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Insulin Sensitivity and β-Cell Function in Protease Inhibitor-Treated and -Naive Human Immunodeficiency Virus-Infected Children

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Previous pediatric studies have failed to demonstrate a clear association between protease inhibitor (PI) therapy and abnormal glucose homeostasis in HIV-infected children. To define more precisely the impact of PI therapy on glucose homeostasis in this population, we performed the insulin-modified frequent-sampling iv glucose tolerance test on 33 PI-treated and 15 PI-naive HIV-infected children. Other investigations included fasting serum lipids; glucose, insulin, and C-peptide; single-slice abdominal computed tomography; and, in a subset of PI-treated children, an oral glucose tolerance test.

There were no differences between the two groups with respect to fasting serum insulin or C-peptide, homeostatic model assessment insulin resistance, or quantitative insulin sensitivity check index. The mean insulin sensitivity index of PI-treated and PI-naive children was 6.93 ± 6.37 and 10.58 ± 12.93 × 10^{-4} min^{-1} [μU/ml]$^{-1}$, respectively ($P = 0.17$). The mean disposition index for the two groups was 1840 ± 1575 and 3708 ± 3005 × 10^{-4} min^{-1} ($P = 0.013$), respectively. After adjusting for potential confounding variables using multiple regression analysis, the insulin sensitivity index and disposition index of PI-treated children were significantly lower than that of PI-naive children ($P = 0.01$ for both). In PI-treated but not PI-naive children, insulin sensitivity correlated inversely with visceral adipose tissue area ($r = -0.43$, $P = 0.01$) and visceral to sc adipose tissue ratio ($r = -0.49$, $P = 0.004$). Mildly impaired glucose tolerance was noted in four of 21 PI-treated subjects tested.

Our results demonstrate not only that PI therapy reduces insulin sensitivity in HIV-infected children but also that it impairs the β-cell response to this reduction in insulin sensitivity and, in a subset of children, leads to the development of impaired glucose tolerance. The presence of insulin resistance, dyslipidemia, and the significant correlation of reduced insulin sensitivity with increased visceral adipose tissue content suggest that PI-containing highly active antiretroviral therapy is associated with the emergence of early features of a metabolic syndrome-like phenotype. (*J Clin Endocrinol Metab* 90: 168–174, 2005)

IN HIV-INFECTED ADULTS, highly active antiretroviral therapy (HAART) has been associated with a spectrum of metabolic abnormalities including impairment of glucose homeostasis, ranging from insulin resistance to impaired glucose tolerance and diabetes mellitus, dyslipidemia, and alterations in body fat distribution (1, 2). The causes of these metabolic abnormalities appear to be multifactorial, involving adverse effects of antiretroviral medications as well as HIV-related viral and immunologic factors (1). The protease inhibitors (PIs) are thought to play an important role in the development of insulin resistance, dyslipidemia, and visceral adipose tissue accumulation, whereas the nucleoside reverse transcriptase inhibitors appear to be the predominant factor leading to development of peripheral lipoatrophy.

The metabolic perturbations associated with antiretroviral therapy in general, and PI therapy in particular, have not been characterized in children to the same extent as in adults. Nevertheless, there is evidence that hypercholesterolemia, hypertriglyceridemia, peripheral lipoatrophy, and intraabdominal adipose tissue accumulation are relatively common abnormalities in HIV-infected children (3–10). In contrast to adults, insulin resistance, impaired glucose tolerance, and diabetes mellitus appear to be a relatively uncommon finding in children (3–5, 11). However, the methodologies used in previously published pediatric studies were not of sufficient sensitivity to detect early disturbances in glucose homeostasis. To define more precisely the impact of PI therapy on glucose homeostasis in HIV-infected children, we performed the frequent-sampling iv glucose-tolerance test (FSIVGTT) on PI-treated and PI-naive HIV-infected children. Fasting measures of glucose homeostasis, serum lipids, and abdominal adipose tissue distribution were also evaluated. A subgroup of PI-treated children also underwent an oral glucose tolerance test (OGTT).

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Abbreviations: BMI, Body mass index; FSIVGTT, frequent sampling iv glucose tolerance test; HAART, highly active antiretroviral therapy; HOMA-IR, homeostatic model assessment-insulin resistance; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; PI, protease inhibitor; QUICKI, quantitative insulin sensitivity check index.

Subjects and Methods

Subjects

Subjects were recruited from among HIV-infected children followed up by the Division of Infectious Diseases at the Hospital for Sick Children, Toronto, and British Columbia Children's Hospital in Vancouver

between August 1999 and March 2002. Subjects were eligible for inclusion in this cross-sectional study if they were between 3 and 18 yr of age and if there had been no change in their clinical and/or immunological HIV classification status during the preceding 6 months. A minimum of 3 months of PI therapy was required for inclusion in the PI-treated group. Exclusion criteria included: 1) presence of a chronic illness in addition to HIV, such as familial hypercholesterolemia, that could significantly affect one or more of the outcome measures; 2) inability of the family and/or patient to comply with the study protocol due to difficult social circumstances, general unreliability of the patient and/or guardian with respect to medical care, or a significant underlying clinical illness in the child, such as HIV encephalopathy that would compromise his/her ability to cooperate with study requirements; 3) high-dose or prolonged glucocorticoid therapy during the 30 d before performance of study investigations; 4) birth control pill administration; or 5) poor adherence to antiretroviral therapy based on history and prescription renewal information.

Ethical approval for this study was obtained from the Research Ethics Boards of the Hospital for Sick Children, Toronto, and the University of British Columbia and British Columbia Children's Hospital in Vancouver. Written voluntary informed consent was obtained from all children 16 yr of age or older. For younger children, written voluntary informed consent was obtained from the child's legal guardian; assent was obtained for all children between 6 and 16 yr of age deemed capable.

Protocol

Study investigations were completed during two clinic visits. The FSIVGTT, fasting blood work, and computed tomography of the abdomen were performed during the first visit. Baseline and demographic characteristics were recorded at this time. The OGTT, administered to a subset of PI-treated children, was performed within 12 months of the first study visit.

Clinical and demographic characteristics. The age, sex, ethnicity (Caucasian, Black, other), clinical and immunological HIV disease category, CD4 count, viral load, past and current antiretroviral medications, height, weight, Tanner stage, and waist to hip circumference ratio were recorded. In those receiving PI therapy, the duration of such therapy was documented. Height was measured using a wall-mounted stadiometer and weight using a standard balance scale. Body mass index (BMI) was calculated in standard fashion (weight in kilograms divided by the square of the height in meters). BMI SD score was used as an age- and sex-adjusted measure of obesity (12). Pubertal developmental stage was evaluated using Marshall and Tanner criteria (13, 14). Waist and hip circumference were measured in duplicate to the nearest millimeter using a plastic tape measure. Measurement of waist circumference was performed at the level of the umbilicus and that of the hip at the level of the greater trochanter and symphysis pubis.

FSIVGTT. Insulin sensitivity, acute insulin response, and glucose effectiveness were determined from the insulin-modified FSIVGTT (15). Briefly, a single iv line connected to a three-way stopcock was inserted into the antecubital vein after a 10-h overnight fast. Patients were instructed to take their morning medications with water in accordance with their usual schedule. An iv bolus of 0.3 g/kg of 50% glucose solution was administered over 30 sec after obtaining two separate baseline samples for measurement of fasting glucose and insulin (−5 and −1 min). Twenty minutes after the bolus infusion of glucose, a bolus of 0.03 U/kg regular human insulin (1.0 U/ml solution) was administered iv. Sequential blood samples for measurement of serum glucose and insulin were drawn at 2, 4, 6, 8, 10, 13, 16, 19, 22, 24, 27, 30, 40, 50, 70, 90, and 120 min after the bolus infusion of glucose. All blood samples were immediately stored on ice and transported to the laboratory at which they were centrifuged and the serum stored at −20 C for measurement at a later date. Serum glucose and insulin levels were determined for all 19 blood samples; serum glucose was measured by the glucose oxidase method (Synchron CX3 Delta Clinical System, Beckman Coulter, Inc., Fullerton, CA) and serum insulin using a double-antibody RIA (Insulin RIA 100, Pharmacia & Upjohn, Stockholm, Sweden). The MINMOD computer program (version 3, Richard N. Bergman) was used to determine the insulin sensitivity index, acute insulin response, and glucose effectiveness (16). The disposition index was defined as the product of acute insulin response and insulin sensitivity (17, 18). The glucose disposal coefficient was defined as the rate of change in serum glucose that occurred between 10 and 20 min after bolus infusion of glucose.

Fasting serum lipids and abdominal adipose tissue distribution. Fasting serum total, low-density lipoprotein (LDL) and high-density lipoprotein cholesterol, triglycerides and C-peptide, homeostatic model assessment-insulin resistance (HOMA-IR), and quantitative insulin sensitivity check index (QUICKI) were determined from the first fasting blood sample and abdominal adipose tissue distribution using single-slice (10 mm thickness) computed tomography at the level of the umbilicus as previously described (3). HOMA-IR and QUICKI were derived from fasting glucose and insulin measurements in standard fashion (19–21).

OGTT. A subset of PI-treated subjects (n = 21) had an OGTT within 12 months of completing the insulin-modified FSIVGTT. Twelve PI-treated subjects declined the test; in all 12 cases, this was due to their reluctance to undergo an additional test that required overnight fasting, iv access, and several hours of observation. There were no statistically significant differences between those who did and those who did not undergo an OGTT with respect to age, Tanner stage, sex, BMI, ethnic background, viral load, CD4 count, HIV clinical stage, HIV immunologic stage, stavudine exposure, or duration of PI therapy or antiretroviral therapy. An iv line was placed after a 10-h overnight fast. At the time of iv line insertion, a blood sample was drawn for measurement of fasting serum glucose and insulin. An oral glucose solution (Glucodex, 75 g/300 ml, Technilab Rougier Altimed Nadeau, Mirabel, Québec) was then administered in a dose of 1.75 g/kg body weight (maximum dose 75 g). Blood samples were obtained for measurement of glucose and insulin 30, 60, 90, and 120 min after administering the oral glucose solution. Impaired glucose tolerance and diabetes mellitus were defined in accordance with the criteria set forth by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (22).

Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC) and graphical analysis using GraphPad Prism statistical software (GraphPad Software, Inc., San Diego, CA). The PI-treated and PI-naive HIV-infected cohorts were compared with respect to baseline characteristics and outcome variables using the two-tail Student's t test for continuous variables and the χ^2 statistic for dichotomous variables. Continuous variables with skewed, nonnormal distributions were log transformed before analysis. Spearman correlation coefficients were used to assess correlation between variables. Stepwise multiple linear regression analysis was used to determine the independent effect of PI therapy and other variables on glucose homeostasis outcomes. The best-fitted regression model was selected on the bases of adjusted R^2 and the Cp statistic.

Results

Clinical and demographic characteristics

Sixty-three children met the inclusion criteria. Eight were excluded, two because of clinically unstable HIV disease, one because of poor compliance with therapy, and five because of inability to comply with the study protocol. Forty-eight of the remaining 55 children were enrolled; 33 were PI treated and 15 PI naive. The mean duration of PI therapy in the former group was 26.0 ± 14.5 months (range 8–59 months). All PI-treated subjects were receiving HAART that included, in addition to the PI, at least two other non-PI antiretroviral drugs. Protease inhibitor therapy consisted of ritonavir (n = 10), nelfinavir (n = 14), indinavir (n = 2), lopinavir/ritonavir (n = 5), ritonavir + nelfinavir (n = 1), and nelfinavir + saquinavir (n = 1). In the PI-naive group, three were antiretroviral naive, 10 were on dual-nucleoside reverse transcriptase inhibitor therapy, and two were on HAART consisting of two nucleoside reverse transcriptase inhibitors and a nonnucleoside reverse transcriptase inhibitor. Among PI-treated subjects, three were classified

as asymptomatic (class N), eight as mildly symptomatic (class A), eight as moderately symptomatic (class B), and 14 as severely symptomatic (class C) in accordance with the revised pediatric clinical classification system of the Centers for Disease Control and Prevention (23). In the PI-naive group, there were five asymptomatic (class N), five mildly symptomatic (class A), three moderately symptomatic (class B), and two severely symptomatic (class C) children.

Clinical and demographic characteristics of PI-treated and PI-naive HIV-infected children are depicted in Table 1. The two groups were similar with respect to age, sex, ethnic background, Tanner stage, and BMI. In the PI-treated group, 55% were black, 33% were Caucasian, and 12% were of other ethnic groups. The corresponding proportions in the PI-naive group were 73, 20, and 7%, respectively. Among PI-treated subjects, 73% were prepubertal, 9% were Tanner stage 2 or 3, and 18% were Tanner stages 4 or 5. The corresponding proportions in the PI-naive group were 67, 20, and 13%, respectively. The duration of antiretroviral therapy was significantly longer in the PI-treated group ($P = 0.03$). PI-treated children were also more likely to have been exposed to stavudine ($P < 0.0001$), and they tended to have more advanced HIV disease, as determined by HIV clinical category ($P = 0.06$) and HIV immunologic category ($P = 0.12$). On the other hand, the absolute CD4 count ($P = 0.17$) and CD4 percent ($P = 0.27$) was slightly higher and the viral load slightly lower ($P = 0.32$) in the PI-treated group.

Insulin-modified FSIVGTT

Indices of glucose homeostasis for both PI-treated and PI-naive HIV-infected children are shown in Tables 2 and 3. The fasting serum glucose, insulin and C-peptide, HOMA-IR, and QUICKI of the two groups were similar (Table 2). The mean disposition index of PI-treated children was 50% lower than that of PI-naive children ($P = 0.013$; Table 3). No statistically significant differences were observed between the two groups with respect to insulin sensitivity, glucose effectiveness, acute insulin response, or glucose disposal coefficient. Interestingly, the acute insulin response of PI-treated children was 11% lower than that of PI-naive children despite an insulin sensitivity that was 34% lower in the former group. The hyperbolic curve relating insulin sensitivity with acute insulin response for the PI-treated cohort was shifted downward and to the left in comparison with that of the PI-naive cohort (Fig. 1).

Multiple linear regression analysis was used to determine the independent effect of PI therapy and define the important predictor variables with respect to insulin sensitivity, glucose effectiveness, acute insulin response, disposition index, and glucose disposal coefficient. All potential explanatory variables listed in Table 1 were included in the model-building process. After adjusting for potential confounding variables, the insulin sensitivity index and disposition index for PI-treated children were significantly lower than that for PI-naive children (Table 4). No significant differences in acute insulin response, glucose effectiveness, or glucose disposal

TABLE 2. Unadjusted comparison of fasting glucose homeostasis parameters

Parameter	PI-treated (n = 33) Mean ± SD	PI-naive (n = 15) Mean ± SD	P^a
Glucose (mg/dl)[b]	81.6 ± 9.4	80.0 ± 7.0	0.55
Insulin (μU/ml)[b]	9.08 ± 5.89	8.48 ± 4.38	0.81
C-peptide (ng/ml)[b]	1.28 ± 0.89	0.97 ± 0.45	0.38
HOMA-IR[c]	1.55 ± 1.08	1.42 ± 0.67	0.68
QUICKI[d]	0.370 ± 0.031	0.370 ± 0.029	0.99

[a] Two-tail Student's *t* test for all variables; comparisons performed on log-transformed data for nonnormally distributed variables.

[b] Conversion to Systeme International units: glucose (millimoles per liter) = glucose (milligrams per deciliter) × 0.05551; insulin (picomoles per liter) = insulin (microunits per milliliter) × 6.0; and C-peptide (picomoles per liter) = C-peptide (nanograms per milliliter) × 331.

[c] HOMA-IR = [fasting insulin (microunits per milliliter) × fasting glucose (millimoles per liter)]/22.5 (19).

[d] QUICKI = $1/[\log(I_0) + \log(G_0)]$ where I_0 is fasting insulin in microunits per milliliter and G_0 is fasting glucose in milligrams per deciliter (21).

TABLE 1. Clinical and demographic characteristics[a]

Parameter	PI-treated (n = 33)	PI-naive (n = 15)	P^b
Age (yr)	9.1 ± 4.3	8.7 ± 3.7	0.75
Sex (% female)	42	47	1.00
Ethnicity (% Caucasian)	33	20	0.50
Tanner stage (% Tanner 1)	73	67	0.74
Viral load ($\log_{10}$[copies/ml])	4.02 ± 4.45	4.28 ± 4.45	0.32
Absolute CD4 (cells/mm^3)	932 ± 579	706 ± 368	0.17
CD4 %	28.8 ± 9.5	25.5 ± 9.8	0.27
HIV clinical category (% category C)[c]	42	13	0.06
HIV immunologic category (% category 3)[b]	45	20	0.12
Stavudine exposure (%)[d]	88	27	<0.0001
Duration of stavudine exposure (months)	20.6 ± 16.2	7.5 ± 14.4	0.01
Duration of antiretroviral therapy (months)	53.0 ± 25.5	36.1 ± 20.5	0.03
Waist-hip ratio	0.92 ± 0.06	0.89 ± 0.04	0.09
BMI (kg/m^2)	17.9 ± 3.8	18.2 ± 3.5	0.74
Age adjusted BMI score	0.6 ± 1.5	1.1 ± 2.1	0.34
Visceral adipose tissue area (mm^2)	1920 ± 2240	1523 ± 799	0.37
Subcutaneous adipose tissue area (mm^2)	6904 ± 6883	8590 ± 7582	0.45
Visceral/sc adipose tissue ratio	0.298 ± 0.199	0.239 ± 0.105	0.18

[a] Reported as mean ± SD for continuous variables and percent for dichotomous variables.

[b] Two-tail Student's *t* test for continuous variables and Fischer's exact test for dichotomous variables.

[c] Based on the revised pediatric Centers for Disease Control and Prevention HIV clinical staging system (23).

[d] Defined by past or present stavudine exposure.

TABLE 3. Unadjusted comparison of glucose homeostasis parameters derived from the FSIVGTT

Parameter	PI-treated (n = 33) Mean ± SD	PI-naive (n = 15) Mean ± SD	P^a
Insulin sensitivity index ($\times 10^{-4}$ min^{-1} [μU/ml]$^{-1}$)^b	6.93 ± 6.37	10.58 ± 12.93	0.17
Acute insulin response (μU/ml)^b	486.6 ± 566.6	544.2 ± 471.2	0.29
Glucose effectiveness (10^{-2} min^{-1})	3.90 ± 1.56	3.56 ± 1.30	0.44
Disposition index ($\times 10^{-4}$ min^{-1})	1840 ± 1575	3708 ± 3005	0.013
Glucose disposal coefficient (10^{-2} [mg/dl] min^{-1})^b	2.71 ± 0.31	2.69 ± 0.16	0.89

[a] Two-tail Student's *t* test for all variables; comparisons performed on log-transformed data for nonnormally distributed variables.
[b] Conversion to Systeme International units: insulin sensitivity index ($\times 10^{-4}$ min^{-1} [picomoles per liter]$^{-1}$) = insulin sensitivity index ($\times 10^{-4}$ min^{-1} [microunits per milliliter]$^{-1}$) $\times$ 0.167; acute insulin response (picomoles per liter) = acute insulin response (microunits per milliliter) $\times$ 6.0; glucose disposal coefficient (millimoles per liter) = glucose disposal coefficient (milligrams per deciliter) $\times$ 0.05551.

coefficient were detected in the adjusted analysis (data not shown). Explanatory variables significantly associated with insulin sensitivity in the final multiple regression model included PI therapy, CD4 percent, age, stavudine exposure, and duration of antiretroviral therapy (Table 4). Tanner stage, although strongly associated with the insulin sensitivity index on univariate analysis (r = −0.48, $P = 0.0006$), was excluded from the final model due to its high degree of correlation with age (r = 0.77, $P < 0.0001$); the more significant association of the insulin sensitivity index with age (r = −0.58, $P < 0.0001$), compared with Tanner stage; and the better overall model fit with age. With respect to disposition index, PI therapy and ethnic background were the only significant explanatory variables.

OGTT

In accordance with the recommendations of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (22), none of the 21 PI-treated children tested had diabetes mellitus. Impaired glucose tolerance was demonstrated in four children; in one, the fasting blood glucose was more than 110 mg/dl (6.1 mmol/liter) but less than 126 mg/dl (7 mmol/liter), and in three, the 2-h glucose was more than 140 mg/dl (7.8 mmol/liter) but less than 200 mg/dl (11.1 mmol/liter). None of these children were obese and three of four were prepubertal. The insulin sensitivity index for these four children was low, ranging from 1.08 to 3.66 $\times$ 10^{-4}min^{-1}[μU/ml]$^{-1}$. The insulin sensitivity index correlated moderately well with the Matsuda index (24) derived from the OGTT (r = 0.60, $P = 0.004$). The correlation of the insulin sensitivity index with fasting serum insulin, HOMA-IR, and QUICKI (r = −0.38, $P = 0.08$; r = −0.40, $P = 0.08$; and r = 0.40, $P = 0.07$, respectively) was not as high as that with the Matsuda index.

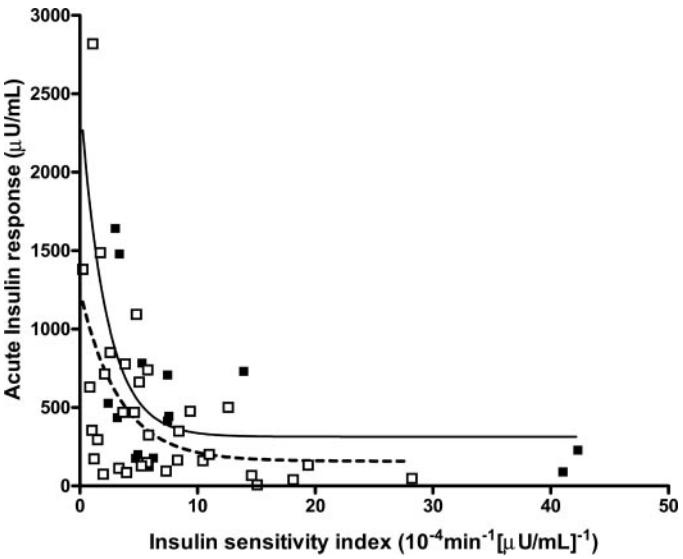

FIG. 1. Hyperbolic insulin sensitivity/secretion curve according to PI treatment category. The curve for PI-treated children (*open squares*, *broken regression line*) is shifted downward and to the left, compared with that of PI-naive children (*solid squares*, *solid regression line*). Insulin sensitivity index [$\times 10^{-4}$ min^{-1} (picomoles per liter)$^{-1}$] = insulin sensitivity index ($\times 10^{-4}$ min^{-1} [microunits per milliliter]$^{-1}$) $\times$ 0.167; AIR (picomoles per liter) = AIR (microunits per milliliter) $\times$ 6. AIR, Acute insulin response.

Fasting serum lipids and abdominal adipose tissue content and their correlation with insulin sensitivity and fasting serum insulin

Fasting serum lipids and computed tomographic measures of abdominal adipose tissue distribution of most of the patients in the present study were included in a previous report (3). In the present cohort, the mean total cholesterol of PI-treated subjects was significantly higher than that of PI-naive subjects (204.6 ± 59.2 and 151.9 ± 29.5 mg/dl, respectively; $P = 0.0002$; multiply by 0.0259 to convert to Systeme International units). PI-treated subjects also had significantly higher mean LDL cholesterol (131.2 ± 52.5 and 90.2 ± 27.0 mg/dl, respectively; $P = 0.001$; multiply by 0.0259 to convert to Systeme International units) and triglycerides (160.4 ± 116.9 and 95.9 ± 45.8 mg/dl, respectively; $P = 0.009$; multiply by 0.0113 to convert to Systeme International units). The mean serum high-density lipoprotein cholesterol of the two

TABLE 4. Final multiple regression models for insulin sensitivity and disposition index[a]

Outcome variable	Explanatory variables	Direction of effect	*P*	Model adjusted R^2
Insulin sensitivity	PI therapy	−	0.0100	0.44
	CD4 %	+	0.0047	
	Age	−	0.0048	
	D4T exposure	+	0.0249	
	Duration of ART	−	0.0490	
Disposition index	PI therapy	−	0.0112	0.25
	Caucasian race	−	0.0132	
	CD4 %	+	0.1077	

[a] The effect of PI therapy on acute insulin response, glucose effectiveness, and glucose disposal coefficient remained nonsignificant after adjusting for other variables. −, Negative; +, positive; ART, antiretroviral therapy.

groups was similar (44.3 ± 11.8 and 42.7 ± 12.1 mg/dl, respectively; $P = 0.7$; multiply by 0.0259 to convert to Systeme International units). The mean visceral adipose tissue area and visceral to sc adipose tissue ratio of PI-treated subjects were slightly higher than that of PI-naive subjects, although not significantly so (Table 1).

The correlation of insulin sensitivity and fasting serum insulin with fasting lipids and abdominal adipose tissue content measures are shown in Table 5. In PI-treated children, insulin sensitivity correlated inversely with visceral adipose tissue area ($P = 0.01$) and visceral to sc adipose tissue ratio ($P = 0.004$). In this same group, fasting serum insulin correlated positively with total, visceral, and sc adipose tissue areas ($P < 0.05$ for all) and BMI ($P = 0.008$). In PI-naive children, fasting serum insulin correlated positively with BMI ($P = 0.02$) and fasting serum triglycerides ($P = 0.03$) and inversely with visceral to sc adipose tissue ratio ($P = 0.05$).

Discussion

Previously published pediatric studies failed to demonstrate an association between PI use and the development of insulin resistance, impaired glucose tolerance, or diabetes mellitus (3–5, 11). In the study by Jaquet *et al.* (11), all 39 children who underwent OGTT, including 31 who were receiving PI therapy, had normal glucose tolerance. No significant differences in fasting serum insulin, proinsulin or C-peptide, insulin to glucose ratio, or HOMA-IR were detected between PI-treated and PI-naive children in any of these studies (3–5, 7). In the present study, the insulin sensitivity index (derived from the FSIVGTT) of PI-treated subjects was significantly lower than that of PI-naive subjects, after adjusting for potential confounding variables, despite no discernible differences in fasting serum insulin or C-peptide, HOMA-IR, or QUICKI. The apparent discrepancy noted between the FSIVGTT results and those from fasting serum insulin and C-peptide, HOMA-IR, and QUICKI may relate to the different aspects of insulin sensitivity assessed by these tests, namely stimulated insulin sensitivity for the FSIVGTT *vs.* basal insulin sensitivity for the other measures and perhaps to the capacity of the FSIVGTT to detect a relatively early disturbance in glucose homeostasis. Furthermore, the FSIVGTT has been shown to correlate highly with the accepted gold standard for assessment of insulin resistance, the glucose clamp technique (25, 26). Our results suggest that, in HIV-infected children, PI therapy leads to development of insulin resistance in a manner similar to that observed in adults, but this effect is more difficult to detect, perhaps due to the inherently higher insulin sensitivity of children, compared with adults (27, 28).

Under normal physiologic conditions, reduction in insulin sensitivity would be expected to result in a compensatory increase in insulin secretion and maintenance of a constant disposition index and, by extension, maintenance of normal glucose tolerance (17, 18). In our cohort, the insulin sensitivity/secretion curve for PI-treated children was shifted downward and to the left of that for PI-naive children (Fig. 1). In the unadjusted analysis, the mean acute insulin response and disposition index were 11 and 50% lower in PI-treated, compared with PI-naive, children, despite an insulin sensitivity that was 34% lower in the former group (Table 3). After adjusting for potential confounding variables in the multiple regression analysis, the reduction in insulin sensitivity and disposition index associated with PI therapy was statistically significant (Table 4). These observations suggest that, in HIV-infected children, the β-cell response to a reduction in insulin sensitivity is impaired by PI therapy. The significance of this observation is further supported by the finding of mildly impaired glucose tolerance in four of 21 PI-treated children. Taken together, these results suggest that PI-treated HIV-infected children are at risk for the development of type 2 diabetes mellitus.

Clinical studies in HIV-infected adults and *in vitro* mechanistic studies support our finding of PI-induced derangement of glucose homeostasis that involves both a reduction in insulin sensitivity and failure of the pancreatic β-cell response. Reduced insulin sensitivity has been demonstrated among PI-treated HIV-infected adults in cross-sectional, prospective longitudinal and randomized, double blind, crossover studies using both the hyperinsulinemic euglycemic clamp (29, 30) and FSIVGTT (31). This reduction in insulin sensitivity appears, at least in part, to involve a defect of glucose transport. *In vitro*, the PIs have been shown to cause selective inhibition of GLUT-4 (32) and *in vivo*, a reduction in total body glucose disposal, total oxidative glucose disposal, and total nonoxidative glucose disposal has been linked to impaired glucose transport and phosphorylation by skeletal muscle (33, 34). In our study, the glucose effectiveness of PI-treated and PI-naive HIV-infected children was similar,

TABLE 5. Correlation of insulin sensitivity and fasting serum insulin with serum lipids and abdominal adipose tissue content

Parameter	Insulin sensitivity index [r (*P* value)][a]		Fasting serum insulin [r (*P* value)][a]	
	PI-treated (n = 33)	PI-naive (n = 15)	PI-treated (n = 33)	PI-naive (n = 15)
Total cholesterol	0.19 (0.29)	−0.01 (0.97)	−0.11 (0.53)	0.27 (0.32)
LDL cholesterol	0.19 (0.30)	0.04 (0.88)	−0.21 (0.26)	0.26 (0.33)
HDL cholesterol	0.31 (0.08)	−0.004 (0.99)	−0.30 (0.09)	−0.18 (0.51)
Triglycerides	−0.15 (0.41)	0.25 (0.38)	0.18 (0.32)	0.57 (0.03)
BMI	−0.17 (0.34)	−0.10 (0.72)	0.46 (0.008)	0.60 (0.02)
Total adipose tissue area	−0.14 (0.45)	−0.28 (0.32)	0.43 (0.01)	0.47 (0.08)
Visceral adipose tissue area	−0.43 (0.01)	−0.21 (0.45)	0.38 (0.03)	0.35 (0.20)
Subcutaneous adipose tissue area	−0.12 (0.50)	−0.28 (0.32)	0.44 (0.01)	0.47 (0.08)
V/SC adipose tissue ratio[b]	−0.49 (0.004)	0.18 (0.50)	0.04 (0.82)	−0.52 (0.05)

[a] r refers to Spearman correlation coefficient.
[b] V/SC, Visceral/subcutaneous.

indicating that noninsulin-dependent glucose disposal is not impaired by the PIs. With respect to pancreatic β-cell function, the lack of an appropriate increase in insulin secretion in response to a decline in insulin sensitivity, as measured by the acute insulin response during an iv glucose tolerance test, has been observed in one prospective trial of indinavir-treated adults (31). Recently the PIs were shown to cause acute impairment of glucose-stimulated insulin secretion in rodent islet cell culture, providing a potential explanation for PI-induced inhibition of β-cell function in PI-treated HIV-infected individuals (35).

Our results suggest that routine monitoring of glucose homeostasis may be necessary for all PI-treated HIV-infected children. Unfortunately, the findings of this study as well as other pediatric studies indicate that fasting serum insulin, insulin to glucose ratio, HOMA-IR, or QUICKI is not adequately sensitive to reliably accomplish this goal for the individual pediatric patient (3–5). On the other hand, routine performance of the FSIVGTT is not practical because of the complexity and invasive nature of this methodology. Our results suggest that the OGTT may be a reasonable alternative for the routine assessment of insulin sensitivity in these children. The Matsuda index, derived from the OGTT (24), correlated well with the insulin sensitivity index ($r = 0.60$, $P = 0.004$). In contrast, the correlation of HOMA-IR and QUICKI with the FSIVGTT-derived insulin sensitivity index was no better than that of fasting serum insulin, a finding that is consistent with previously published data (20).

The similarity of the metabolic perturbations associated with antiretroviral therapy in HIV-infected adults and those of the metabolic syndrome (36, 37) support the possibility that HIV-infected individuals manifesting these abnormalities may be at increased risk of developing not only diabetes mellitus but also premature atherosclerotic cardiovascular disease. Premature coronary artery disease has been reported among dyslipidemic PI-treated HIV-infected adults (38), but a clear causal link with PI therapy awaits the outcome of ongoing long-term prospective studies. In the present study, PI therapy was associated with elevated levels of total cholesterol, LDL cholesterol, and triglycerides, a finding that has been consistently observed in previously published pediatric studies (3–6, 10). With respect to adipose tissue distribution, visceral adipose tissue area and visceral to sc adipose tissue ratio were higher in PI-treated, compared with PI-naive, subjects in our cohort, albeit not significantly so. In addition, visceral adipose tissue area and visceral to sc adipose tissue ratio were inversely associated with insulin sensitivity in PI-treated but not PI-naive subjects. In previously published pediatric studies, intraabdominal adipose tissue accumulation and/or peripheral lipoatrophy (measured by dual-energy x-ray absorptiometry and/or magnetic resonance imaging) were observed in 29–84% of HAART-treated HIV-infected children (8, 9, 39). Taken together, these data suggest that in HIV-infected children, PI-containing HAART is associated with an atherogenic dyslipidemia and possibly intraabdominal adipose tissue accumulation.

The main limitation of the present study was its cross-sectional design. Protease inhibitor-treated subjects had received antiretroviral therapy for a longer period of time, were more likely to have been exposed to stavudine, and tended to have more advanced HIV disease (as defined by clinical and immunologic category), compared with PI-naive children. In addition, it is possible that the two groups differed in other important yet unforeseen ways. The potential bias related to inequality of study groups from known potential confounding variables was minimized through the use of multiple regression analysis. Importantly, the two groups did not differ with respect to several variables known to affect glucose homeostasis including age, Tanner stage, BMI, sex, and ethnic background. Recruitment bias was minimized by approaching all eligible children and the high uptake of the study.

The small sample size may have limited our ability to demonstrate some important differences between PI-treated and PI-naive subjects and detect all but the strongest of associations between variables included in the analysis. Thus, the failure to demonstrate a significant difference in visceral adipose tissue content between PI-treated and PI-naive subjects may have been due to a lack of power. Similarly, whereas we did not encounter major model building problems due to collinearity, a moderate degree of collinearity between some of the variables cannot be excluded. Larger studies would be needed to tease out the complex web of associations that may exist between the various potential explanatory and outcome variables.

The impact of PI therapy on the risk of impaired glucose tolerance in HIV-infected children cannot be ascertained with certainty from the present study because none of the PI-naive subjects and only 21 of 33 PI-treated subjects underwent OGTT. The OGTT was offered to PI-treated subjects, subsequent to their enrollment and completion of the FSIVGTT, because of the association of PI therapy with impaired glucose tolerance and diabetes mellitus in adults (40, 41). We believe that our OGTT results are generalizable to our PI-treated patient population as a whole because of the high uptake rate of the study, the fact that all 33 PI-treated children were approached for performance of the OGTT, and because those who did and did not undergo the OGTT did not differ with respect to potential confounding variables such as age, Tanner stage, BMI, sex, ethnic background, and a variety of HIV-related factors.

In conclusion, our results demonstrate that not only does PI therapy reduce insulin sensitivity in HIV-infected children but also that it also impairs the β-cell response to this reduction in insulin sensitivity and, in a subset of children, leads to the development of impaired glucose tolerance. Furthermore, we found a significant association of PI therapy with an atherogenic dyslipidemia and a correlation, in PI-treated children, of reduced insulin sensitivity with increased visceral adipose tissue content. Taken together, these observations suggest that PI-containing HAART is associated with the emergence of early features of a metabolic syndrome-like phenotype. Whereas the long-term impact of these metabolic abnormalities remains to be determined, it is clear that close monitoring of glucose homeostasis and serum lipids is warranted for all PI-treated HIV-infected children. The most appropriate test to use in following glucose homeostasis in the clinical setting requires further study. We believe that the OGTT deserves further examination in this regard because of its ability to detect early changes in glucose tolerance and provide a measure of insulin sensitivity.

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