A cohort study. Lancet 353: 2093-2099, 1999
10. Behrens G, Dejam A, Schmidt H, et al: Impaired glucose tolerance, beta cell function and fat metabolism in HIV patients under treatment with protease inhibitors. AIDS 13:F63-70, 1999
11. Hardin DS, LeBlanc A, Young D, et al: Increased leucine turnover and insulin resistance in men with advanced HIV infection. J Investig Med 47:405-413, 1999
12. Dever LL, Oruwari PA, Figueroa WE, et al: Hyperglycemia associated with protease inhibitors in an urban HIV-infected minority patient population. Ann Pharmacother 34:580-584, 2000
13. Walli R, Herfort O, Michl GM, et al: Treatment with protease inhibitors associated with peripheral insulin resistance and impaired oral glucose tolerance in HIV-infected patients. AIDS 12:F167-173, 1998
14. Kaufman MB, Simionatto C: A review of protease inhibitor-induced hyperglycemia. Pharmacotherapy 19:114-117, 1999
15. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 21:S5-S19, 1998
16. Grunfeld C, Kotler P, Shigenaga JK, et al: Circulating interferon-alpha levels and hypertriglyceridemia in the acquired immunodeficiency syndrome. Am J Med 90:154-162, 1991
17. Crook MA, Mir N: Abnormal fats and the acquired immunodeficiency syndrome: Is there a problem and what should we do about it? Int J STD AIDS 10:353-356, 1999
18. Kotler DP, Rosenbaum K, Wang J, et al: Studies of body composition and fat distribution in HIV-infected and control subjects. J Acquir Immune Defic Syndr Hum Retrovirol 20:228-237, 1999
19. Acheson KJ, Thelin A, Ravussin E, et al: Contribution of 500 g naturally labeled ^{13}C dextrin maltose to total carbohydrate utilization and the effect of the antecedent diet, in man. Am J Clin Nutr 41:881-890, 1985
20. Folch N, Péronnet F, Massicotte D, et al: Metabolic response to small and large ^{13}C-labelled pasta meals following rest or exercise in man. Br J Nutr 85:1-10, 2001
21. Acheson KJ, Schutz Y, Bessard T, et al: Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man. Am J Clin Nutr 48:240-247, 1988
22. Macallan DC: Metabolic syndrome in human immunodeficiency virus infection. Horm Res 55:36-41, 2001 (suppl 1)
23. Livesey, G, Elia M: Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. Am J Clin Nutr 47:608-628, 1988
24. Elia M, Livesey G: Theory and validity of indirect calorimetry during net lipid synthesis. Am J Clin Nutr 47:591-607, 1988
25. Normand S, Pachiaudi C, Khalfallah Y, et al: ^{13}C appearance in plasma glucose and breath CO_2 during feeding with naturally ^{13}C-enriched starchy food in normal humans. Am J Clin Nutr 55:430-435, 1992
26. Schneiter P, Di Vetta V, Jéquier E, et al: Effect of physical exercise on glycogen turnover and net substrate utilization according to the nutritional state. Am J Physiol 269:E1031-E1036, 1995
27. Hommes MJ, Romijn JA, Endert E, et al: Basal fuel homoeostasis in symptomatic human immunodeficiency virus infection. Clin Sci 80:359-365, 1991
28. Yki-Järvinen H, Sammlkorpi K, Koivisto VA, et al: Severity, duration and mechanisms of insulin resistance during acute infections. J Clin Endocrinol Metab 69:317-323, 1989
29. Vigouroux C, Gharakhanian S, Salhi Y, et al: Diabetes, insulin resistance and dyslipidaemia in lipodystrophic HIV-infected patients on highly active antiretroviral therapy (HAART). Diabetes Metab 25:225-232, 1999
30. Burelle Y, Péronnet F, Charpentier S, et al: Oxidation of an oral [^{13}C]glucose load at rest and prolonged exercise in trained and sedentary subjects. J Appl Physiol 86:52-60, 1999
31. Shaw AJ, McLean KA, Evans BA: Disorders of fat distribution in HIV infection. Int J STD AIDS 9:595-599, 1998
32. Gervasoni C, Ridolfo AL, Trifirò G, et al: Redistribution of body fat in HIV-infected women undergoing combined antiretroviral therapy. AIDS 13:465-471, 1999
33. Carr A, Miller J, Law M, et al: A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: Contribution to protease inhibitor-related lipodystrophy syndrome. AIDS 14:F25-32, 2000
34. Hadigan C, Miller K, Corcoran C, et al: Fasting hyperinsulinemia and changes in regional body composition in human immunodeficiency virus-infected women. J Clin Endocrinol Metab 84:1932-1937, 1999
35. Hadigan C, Corcoran C, Stanley T, Piecuch S, et al: Fasting hyperinsulinemia in human immunodeficiency virus-infected men: Relationship to body composition, gonadal function, and protease inhibitor use. J Clin Endocrinol Metab 85:35-41, 2000