Mechanisms for the Deterioration in Glucose Tolerance Associated With HIV Protease Inhibitor Regimens

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The mechanisms responsible for the deterioration in glucose tolerance associated with protease inhibitor–containing regimens in HIV infection are unclear. Insulin resistance has been implicated as a major factor, but the affected tissues have not been identified. Furthermore, β-cell function has not been evaluated in detail. The present study was therefore undertaken to assess the effects of protease inhibitor–containing regimens on hepatic, muscle, and adipose tissue insulin sensitivity as well as pancreatic β-cell function. We evaluated β-cell function in addition to glucose production, glucose disposal, and free fatty acid (FFA) turnover using the hyperglycemic clamp technique in combination with isotopic measurements in 13 HIV-infected patients before and after 12 weeks of treatment and in 14 normal healthy volunteers. β-Cell function and insulin sensitivity were also assessed by homeostasis model assessment (HOMA). Treatment increased fasting plasma glucose concentrations in all subjects ($P < 0.001$). Insulin sensitivity as assessed by HOMA and clamp experiments decreased by ~50% ($P < 0.003$). Postabsorptive glucose production was appropriately suppressed for the prevailing hyperinsulinemia, whereas glucose clearance was reduced ($P < 0.001$). β-Cell function decreased by ~50% ($P = 0.002$), as assessed by HOMA, and first-phase insulin release decreased by ~25%, as assessed by clamp data ($P = 0.002$). Plasma FFA turnover and clearance both increased significantly ($P < 0.001$). No differences at baseline or in responses after treatment were observed between drug naïve patients who were started on a nucleoside reverse transcriptase inhibitor (NRTI) plus a protease inhibitor and patients who had been on long-term NRTI treatment and had a protease inhibitor added. The present study indicates that protease inhibitor–containing regimens impair glucose tolerance in HIV-infected patients by two mechanisms: *1*) inducement of peripheral insulin resistance in skeletal muscle and adipose tissue and *2*) impairment of the ability of the β-cell to compensate. *Diabetes* 52: 918–925, 2003

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CT, computer tomography; FFA, free fatty acid; HLS, HIV-lipodystrophy syndrome; HOMA, homeostasis model assessment; HPLC, high-performance liquid chromatography; IGT, impaired glucose tolerance; IS_{HOMA}, insulin sensitivity measured by HOMA; NRTI, nucleoside reverse transcriptase inhibitor; SA, specific activity; $SECR_{homa}$, β-cell function measured by HOMA.

Use of protease inhibitors has remarkably improved long-term survival after HIV infection (1,2). However, up to 60% of HIV-infected patients treated with these agents develop either impaired glucose tolerance (IGT) or type 2 diabetes (3–6), and it now appears to be well established that regimens including protease inhibitors are associated with insulin resistance (2,5,7,8). Noor et al. (9,10) have shown that acute and 4-week protease inhibitor exposure of normal volunteers reduces glucose disposal during euglycemic-hyperinsulinemic clamp experiments. Moreover, in vitro studies have demonstrated that protease inhibitors reduce insulin-stimulated glucose uptake in adipocytes and skeletal muscle (11,12).

Knowledge of the mechanisms responsible for deterioration in glucose tolerance during protease inhibitor–containing regimens is still incomplete. It is unclear whether protease inhibitors adversely affect pancreatic β-cell function (4,8) and what effect they have on glucose production and free fatty acid (FFA) turnover. Protease inhibitors are aspartate endopeptidase inhibitors (13–15). Because an aspartate endopeptidase is involved in converting proinsulin to insulin, the observation that plasma proinsulin levels are increased in protease inhibitor–treated patients (5) suggests that these drugs may directly impair pancreatic β-cell function. Increased plasma FFA levels have been found in protease inhibitor–treated patients (16), and it is not known whether this is due to increased release or decreased utilization.

This study was undertaken to prospectively assess the effect of protease inhibitor–based treatment regimens on pancreatic β-cell function and to determine the sites of insulin resistance. We studied 13 HIV-infected subjects with normal glucose tolerance before and after 12 weeks of protease inhibitor–based treatment and 14 healthy normal volunteers using hyperglycemic clamp experiments in combination with isotopic determination of glucose and FFA turnover. Eight of the patients had been on a chronic regimen containing nucleoside reverse transcriptase inhibitors (NRTIs) to which a protease inhibitor was added, and five patients were drug naïve and were started on a combination of an NRTI and a protease inhibitor.

RESEARCH DESIGN AND METHODS

Informed written consent was obtained from 13 HIV-infected otherwise healthy subjects and 14 HIV-negative normal volunteers after the protocol had been approved by the University of Rochester Institutional Review Board. Subjects were recruited in the Infectious Disease Clinic based on plans for

TABLE 1
Antiviral therapy before and after starting protease inhibitors

Subject	Pre-PI treatment	Post-PI treatment	Age	Sex	Race
1	Nevirapine, stavudine, lamivudine	Stavudine, lamivudine, nelfinavir	53	M	AA
2	Efavirenz, abacavir, stavudine	Lamivudine, zidovudine, nelfinavir	35	F	C
3	Nevirapine, stavudine, lamivudine	Lamivudine, stavudine, nelfinavir	46	F	H
4	Nevirapine, stavudine, lamivudine	Stavudine, lamivudine, indinavir, ritonavir	47	F	AA
5	Efavirenz, abacavir, stavudine	Abacavir, stavudine, saquinavir	38	F	AA
6	Stavudine, lamivudine, nevirapine	Stavudine, lamivudine, saquinavir, ritonavir	31	M	AA
7	Stavudine, lamivudine, efavirenz	Stavudine, lamivudine, nelfinavir	40	F	C
8	Nevirapine, stavudine, lamivudine	Stavudine, lamivudine, nelfinavir	50	M	C
9	None	Stavudine, lamivudine, nelfinavir	47	M	AA
10	None	Stavudine, lamivudine, nelfinavir	46	M	H
11	None	Stavudine, lamivudine, nelfinavir	32	F	AA
12	None	Zidovudine, lamivudine, nelfinavir	39	M	AA
13	None	Lopinavir, efavirenz, lamivudine, ritonavir	38	F	C
Normal volunteers (N = 14)	—	—	42 ± 3	6F/8M	3AA/10C/1H

Data are means ± SD unless otherwise indicated. AA, African-American; C, Caucasian; H, Hispanic; PI, protease inhibitor.

initiation of protease inhibitor treatment. All subjects had normal routine laboratory screening results including normal liver function tests and urinalysis. None of the subjects had a family history of diabetes. Normal glucose tolerance had been ascertained by fasting plasma glucose levels <6 mmol/l and plasma glucose levels <7.8 mmol/l 2 h after a 75-g oral glucose load (17). The drug that patients received was based on the clinical judgment of their physician. Table 1 gives the drug regimens of individual patients before and after protease inhibitor treatment. Eight subjects (NRTI pretreated) had been on a combination therapy of nucleoside analogues and a non-NRTI for at least 12 months; the NRTIs were continued throughout the study, the non-NRTI was discontinued, and a protease inhibitor was added. Five subjects (NRTI naïve), who were drug naïve, were started on two NRTIs plus a protease inhibitor. Thus, during the treatment phase all patients were on a combination of a protease inhibitor and NRTIs. None of the subjects experienced any subcutaneous fat wasting before or throughout the study. All were studied on three occasions: once before initiation of protease inhibitor treatment and at 6 and 12 weeks thereafter. Only results of those before and at 12 weeks are reported.

Subjects consumed a weight maintenance diet containing 200–300 g carbohydrate at least 3 days before the first hyperglycemic clamp experiment and throughout the whole 12-week study period.

On each study occasion subjects were admitted to the clinical research center at 5:00 P.M. the day before the experiment. Between 6:00 P.M. and 7:00 P.M. a standard dinner (10 kcal/kg: 50% carbohydrate, 35% fat, and 15% protein) was given. At ~5:00 A.M. the following morning, primed-continuous infusions of [6,6-$^2H_2$]glucose (24 μmol/kg, 0.24 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and [9,10-^{3}H]palmitate (0.8 μCi/min) were started via a forearm vein. At 6:00 A.M. a retrograde venous catheter was inserted into a dorsal hand vein and maintained in a thermoregulated box at 65°C to obtain arterialized blood samplings (18). After allowing at least 2 h to achieve isotopic steady state, baseline samples for substrate enrichments, specific activities, plasma glucose, FFAs, glycerol, insulin, glucagon, C-peptide, and proinsulin concentrations were collected at −30, −15, and 0 min. Just before beginning the clamp, subjects took their medications. Subsequently, a primed (150 mg/kg body wt) 20% glucose infusion was given and plasma glucose levels were clamped at 10 mmol/l (180 mg/dl) for 3 h (19).

Plasma insulin, proinsulin, and C-peptide concentrations were measured at 2.5, 5.0, 7.5, 10, 15, 30, 60, 90, 120, 140, 160, and 180 min. Samples for plasma glucose, FFA, glucagon, glycerol concentrations, enrichments, and specific activities (SAs) were collected during the last hour of the clamp at 20-min intervals (120, 140, 160, and 180 min). Plasma glucose was measured at 5-min intervals throughout for adjustments of glucose infusion rates using a glucose analyzer (YSI Glucose Analyzer; Yellow Springs Instruments). Plasma insulin (Linco Research), proinsulin (Linco Research), C-peptide (Diagnostic Product), and glucagon (Linco Research) were measured by standard radioimmunoassays. Plasma FFA levels were measured by an enzymatic calorimetric method (NEFAC; Wako Pure Chemical). Plasma glycerol concentrations were determined by standard microfluorometric assays (20). HbA_{1c} was determined by high-performance liquid chromatography (HPLC).

Plasma [6,6-$^2H_2$]glucose enrichments were measured by gas chromatography mass spectroscopy (21), and plasma palmitate concentrations and SAs were determined by a previously described HPLC technique (22) with coefficients of variation of 3.8 and 2.3%, respectively. Total body fat and fat-free mass was determined by bioimpedance measurements (23).

Calculations. First-phase insulin release was calculated as the sum of the increments of C-peptide above baseline at 2.5, 5.0, 7.5, and 10 min. Second-phase insulin release was calculated as the average C-peptide concentration during the last hour of the clamp (24). Insulin sensitivity index (25) was calculated by dividing the average glucose infusion rate during the last hour of the clamp ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) by the product of the average plasma insulin (pmol/l) and glucose (mmol/l) concentration during the same time interval (25).

β-Cell function and insulin sensitivity were also assessed by using HOMA as previously described (26). Insulin sensitivity (IS_{HOMA}) was calculated as $IS_{HOMA} = 1/(135 \times \text{insulin} \times \text{glucose})$, i.e., the inverse of insulin resistance. β-Cell function ($SECR_{homa}$) was calculated as $SECR_{homa}$ = C-peptide × 3.33/(GLUC − 3.5), where C-peptide (pmol/l) represents the average fasting plasma C-peptide concentration and GLUC (mmol/l) the average fasting plasma glucose concentration (27). C-peptide was used in place of the originally proposed plasma insulin because of changes in insulin metabolism induced by protease inhibitor treatment as described below. To evaluate the appropriateness of changes in β-cell function in relation to changes in insulin sensitivity, the disposition index (28) was calculated as the product of β-cell function and insulin sensitivity determined during the clamp experiments.

During the basal state before the clamp, rates of appearance and disappearance (turnover) of FFA were calculated using the steady-state equation for the infusion of radioactive isotopes Ra(Rd) = F/SA (29), where Ra represents the rate of appearance, Rd the rate of disappearance, F the infusion rate of tracer ($dpm \cdot min^{-1} \cdot kg^{-1}$), and SA the specific activity ($dpm \cdot \mu mol^{-1}$). For glucose turnover, isotope enrichments (%) were substituted for SAs and corrected for the proportion of infused [6,6-$^2H_2$]glucose by subtracting its infusion rate from Ra(Rd). Glucose enrichments and palmitate SAs were not significantly different from one another, indicating that isotopic steady state had been achieved. Because there is evidence that NRTIs may be associated with insulin resistance (30), subjects were divided into two groups. Group 1 (NRTI pretreated, n = 8) had been on an NRTI and had a protease inhibitor added; group 2 (NRTI naïve, n = 5) had been drug naïve and were started on an NRTI and a protease inhibitor. The data of these groups were compared in an attempt to factor out the effects of protease inhibitors. Furthermore, baseline data of NRTI ± pretreated volunteers was compared with those of 14 healthy subjects to determine whether NRTI pretreatment had effects on insulin sensitivity and β-cell function. Unless specified otherwise, data are given as the mean ± SE and were initially evaluated by ANOVA for repeated measurements followed by Wilcoxon's matched pairs test comparing baseline data with results after 12 weeks of treatment. Mann-Whitney tests were used to compare changes between the groups during the treatment period as well as baseline data. Correlations between variables were performed using Spearman's regression analysis.

TABLE 2
Body composition, viral load, and CD_4 counts

	Baseline	12 weeks	*P* vs. baseline
BMI (kg/m^2)			
NRTI pretreated	29.7 ± 2.1	29.9 ± 1.7	0.93
NRTI naïve	26.7 ± 1.6	27.3 ± 2.6	0.92
Both	29.0 ± 1.8	28.9 ± 1.5	0.81
Normal volunteers	28.2 ± 0.8		
P vs. groups	0.34		
Lean body mass (kg)			
NRTI pretreated	56.4 ± 3.8	55.9 ± 3.6	
NRTI naïve	58.9 ± 3.1	60.5 ± 4.0	
Both	57.4 ± 2.6	57.7 ± 2.7	0.69
Normal volunteers	60.1 ± 4.9		
P vs. groups	0.62		
Body fat (kg)			
NRTI pretreated	33.0 ± 5.6	33.2 ± 5.5	
NRTI naïve	16.5 ± 2.9	18.2 ± 3.0	
Both	26.7 ± 4.9	27.4 ± 4.0	0.03
Normal volunteers	23.2 ± 2.6		
P vs. groups	0.048		
CD_4 counts			
NRTI pretreated	393 ± 50		
NRTI naïve	311 ± 51		
Both	361 ± 37		
P vs. groups	0.73		
Viral load			
NRTI pretreated	3638 ± 1588	256 ± 119	<0.001
NRTI naïve	2814 ± 1267	511 ± 205	<0.001
Both	3321 ± 1060	355 ± 108	<0.001
P vs. groups	0.82		

Data are means ± SD.

TABLE 3
HbA_{1c}, fasting plasma glucose, insulin, glucagon, C-peptide, and proinsulin

	Before	12 weeks	*P* vs. baseline
HbA_{1c} (%)			
NRTI pretreated	5.5 ± 0.3	—	
NRTI naïve	5.8 ± 0.3	—	
Both	5.6 ± 0.2	—	
Normal volunteers	5.8 ± 0.3		
P vs. groups	0.91		
Glucose (mmol/l)			
NRTI pretreated	4.58 ± 0.23	5.36 ± 0.13	
NRTI naïve	4.87 ± 0.25	5.69 ± 0.14	
Both	4.69 ± 0.11	5.49 ± 0.11	0.001
Normal volunteers	4.96 ± 0.11		
P vs. groups	0.59		
Plasma insulin (pmol/l)			
NRTI pretreated	108 ± 17	125 ± 17	
NRTI naïve	61 ± 12	87 ± 9	
Both	90 ± 13	111 ± 11	0.001
Normal volunteers	72 ± 12		
P vs. groups	0.08		
Plasma glucagon (pg/ml)			
NRTI pretreated	80 ± 4	80 ± 5	
NRTI naïve	78 ± 7	71 ± 3	
Both	79 ± 3	77 ± 4	0.69
Normal volunteers	70 ± 6		
P vs. groups	0.98		
Plasma C-peptide (nmol/l)			
NRTI pretreated	0.77 ± 0.13	0.73 ± 0.08	
NRTI naïve	0.51 ± 0.09	0.61 ± 0.06	
Both	0.67 ± 0.09	0.68 ± 0.05	0.84
Normal volunteers	0.54 ± 0.06		
P vs. groups	0.34		
Plasma proinsulin (pmol/l)			
NRTI pretreated	13.3 ± 2.1	13.0 ± 1.9	
NRTI naïve	11.1 ± 1.6	12.0 ± 1.4	
Both	12.4 ± 1.4	12.6 ± 1.2	0.61
Normal volunteers	12.0 ± 1.6		
P vs. groups	0.87		

Data are means ± SD.

RESULTS

Body composition, waist-to-hip ratio, viral loads, and CD_4 counts

Baseline characteristics of the groups did not differ except that the NRTI-naïve subjects had lower initial body fat. During 12 weeks of treatment, BMI, total body fat, and lean body mass did not change in either group; viral load decreased by >80% in both groups ($P < 0.001$) (Table 2).

HbA_{1c}, fasting plasma glucose, insulin, glucagon, C-peptide, proinsulin, glycerol, FFAs, and plasma lipids

Baseline values, which were comparable with those of the normal volunteers, and responses during treatment in NRTI-pretreated and NRTI-naïve groups were not significantly different (Tables 3 and 4).

After 12 weeks of treatment, fasting plasma glucose increased in all subjects ($P < 0.001$). Fasting plasma insulin also increased ($P = 0.001$). In contrast, fasting plasma C-peptide and proinsulin did not change (both $P > 0.61$). Consequently, both the plasma C-peptide–to–insulin ratio and the plasma proinsulin–to–insulin ratio decreased (both $P = 0.007$). The plasma C-peptide–to–proinsulin ratio and plasma glucagon remained unchanged ($P = 0.59$ and 0.69, respectively).

Fasting plasma glycerol increased ($P = 0.02$), whereas plasma FFAs, triglycerides, and total and HDL cholesterol remained unchanged (all $P > 0.13$). However, plasma LDL cholesterol increased slightly but significantly ($P = 0.03$).

β-Cell function and insulin sensitivity

HOMA. Baseline values and responses to treatment were not significantly different in NRTI-pretreated and NRTI-naïve subjects. At baseline, both groups had comparable insulin sensitivity and β-cell function with those of normal volunteers. After 12 weeks of treatment, both insulin sensitivity and pancreatic β-cell function decreased significantly ($P = 0.002$ and 0.003, respectively) (Tables 5 and 6 and Fig. 1).

Hyperglycemic clamp experiments

Insulin sensitivity. Plasma glucose, insulin, C-peptide, and proinsulin concentrations and glucose infusion rates are given in Fig. 1. Plasma glucose levels were not significantly different in clamps performed at baseline and 12 weeks in all groups. The insulin sensitivity index calculated by dividing the glucose infusion rates by the product of plasma glucose and insulin concentrations were comparable with those of the normal volunteers, and responses to treatment were not significantly different in NRTI-pretreated and NRTI-naïve subjects. The insulin sensitivity index decreased significantly after treatment ($P < 0.001$).

β-Cell function. At baseline, both first- and second-phase insulin release was normal in both patient groups. After treatment, first-phase insulin release decreased signifi-

TABLE 4
Plasma glycerol, FFAs, triglycerides, total cholesterol, and LDL and HDL cholesterol

	Before	12 weeks	*P* vs. baseline
Plasma glycerol (μmol/l)			
NRTI pretreated	86.2 ± 8.4	106 ± 10	
NRTI naïve	64.3 ± 8.3	124.6 ± 29	
Both	77.7 ± 6.6	113 ± 13	0.02
Normal volunteers	76.2 ± 7.2		
P vs. groups	0.08		
Plasma FFA (μmol/l)			
NRTI pretreated	595 ± 54	601 ± 32	
NRTI naïve	458 ± 72	664 ± 136	
Both	542 ± 46	625 ± 53	0.33
Normal volunteers	474 ± 32		
P vs. groups	0.26		
Total cholesterol (mg/dl)			
NRTI pretreated	123 ± 7	127 ± 8	
NRTI naïve	123 ± 12	135 ± 10	
Both	123 ± 6	130 ± 6	0.42
P vs. groups	0.98		
HDL (mg/dl)			
NRTI pretreated	41 ± 5	37 ± 4	
NRTI naïve	37 ± 6	36 ± 3	
Both	39 ± 3	37 ± 2	0.78
P vs. groups	0.81		
LDL (mg/dl)			
NRTI pretreated	60 ± 5	69 ± 6	
NRTI naïve	74 ± 12	89 ± 9	
Both	66 ± 5	77 ± 6	0.03
P vs. groups	0.92		
Triglycerides (mg/dl)			
NRTI pretreated	103 ± 11	131 ± 18	
NRTI naïve	78 ± 9	94 ± 9	
Both	95 ± 8	110 ± 13	0.13
P vs. groups	0.35		

Data are means ± SD.

cantly in both groups to a comparable extent ($P = 0.002$). Second-phase insulin release did not change in either group. However, the disposition index, calculated from second-phase insulin release, decreased significantly and to comparable extents in both groups ($P < 0.001$), indicating lack of appropriate β-cell compensation.

Systemic glucose and FFA turnovers and clearances. Baseline values and responses to treatment did not differ among the patient groups. Fasting plasma glucose turnover, which was normal at baseline, decreased significantly after treatment ($P = 0.002$). The product of basal plasma insulin and glucose turnover, an index of the appropriateness of endogenous glucose production for the prevailing insulinemia, remained unchanged in both groups, suggesting appropriate suppression of endogenous glucose production. Glucose clearance, which was normal at baseline, decreased significantly ($P < 0.001$), indicating peripheral insulin resistance. Although plasma FFA concentrations did not change as indicated above, both fasting plasma FFA turnover and clearance increased significantly after treatment (both $P < 0.001$) (Table 7).

DISCUSSION

This study was undertaken to assess the effect of protease inhibitor–based treatment on pancreatic β-cell function and to determine mechanisms and sites of insulin resistance induced by these agents. We studied 13 asymptomatic HIV-infected individuals before and after addition of a protease inhibitor, mainly nelfinavir ($n = 8$). Eight of the subjects had already been on NRTI-containing antiviral regimens and the remaining five had been drug naïve, but for clinical reasons were started on both a protease inhibitor and NRTI. Some studies have suggested that NRTIs may cause insulin resistance, whereas others have not (30–32). To factor out the effect of protease inhibitor and NRTI treatment, we analyzed these groups separately. At baseline, β-cell function and insulin sensitivity did not differ between groups or when compared with a group of matched normal volunteers. This suggests either that the previous long-term NRTI treatment had not affected either β-cell function or insulin sensitivity or that, if it had, its effect was too small to be detected given the number of subjects studied. Responses during treatment with either addition of a protease inhibitor alone or with the addition of both a protease inhibitor and NRTIs were comparable. This suggests that the effects we observed when adding a protease inhibitor to long-term NRTI treatment or when starting both types of agents were primarily those of the protease inhibitors as previously suggested (7).

After 12 weeks of treatment, we found a deterioration in glucose tolerance in all subjects, as indicated by increased fasting plasma glucose levels. Insulin sensitivity decreased

TABLE 5
HOMA assessment of β-cell function and insulin sensitivity

	Baseline	12 weeks	*P* vs. baseline
β-Cell function (pmol/l · mmol/l)			
NRTI pretreated	2,484 ± 375	1,379 ± 189	
NRTI naïve	1,352 ± 263	937 ± 77	
Both	2,049 ± 291	1,209 ± 132	0.002
Normal volunteers	1,288 ± 123		
P vs. groups	0.10		
Insulin sensitivity (pmol/l^{-1} · mmol/l^{-1})			
NRTI pretreated	0.49 ± 0.24	0.24 ± 0.05	
NRTI naïve	0.56 ± 0.13	0.29 ± 0.04	
Both	0.51 ± 0.15	0.26 ± 0.03	0.003
Normal volunteers	0.50 ± 0.07		
P vs. groups	0.98		

Data are means ± SD.

TABLE 6
Hyperglycemic clamp assessment of β-cell function and insulin

	Baseline	12 weeks	*P* vs. baseline
First-phase insulin release (nmol/l)			
NRTI pretreated	6.4 ± 1.1	5.0 ± 1.0	
NRTI naïve	5.2 ± 0.6	3.6 ± 0.4	
Both	5.9 ± 0.8	4.5 ± 0.7	0.002
Normal volunteers	4.6 ± 0.6		
P vs. groups	0.85		
Second-phase insulin release (nmol/l)			
NRTI pretreated	3.6 ± 0.6	3.8 ± 0.7	
NRTI naïve	3.2 ± 0.6	2.9 ± 0.3	
Both	3.5 ± 0.5	3.5 ± 0.5	0.98
Normal volunteers	2.8 ± 0.3		
P vs. groups	0.25		
Glucose infusion rates ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
NRTI pretreated	44 ± 3	36 ± 4	
NRTI naïve	54 ± 5	37 ± 6	
Both	48 ± 3	36 ± 3	
Normal volunteers	50 ± 6		$p < 0.001$
P vs. groups	0.83		
Plasma insulin (pmol/l)			
NRTI pretreated	836 ± 204	1,184 ± 298	
NRTI naïve	453 ± 61	654 ± 127	
Both	688 ± 136	980 ± 199	<0.001
Normal volunteers	473 ± 119		
P vs. groups	0.83		
Insulin sensitivity index ($ml \cdot kg^{-1} \cdot min^{-1}$)			
NRTI pretreated	9.0 ± 2.9	5.2 ± 1.6	
NRTI naïve	13.7 ± 2.3	7.5 ± 2.2	
Both	10.8 ± 2.0	6.1 ± 1.3	
Normal volunteers	14.1 ± 2.1		<0.001
P vs. groups	0.32		
Disposition index ($ml \cdot pmol/l \cdot kg^{-1} \cdot min^{-1} \cdot nmol/l^{-1}$)			
NRTI pretreated	24.9 ± 5.4	14.0 ± 1.8	
NRTI naïve	48.2 ± 15.8	22.6 ± 7.8	
Both	33.8 ± 7.3	17.3 ± 3.2	<0.001
Normal volunteers	33.6 ± 4.6		
P vs. groups	0.26		

Data are means ± SD.

by 40–50% as determined by HOMA and the hyperglycemic clamp technique. Overall pancreatic β-cell function, as assessed by HOMA, and first-phase insulin release, assessed during hyperglycemic clamp experiments, decreased significantly. Second-phase insulin release during the hyperglycemic clamp experiments was not reduced in an absolute sense but with use of the disposition index, an assessment of the appropriateness of β-cell function for a given degree of insulin resistance, it was found to be reduced significantly. Thus, it appears that first-phase insulin release was more severely affected than second-phase insulin release. Our results thus confirm that protease inhibitor treatment of HIV-infected individuals is associated with insulin resistance (2,7,33); furthermore, our results demonstrate that this insulin resistance is not accompanied by an appropriate compensatory increase in insulin secretion. Indeed, there was a strong correlation between deterioration of the disposition index and the increase in fasting plasma glucose concentrations (r = 0.645).

Various protease inhibitors (indinavir, nelfinavir, lopinovir, saquinavir, and ritonavir) have been reported to cause insulin resistance either in vivo or in vitro (1,5,12,34,35). It has been suggested that inducement of insulin resistance by protease inhibitors may be a class effect (33), although these agents may differ in the extent to which they affect insulin sensitivity (7). However, our study was not designed or powered to detect differences in magnitude among these agents, but rather to assess the mechanisms responsible for the associated deterioration in glucose tolerance. Since most of our subjects received nelfinavir, the conclusion of a class effect must be drawn with caution.

Regarding the mechanisms/sites of insulin resistance induced by protease inhibitor treatment, we found a reduction in glucose disposal, whereas glucose production appeared to be appropriately reduced for the prevailing plasma insulin concentration. Under euglycemic-hyperinsulinemic clamp conditions, most glucose disposal occurs in skeletal muscle (25). Indinavir has been shown to inhibit muscle glucose transport (36). Our results thus provide additional evidence that these agents reduce skeletal muscle insulin sensitivity.

Furthermore, our findings suggest that there was also insulin resistance in adipose tissue. Although plasma FFA concentrations did not change, plasma glycerol concentra-

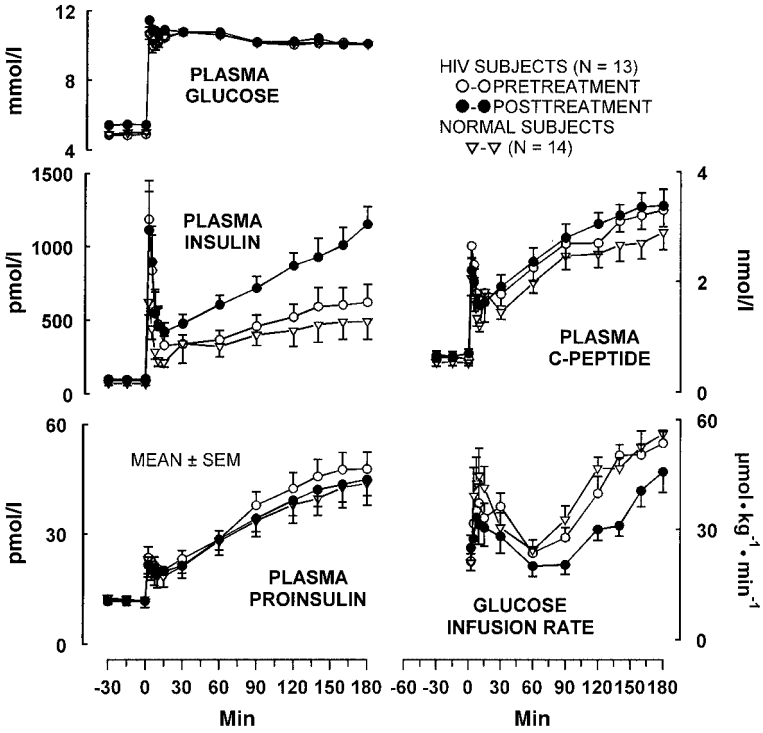

FIG. 1. Plasma glucose, insulin, C-peptide, and proinsulin concentrations and glucose infusion rates in HIV subjects pre- and posttreatment and in normal volunteers.

tions and plasma FFA turnover increased. Because this occurred in the face of increased plasma insulin levels, these observations provide evidence for impaired suppression of lipolysis by insulin. The latter is consistent with recent reports that nelfinavir, saquinavir, and ritonavir increase lipolysis (11,35,37).

We found no alteration of plasma lipids except for an increase in LDL cholesterol. Previous studies have found no effect of indinavir and ritonavir on LDL cholesterol in normal volunteers, whereas in HIV-infected individuals protease inhibitors appear to consistently increase LDL cholesterol and have variable effects on triglycerides (9,38,39).

Recently, the HIV-lipodystrophy syndrome (HLS), a condition characterized by changes in body fat redistribution, peripheral and facial fat loss, dyslipidemia, and insulin resistance has been associated with protease inhibitor and NRTI therapy (40). It has been suggested that the changes in lipid metabolism associated with the HLS may be at least partially responsible for a deterioration in glucose tolerance (41). We did not find any significant changes in body fat content and lean body mass or any clinical signs of fat redistribution in our patients either before or after treatment. However, we did not assess changes in body fat distribution with magnetic resonance imaging or computer tomography (CT). Thus, we cannot exclude that subtle changes in body composition might have occurred. However, changes in glucose tolerance have been reported to take place with protease inhibitors without detectable changes in body composition determined by CT (9). However, we did find an increase in FFA turnover and clearance after treatment. Recently, Sekhar et al. (42) provided strong evidence that the HLS is associated with increased FFA turnover and suggested that this reflected ongoing fat redistribution. Thus, the increased FFA turnover that we found in the absence of obvious lipodystrophy may be an early indicator for changes in lipid metabolism, which might eventually lead to the development of lipodystrophy. On the other hand, the increased FFA turnover may simply reflect insulin resistance accompanying the treatment unrelated to the HLS. Nevertheless, the insulin resistance in type 2 diabetes is characterized by normal FFA turnover and reduced FFA clearance (43,44).

The molecular mechanism for the deterioration in pancreatic β-cell function found in the present study and in a preliminary report in nelfinavir-treated rats (45) remains to be elucidated. It had been initially speculated that protease inhibitors may impair β-cell function by inhibiting one of the endopeptidases involved in the cleavage of proinsulin to insulin (4), because increased plasma insulin–to–proinsulin ratios were found in protease inhibitor–treated patients (5). In the present studies we found the opposite. Moreover, subsequent studies (46) in which islet cells were incubated with protease inhibitors did not show any adverse effect on the conversion of proinsulin to insulin. Thus, endopeptidase inhibition does not seem to be involved.

There is evidence, however, that insulin itself may be important for maintaining normal β-cell function based on the finding of reduced insulin release in mice with knockout of the insulin receptor and insulin receptor substrate-2 (47). Conceivably, therefore, the same mechanism by which protease inhibitor–containing regimens cause insulin resistance in the peripheral tissues might be operative in the pancreatic β-cell.

It is noteworthy that in the present study the ratio of plasma proinsulin to plasma insulin and the ratio of plasma C-peptide to plasma insulin both decreased significantly with treatment, whereas the ratio of plasma proinsulin to plasma C-peptide was unaltered. Because insulin and C-peptide are secreted from the β-cell in equimolar amounts, changes in the ratio of plasma C-peptide to plasma insulin would reflect a change in the clearance of insulin or C-peptide. The above findings are therefore

TABLE 7
Baseline systemic glucose and FFA turnover and clearance

	Before	12 weeks	*P* vs. baseline
Glucose turnover (μmol · kg^{-1} · min^{-1})			
NRTI pretreated	9.6 ± 0.8	8.4 ± 0.7	
NRTI naïve	11.8 ± 0.4	9.2 ± 1.0	
Both	10.3 ± 0.5	8.7 ± 0.6	0.002
Normal volunteers	11.3 ± 0.5		
P vs. groups	0.08		
Glucose turnover × plasma insulin (μmol · kg^{-1} · min^{-1} · pmol/l^{-1})			
NRTI pretreated	1042 ± 201	1073 ± 175	
NRTI naïve	706 ± 131	792 ± 101	
Both	913 ± 138	965 ± 117	0.55
Normal volunteers	826 ± 143		
P vs. groups	0.34		
Glucose clearance (ml · kg^{-1} · min^{-1})			
NRTI pretreated	2.10 ± 0.17	1.58 ± 0.14	
NRTI naïve	2.44 ± 0.13	1.61 ± 0.16	
Both	2.21 ± 0.13	1.59 ± 0.10	<0.001
Normal volunteers	2.31 ± 0.11		
P vs. groups	0.93		
FFA turnover			
NRTI pretreated	2.68 ± 0.25	4.80 ± 0.42	
NRTI naïve	3.12 ± 0.31	5.89 ± 0.90	
Both	2.85 ± 0.20	5.22 ± 0.44	<0.001
Normal volunteers	3.54 ± 0.3		
P vs. groups	0.15		
FFA clearance (ml · kg^{-1} · min^{-1})			
NRTI pretreated	4.79 ± 0.66	8.16 ± 0.79	
NRTI naïve	7.26 ± 1.02	9.20 ± 0.86	
Both	5.74 ± 0.64	8.56 ± 0.58	0.001
Normal volunteers	7.90 ± 0.94		
P vs. groups	0.06		

Data are means ± SD.

consistent with the conclusion that insulin clearance had been reduced. If C-peptide clearance been altered, one would expect to find a change in the plasma proinsulin–to–plasma C-peptide ratio. The practical significance of this finding is that changes in plasma insulin concentrations cannot be used as an index of β-cell function during treatment with protease inhibitor–containing regimens.

In conclusion, the present study indicates that protease inhibitor–containing regimens impair glucose tolerance in HIV-infected patients by two mechanisms: *1*) inducement of peripheral insulin resistance in skeletal muscle and adipose tissue and *2*) reduction in pancreatic β-cell function. There are several clinical implications of this study. First, use of these regimens may cause deterioration of glycemic control in patients with preexisting diabetes. Second, because these regimens appear to induce insulin resistance predominantly in skeletal muscle and adipose tissue, antidiabetic agents that exert their major action on peripheral tissues rather than on the liver (e.g., thiazolidinediones) would appear advantageous (48). Third, because first-phase insulin release appears to be more severely impaired than second-phase insulin release, secretagogues such as the meglitinides, which primarily improve first-phase insulin release (49), may be preferable to sulfonylureas, which apparently only affect second-phase insulin release (49).

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