Metabolic syndrome and serum fatty acid patterns in serum phospholipids in hypertriglyceridemic persons with human immunodeficiency virus[1–3]

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ABSTRACT

Background: HIV infection and its treatment are associated with abnormal lipid profiles. High triglyceride concentrations and low HDL-cholesterol concentrations are the most common health abnormalities and raise concerns about an increased risk of cardiovascular disease.

Objective: We compared the fatty acid patterns of serum phospholipids between persons with HIV and non-HIV controls to determine whether there are differences that explain the elevated triglyceride concentrations, insulin resistance, and inflammation that are part of the metabolic syndrome in patients with HIV.

Design: Thirty-nine persons with HIV and elevated serum triglycerides (>150 mg/dL) and/or indicators of insulin resistance were recruited to examine fatty acid profiles in serum phospholipid fractions relative to those of 2 control groups without HIV ($n = 31$).

Results: Higher concentrations of 16:1 and 18:0 fatty acids in the phospholipid fraction indicated increased lipogenesis in the HIV patients and in the non-HIV controls at risk of the metabolic syndrome. However, the subjects with HIV had higher concentrations of both n–6 (omega-6) and n–3 fatty acids of higher elongation and desaturation levels, which indicated a greater promotion of these pathways in this population. The nanomolar percentage (%nmol) arachidonic acid was the same in all 3 groups.

Conclusions: Persons with and without HIV, at risk of the metabolic syndrome, show indications of increased lipogenesis, more so in subjects with HIV taking medication. Higher proportions of distal elongation and desaturation fatty acid products were seen only in the phospholipids fatty acid fraction of the subjects with HIV. *Am J Clin Nutr* 2009;89:1180–7.

INTRODUCTION

Alterations in lipid metabolism were reported early in the HIV epidemic (1–3). Three of these studies (4–6) investigated specific changes in blood fatty acid patterns in HIV. Begin et al (4) reported decreases in polyunsaturated fatty acids (PUFAs), especially of the n–3 series in both plasma total lipids and phospholipid fatty acids. Constans et al (5) measured total plasma lipid and red blood cell fatty acids and also reported low PUFA concentrations and diunsaturated fatty acid classes in these 2 blood fractions, but did not fractionate the individual fatty acids. A third study (6), which measured total serum fatty acid con-

centrations, reported the opposite results, ie, higher PUFA concentrations in persons with HIV than in controls. Reports of elevated concentrations of serum triglycerides and total cholesterol and low concentrations of HDL cholesterol in HIV patients began to appear in the literature with the advent of highly active antiretroviral therapy (HAART) (7–10). It remained unclear whether these changes were due to the long-term effects of the disease and/or to HAART.

In 2004, the National Heart, Blood, and Lung Institute/American Heart Association defined the components of the metabolic syndrome as follows: *1*) fasting glucose ≥ 100 mg/dL, *2*) triglycerides >150 mg/dL, *3*) HDL cholesterol <40 mg/dL in men and <50 mg/dL in women, *4*) blood pressure >130/>85 mm Hg, and *5*) abdominal obesity, defined as a waist circumference >102 cm in men and > 88 cm in women (11). A diagnosis of the metabolic syndrome can be applied to subjects with ≥3 of these indicators. The World Health Organization criteria for the metabolic syndrome includes the presence of insulin resistance as a primary risk factor, using any 1 of 4 criteria (11). The QUICKI (Quantitative Insulin Sensitivity Check Index) is not one of these criteria but can readily be determined from fasting serum insulin and glucose concentrations and is considered a good indicator of insulin resistance (12). An important factor in the development of insulin resistance in humans is an increased intake in dietary saturated fatty acids (SFAs) and a resultant increase in serum SFA concentrations (13, 14). The

fatty acid pattern of high SFA concentrations in plasma lipids is consistently associated with the presence and development of insulin resistance and the metabolic syndrome (15–18). Nutrition intervention studies have shown that increasing PUFA intakes result in improved insulin sensitivity (19–21). The typical plasma fatty acid pattern in individuals with the metabolic syndrome is characterized by elevated concentrations of SFAs, especially palmitic acid, and reduced concentrations of the essential fatty acid linoleic acid (18:2n–6). These differences are likely due to differences in dietary intake (13).

Fatty acid profiles of serum lipids in HIV patients receiving HAART, with documented hypertriglyceridemia or decreased insulin sensitivity as evidence of the metabolic syndrome, have not been reported to our knowledge. Our study was conducted to determine individual fatty acid concentrations in serum phospholipids in persons with HIV and hypertriglyceridemia or decreased insulin sensitivity and to compare concentrations of individual fatty acids and representative fatty acid classes with concentrations in non-HIV healthy controls and non-HIV subjects with elevated serum triglycerides and/or decreased insulin sensitivity.

SUBJECTS AND METHODS

Subjects

Thirty-nine subjects with HIV were recruited between July 2003 and March 2006. The eligibility criteria included HIV infection, age >18 y, a body mass index (BMI; in kg/m^2) between 19 and 30, and a fasting plasma triglyceride concentration >150 mg/dL at any time over the past year or a QUICKI score of <0.35 (12). Subjects also had to be free of any opportunistic infection, have no history of active injection drug use, and not have taken any n–3 fatty acid supplements for ≥3 mo before starting the study. A medical history was taken, which included a list of current antiretroviral medications and other medications and vitamin supplements used. A 3-d food record was collected at baseline with prior detailed instruction and documentation afterward.

Thirty participants in the HIV group were men, and 9 were women. Eighteen (46%) of the 39 subjects were receiving ritonavir-based HAART, 16 (41%) were receiving other HAART regimens that did not include a protease inhibitor, and 5 (13%) had not taken any ART medications for ≥1 mo. Eighteen of our subjects had elevated serum triglycerides, 8 were insulin resistant as defined by a QUICKI score <0.35, and 13 had both characteristics. The eligibility criteria for the study included both elevated serum triglycerides and an abnormal QUICKI score (<0.35) because of their association with insulin resistance using the classic oral glucose tolerance test (11, 12). QUICKI is a mathematical model used to estimate insulin sensitivity as follows: 1/(log of fasting plasma insulin, μU/mL + log of fasting plasma glucose, mg/dL) (12). On the basis of the definition for the metabolic syndrome (11), 13 (33%) of our HIV subjects had the metabolic syndrome and 26 (67%) did not.

The 13 participants in the non-HIV healthy control group had normal serum triglycerides (all <150 mg/dL) and a normal QUICKI score. A second group of 18 non-HIV unhealthy subjects with elevated serum triglycerides (n = 6), an abnormal QUICKI score (n = 7), or both conditions (n = 5) were also recruited.

None of the non-HIV controls had a history of asthma, diabetes, coronary heart disease, stroke, gastrointestinal disease, gallstones, or recent surgery, and all were free of inflammatory disease or infection. None of the subjects had dietary restrictions or were taking medications, and all were weight stable. Written informed consent was obtained from all study participants, and the study protocol was approved by the Tufts–New England Medical Center Institutional Review Board.

Dietary analysis

Dietary intake was obtained from all subjects by using either a 3-d food record in the subjects with HIV or a 24-h dietary recall procedure in the non-HIV controls. The 3-d food records and 24-h dietary recalls were analyzed by using the Nutrition Data System software (version 13a of Food Database 9 and version 28 of the Nutrient Database; Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). The analysis included the total intake of food and supplements.

Blood lipids, glucose, and insulin

All blood was drawn between 0700 and 1000 after a fast of ≥8 h. Insulin was assessed by using a radioimmunoassay (RIA) technique (ADVIA Centaur Immunoassay; Bayer Diagnostics, Tarrytown, NY). QUICKI was determined by using measurements from fasting blood collected at baseline (12). Glucose, total cholesterol, HDL cholesterol, and triglycerides were measured by using the Beckman LX-20 (Beckman Coulter Inc, Fullerton, CA) in the Tufts–New England Medical Center Clinical Laboratory. LDL was calculated by using the Friedewald equation (22). Highly sensitive C-reactive protein was measured by using a turbidimetric in vitro immunoassay (Equal Diagnostics, Exton, PA).

Fatty acid measurement

Blood samples were collected from the study subjects after an 8-h fast into tubes containing EDTA, and the serum was separated within 2 h of collection. Butylated hydroxytoluene (BHT; 40 μL/mL) was added to all samples as an antioxidant. An aliquot was covered by nitrogen gas to protect against the oxidation of fatty acids and was then stored at –80°C until analyzed within 12 mo.

Briefly, an aliquot of 50 μL of a 1 mg/mL solution of diheptadecanoyl phosphatidylcholine (Avanti Polar Lipids Inc, Alabaster, AL) and triheptadecanoyl glycerol (Sigma, St Louis, MO) was added as an internal standard to 500 μL serum before lipid extraction. A liquid-liquid extraction was carried out by using serum with 6 volumes of chloroform:methanol (2:1, vol: vol) centrifuged at 800 × g for 10 min, and the resulting lower phase was aspirated. Lipid extracts from serum samples were fractionated into triglycerides and phospholipids by aminopropyl column chromatography (23). The eluate was evaporated to dryness under nitrogen gas, methylated by adding 0.5 mL methanolic base (Acros Organics, Morris Plains, NJ), and placed in a heating block at 100°C for 3 min. After the sample was cooled on ice, 0.5 mL BF3 reagent (Sigma) was added, and the sample was placed in a heating block at 100°C for 1 min, removed, and allowed to cool on ice. N-Hexane was added (0.5 mL) and incubated at 100°C for 1 min (24). After cooling, 6.5

mL NaCl saturated salt solution was added, and the reaction mixture was centrifuged at 800 × g for 2 min. The upper hexane concentration was aspirated. Fatty acid methyl esters were analyzed by gas chromatography with a Hewlett-Packard 5890A gas chromatograph using Supelcowax-10 (0.25 mm internal diameter column; Supelco, Toronto, Canada). Fatty acid methyl ester peaks were identified by comparing the retention times of standard mixtures against internal standards. Results are expressed as molar percentages of total fatty acid in the sample (%nmol).

Statistical analysis

Means (±SD) were calculated for all continuous variables by study group. To compare mean dietary intakes and fatty acid concentrations between the 3 study groups, generalized estimating equations models with robust SEs were fit by using the normal distribution and identity link (PROC GENMOD in SAS; SAS Institute, Cary, NC). Separate models were fit for each dietary intake and percentage fatty acid outcome. The study group variable was the exposure of interest for each of these models. Overall differences in means were compared between groups by using the type 3 score test. Categorical variables were compared between the 3 study groups by using an overall chi-square or Fisher's exact test when expected cell counts were <5. When overall P values were statistically significant (P < 0.05), pairwise P values were calculated by using a chi-square test for the differences in least-squared means (LSMEANS option in PROC GENMOD). For all pairwise comparisons, Bonferroni-corrected P values were reported to adjust for multiple comparisons between the 3 study groups. All analyses were conducted in SAS (version 9.1).

RESULTS

The mean age of the HIV-positive subjects was 46.4 ± 7.5 y, of the non-HIV healthy controls was 46.4 ± 7.5 y, and of the non-HIV unhealthy controls was 48.2 ± 6.7 y (with elevated serum triglycerides and/or abnormal insulin sensitivity) (**Table 1**). The mean BMI of the HIV subjects was 25.7 ± 2.9, of the non-HIV healthy controls was 23.9 ± 2.7, and of the non-HIV unhealthy controls with elevated serum triglycerides and/or abnormal insulin sensitivity was 28.0 ± 3.9. The non-HIV healthy controls had a mean BMI in the normal range, which was statistically significantly lower than that of the non-HIV unhealthy controls (P = 0.0009), whose mean BMI was in the overweight range (25.0–29.9). The mean BMI for the HIV subjects was in between the 2 control groups and was not significantly different from either group. The mean waist-to-hip ratio of the women in each group was above the cutoff for conferring low risk (<0.75), and there was no significant difference between groups. The mean waist-to-hip ratio in the men was also above the cutoff that confers low risk (<0.85), but that in the non-HIV healthy controls was significantly lower than that in either of the other 2 groups (P ≤ 0.001). There was a significant difference in race between the subjects with HIV and the non-HIV unhealthy controls (P = 0.009), ie, fewer African Americans in the HIV group.

By design, serum triglycerides were significantly lower in the non-HIV healthy controls than in either the HIV-positive subjects or the other non-HIV group (P < 0.001 for both). Fasting glu-

cose concentrations were most elevated in the HIV subjects but were only significantly different from those of the non-HIV healthy controls (P < 0.001). Again by design, fasting insulin concentrations and QUICKI scores were in the normal range and were significantly lower in the non-HIV healthy controls than in the other 2 groups (P ≤ 0.001). HDL-cholesterol values, a risk factor for the metabolic syndrome, were significantly lower in the HIV subjects (31.3 ± 14.6 mg/dL) than in the non-HIV healthy subjects (49.5 ± 17.9 mg/dL; P =0.002), but it was not significantly different from the non-HIV unhealthy controls (38.3 ± 18.0 mg/dL).

Eighteen (46%) of the HIV subjects and 6 (33%) of the non-HIV unhealthy controls with elevated serum triglycerides and/or abnormal insulin sensitivity met the criteria for the metabolic syndrome. A significantly higher proportion (39%) of those in the non-HIV group met the metabolic syndrome criteria for high waist circumference than did the HIV-positive group (18%) and the non-HIV healthy controls (0%); the overall P value was 0.028 (Fisher's exact test). The criteria for high blood pressure and low HDL were equally balanced between the HIV subjects and the non-HIV unhealthy controls.

Dietary intake data for the study subjects obtained via 3-d food records and 24-h dietary recalls are shown in **Table 2**. Of the more standard nutrients, only dietary cholesterol intake was significantly different between study groups; the HIV subjects had significantly higher cholesterol intakes than did the non-HIV healthy controls (453 ± 281 mg/d compared with 233 ± 207 mg/d; P = 0.006). The highest cholesterol intake was found in the non-HIV unhealthy controls (510 ± 782 mg/d), but the high SD observed in this group precluded a statistically significant difference between the 2 non-HIV control groups.

When the individual dietary fatty acids were investigated, significant differences in dietary n–3 fatty acid intakes were found between the diets of these 3 groups. The HIV subjects had consistently higher concentrations of α-linolenic acid (18:3n–3), 22:6n–3 (docosahexaenoic acid; DHA), eicosapentaenoic acid (EPA; 20:5n–3) + DHA, and total n–3 fatty acids than did the non-HIV control groups. The difference in total intakes of n–3 fatty acids was ≈2-fold higher in the HIV subjects than in the non-HIV healthy controls (P ≤ 0.001) or in the non-HIV unhealthy controls (P = 0.006). Vitamin E intake from food alone was also significantly higher in the HIV subjects and was most different between the HIV subjects and the non-HIV unhealthy controls (P ≤ 0.001). Total vitamin E concentrations (food plus supplements) were highest, but not significantly so, in the HIV subjects, which indicated a higher use of vitamin E supplements in that group.

The percentage distribution of serum phospholipid fatty acids in the 3 groups is shown in **Table 3** and indicates significant differences between the HIV subjects and both of the non-HIV control groups. A significantly higher percentage of 16:1, a desaturation product of 16:0, was seen in both the HIV subjects and in the non-HIV unhealthy controls with elevated serum triglycerides and/or abnormal insulin sensitivity than in the non-HIV healthy controls. The percentage of stearic acid (18:0), which is found in the diet but is also an elongation product of 16:0, was significantly different between all groups, with the highest concentrations observed in the HIV subjects followed by the non-HIV unhealthy controls and the non-HIV healthy controls. Significantly lower percentages of 16:0, a precursor of 18:0,

TABLE 1
Demographic characteristics of the HIV patients and of the non-HIV control groups[1]

Characteristic	HIV+ subjects (n = 39)	Non-HIV healthy controls (n = 13)	Non-HIV unhealthy controls[2] (n = 18)	Overall P value[3]	Bonferroni-adjusted P values		
					1 vs 3	1 vs 2	2 vs 3
Age (y)	46.4 ± 7.5[4]	46.4 ± 7.5	48.2 ± 6.7	0.63	—	—	—
Men [n (%)]	30 (77)	8 (62)	14 (78)	0.51	—	—	—
Women [n (%)]	9 (23)	5 (38)	4 (22)	0.50[5]	—	—	—
Race [n (%)][6]							
African American	13 (34)	1 (8)	0 (0)	0.008[5]	0.009[5]	0.21[5]	0.99[5]
White	22 (58)	9 (69)	13 (72)	—	—	—	—
Other or multiple races	3 (8)	3 (23)	5 (28)	—	—	—	—
BMI (kg/m^2)	25.7 ± 2.9	23.9 ± 2.7	28.0 ± 3.9	0.010	0.06	0.09	0.0009
Weight (kg)	77.8 ± 11.3	72.0 ± 8.2	82.3 ± 15.0	0.056	—	—	—
Waist-to-hip ratio							
Women	0.86 ± 0.10	0.77 ± 0.05	0.86 ± 0.14	0.14	—	—	—
Men	0.98 ± 0.08	0.88 ± 0.06	0.99 ± 0.05	0.019	0.99	<0.001	<0.001
TGs (mg/dL)	256.1 ± 237.4	69.6 ± 26.1	166.1 ± 96.0	0.002	0.12	<0.001	<0.001
Fasting glucose (mg/dL)	98.5 ± 17.6	83.9 ± 9.2	89.2 ± 12.5	0.004	0.06	<0.001	0.48
Fasting insulin (μIU/dL)	9.9 ± 6.2	4.9 ± 1.7	12.2 ± 6.0	<0.001	0.51	<0.001	<0.001
QUICKI score	0.35 ± 0.04	0.39 ± 0.02	0.34 ± 0.03	0.001	0.60	<0.001	<0.001
Total cholesterol (mg/dL)	186 ± 41	186 ± 32	208 ± 58	0.34	—	—	—
HDL cholesterol (mg/dL)	31.3 ± 14.6	49.5 ± 17.9	38.3 ± 18.0	0.013	0.42	0.002	0.24
LDL cholesterol (mg/dL)	105.9 ± 46.4	122.2 ± 30.9	136.1 ± 52.3	0.09	—	—	—
C-reactive protein (mg/L)[7]	1.8 ± 1.9	2.3 ± 5.3	3.4 ± 3.1	0.12	—	—	—
CD4 (cells/mm^3)[8]	519 ± 255	—	—	—	—	—	—
HAART use [n (%)]	34 (87)	—	—	—	—	—	—
Metabolic syndrome [n (%)]	18 (46)	0 (0)	6 (33)	0.01	0.99	0.006[5]	0.09[5]
High glucose, >100 mg/dL [n (%)]	15 (38)	0 (0)	2 (11)	0.005[5]	0.12	0.03[5]	0.99[5]
High waist circumference, >102 cm for men and >88 cm for women [n (%)]	7 (18)	0 (0)	7 (39)	0.028[5]	0.33[5]	0.51[5]	0.07[5]
High blood pressure, >130/85 mm Hg [n (%)]	13 (33)	2 (15)	5 (28)	0.46[5]	—	—	—
High serum TGs, >150 mg/dL [n (%)]	26 (67)	0 (0)	11 (61)	<0.001	0.99	<0.001	<0.001[5]
Low HDL, <40 mg/dL for men and <50 mg/dL for women [n (%)]	32 (82)	5 (38)	15 (83)	0.008[5]	0.99[5]	0.02[5]	0.06[5]

[1] QUICKI, Quantitative Insulin Sensitivity Check Index; HAART, highly active antiretroviral therapy; TGs, triglycerides.

[2] Non-HIV unhealthy controls had elevated serum triglycerides (>150 mg/dL) and/or insulin resistance.

[3] Overall P values are from type 3 score statistics for continuous variables and chi-square test for categorical variables unless otherwise noted.

[4] Mean ± SD (all such values).

[5] Fisher's exact test.

[6] One participant was missing race data.

[7] One participant in the non-HIV unhealthy group was missing data.

[8] One participant was missing a CD4 count.

were observed in the HIV subjects than in either of the control groups. The percentage of linoleic acid (18:2n–6), an essential fatty acid that comes from the diet, cannot be synthesized in humans, and is a potential substrate for further elongation and desaturation, was significantly lower in the HIV subjects than in either of the non-HIV control groups ($P = 0.001$).

The essential fatty acid 18:3n–3 was significantly higher in the HIV subjects than in either of the control groups. As shown in Table 2, the dietary intake of this essential fatty acid was also higher in the HIV subjects.

The fatty acid arachidonic acid (20:4n–6), which is important in determining the concentrations of proinflammatory eicosanoids, was not significantly different between any of the 3 study groups. Each of the n–3 fatty acid metabolites—EPA, 22:5n–3 (DPA), and DHA—and the total EPA + DHA and total n–3 fatty acid intakes were significantly higher (%nmol) in the HIV subjects than in either of the non-HIV control groups. There was no difference in percentage levels between the 2 non-HIV control groups. These distal metabolites are present in food but are also elongation and desaturation products in the n–3 pathway. The n–6 fatty acid metabolites dihomo-γ-linolenic acid (20:3n–6; DGLA), 22:4n–6, and 22:5n–6 were also present in significantly higher percentages in the HIV subjects than in either of the control groups. These are all distal elongation and desaturation products of the n–6 pathway.

The percentage of total PUFAs was highest in the HIV subjects, but this appeared to be due to higher concentrations of n–3 fatty acids, because there were no differences in total n–6 fatty acids across the 3 groups. These higher concentrations of n–3 metabolites resulted in a significantly lower n–6/n–3 ratio (by ≈10 units) in the HIV subjects than in either of the non-HIV control groups. Ratios of 21 and 26 were observed in the non-HIV

TABLE 2
Dietary intakes of the HIV patients and of the non-HIV control groups[1]

Characteristic	HIV+ subjects[2] (n = 38)	Non-HIV healthy controls (n = 13)	Non-HIV unhealthy controls[3] (n = 18)	Overall P value[4]	Bonferroni-adjusted P values		
					1 vs 3	1 vs 2	2 vs 3
3-d Food records [n (%)]	21 (55)	0 (0)	0 (0)	—	—	—	—
24-h Dietary recalls [n (%)]	17 (45)	13 (100)	18 (100)	<0.0001	—	—	—
Energy (kcal)	2672 ± 905[5]	2231 ± 842	2429 ± 940	0.26	—	—	—
Energy (kcal/kg)	34.0 ± 10.5	30.7 ± 9.7	30.1 ± 12.5	0.38	—	—	—
Protein (g)	121 ± 51	95 ± 46	108 ± 58	0.23	—	—	—
Protein (%)	18.4 ± 5.5	16.7 ± 3.1	18.2 ± 7.6	0.36	—	—	—
Total fat (g)	106 ± 51	79.6 ± 36.8	91.3 ± 48.2	0.14	—	—	—
Total fat (%)	34.9 ± 8.1	32.7 ± 8.2	32.1 ± 9.2	0.45	—	—	—
Carbohydrate (g)	314 ± 117	287 ± 113	300 ± 115	0.75	—	—	—
Carbohydrate (%)	47.5 ± 11.0	51.5 ± 8.7	51.0 ± 10.8	0.32	—	—	—
Dietary fiber (g)	22.8 ± 10.9	20.8 ± 12.0	19.0 ± 7.4	0.31	—	—	—
Cholesterol (mg)	453 ± 281	233 ± 207	510 ± 782	0.04	0.99	0.006	0.42
Saturated fat (g)	36.4 ± 21.0	27.3 ± 11.9	32.7 ± 19.3	0.18	—	—	—
Saturated fat (%)	11.8 ± 3.9	11.2 ± 2.8	11.4 ± 3.4	0.85	—	—	—
Polyunsaturated fat (g)	22.3 ± 11.7	16.9 ± 11.8	17.4 ± 10.0	0.17	—	—	—
Polyunsaturated fat (%)	7.4 ± 2.7	6.8 ± 2.8	6.2 ± 2.7	0.27	—	—	—
trans Fats (g)	5.2 ± 4.4	3.8 ± 3.0	5.3 ± 4.3	0.40	—	—	—
Dietary 18:3n−3 (g)	2.3 ± 1.7	1.2 ± 0.6	1.4 ± 0.9	0.009	0.024	0.002	0.99
Dietary 20:5n−3 (g)	0.1 ± 0.3	0.02 ± 0.02	0.02 ± 0.03	0.079	—	—	—
Dietary 22:6n−3 (g)	0.2 ± 0.3	0.05 ± 0.07	0.08 ± 0.01	0.025	0.039	0.006	0.99
Dietary 20:5n−3 + 22:6n−3 (g)	0.4 ± 0.6	0.07 ± 0.09	0.1 ± 0.1	0.025	0.03	0.009	0.99
Dietary 18:2n−6 (g)	19.1 ± 9.9	15.3 ± 11.1	15.5 ± 9.4	0.28	—	—	—
Dietary n−6 fatty acids (g)	19.4 ± 10.0	15.5 ± 11.2	15.5 ± 9.3	0.27	—	—	—
Dietary n−3 fatty acids (g)	2.7 ± 2.0	1.3 ± 0.7	1.5 ± 0.9	0.003	0.006	<0.001	0.99
n−6/n−3 Ratio	8.5 ± 3.3	13.9 ± 12.8	11.0 ± 5.2	0.067	—	—	—
Vitamin E (IU)	101 ± 200	25.8 ± 23.1	29.8 ± 57.1	0.095	—	—	—
Vitamin E, not including vitamins and supplements (IU)	20.5 ± 12.8	18.9 ± 19.0	10.4 ± 5.6	0.003	<0.001	0.99	0.30

[1] Nonhealthy controls had elevated serum triglycerides (>150 mg/dL) and/or insulin resistance.

[2] One participant in the HIV group with an implausible dietary intake (>10,000 kcal) was not included but was included in Tables 1 and 3.

[3] Non-HIV unhealthy controls had elevated serum triglycerides (>150 mg/dL) and/or insulin resistance.

[4] Overall P values are from type 3 score statistics for continuous variables and chi-square test for categorical variables.

[5] Mean ± SD (all such values).

healthy controls and in the non-HIV unhealthy controls, respectively, but the difference was not statistically significant. The mead acid/20:4n−6 ratio indicated that none of the groups had indications of essential fatty acid deficiency.

The dietary intakes of specific fatty acids shown in Table 2 indicate that the HIV subjects had significantly higher intakes of total n–3 fatty acids, specifically of 18:3n–3, DHA, and a lower n–6/n–3 ratio. This difference in intake between the study groups could have an effect on the fatty acid composition of serum phospholipids (25, 26). Therefore, we carried out a multivariate analysis to control for the dietary intake of total and specific n–3 fatty acids to see whether this would affect the phospholipids fatty acid profiles seen in Table 3. No changes in the group estimates were observed, and only very small changes were noted in the strength of the statistical significance. However, 45% of the subjects with HIV had 24-h dietary recalls only at baseline, instead of a 3-d food record, and recalls are known to be good for determining group intakes but are not considered accurate for determining individual intakes. We acknowledge that this limits the accuracy of this correction to correct for differences in dietary intakes of specific fatty acids between study groups and is a limitation of the study.

Values (%nmol) of the phospholipid fatty acids 20:3n–6, 22:4n–6, 22:5n–6, EPA, and DHA were examined for trends across the 3 medication categories in the subjects with HIV. There was no significant difference in fatty acid concentrations across the 3 medication groups (P = 0.11, Wilcoxon's rank-sum test). The fatty acid 20:3n–6 was higher in the ritonavir-based HAART group, but there was no statistically significant trend across groups (P = 0.13, Wilcoxon's rank-sum test; data not shown). A lack of an effect of medication on these variables may have been due to the small numbers. Although we collected data from subjects with HIV currently receiving medication, it was not the intention of the study to look at the effect of medications on the outcome variables. We did select subjects who were evaluated as "stable" while taking their current medication and who did not anticipate a change in medicine use over the next 3–6 mo to avoid this possible confounder during the nutrition intervention planned in the subjects with HIV.

Absolute concentrations of phospholipid fatty acids and triglyceride fatty acids (as nmol/mL) in all 3 groups are presented in the final 2 rows of Table 3. The phospholipid fraction is known to help solubilize the triglyceride lipid fraction. The non-HIV healthy control group had nearly equal amounts of phospholipid

TABLE 3
Phospholipid fatty acids at baseline in the HIV patients and in the non-HIV control groups[1]

Characteristic	HIV+ subjects (n = 39)	Non-HIV healthycontrols (n = 13)	Non-HIV unhealthy controls[2] (n = 18)	Overall P value[3]	Bonferroni-adjusted P values		
					1 vs 3	1 vs 2	2 vs 3
14:0 (%nmol)	1.0 ± 3.1[4]	0.6 ± 0.1	0.6 ± 0.2	0.71	—	—	—
16:0 (%nmol)	29.0 ± 2.7	36.5 ± 3.6	34.4 ± 2.7	<0.001	<0.001	<0.001	0.21
16:1n−7 (%nmol)	0.7 ± 0.3	0.4 ± 0.2	0.7 ± 0.4	0.011	0.99	<0.001	0.009
18:0 (%nmol)	14.7 ± 2.1	9.7 ± 2.1	12.0 ± 1.8	<0.001	<0.001	<0.001	0.003
18:1n−9 (%nmol)	12.7 ± 2.5	13.5 ± 1.7	13.2 ± 2.4	0.41	—	—	—
18:2n−6 (%nmol)	21.3 ± 3.1	24.1 ± 4.7	25.0 ± 4.1	0.003	0.001	0.12	0.99
18:3n−3 (%nmol)	0.26 ± 0.15	0.02 ± 0.04	0.04 ± 0.09	<0.001	<0.001	<0.001	0.99
20:3n−9 (%nmol)	0.26 ± 0.16	0.00 ± 0.00	0.03 ± 0.09	<0.001	<0.001	<0.001	0.60
20:3n−6 (%nmol)	3.9 ± 1.4	1.8 ± 1.3	1.1 ± 1.1	<0.001	<0.001	<0.001	0.30
20:4n−6 (%nmol)	11.5 ± 2.7	11.3 ± 2.1	10.8 ± 2.9	0.61	—	—	—
20:5n−3 (%nmol)	0.8 ± 0.7	0.2 ± 0.6	0.2 ± 0.3	<0.001	<0.001	0.012	0.99
22:4n−6 (%nmol)	0.37 ± 0.17	0.13 ± 0.12	0.12 ± 0.12	<0.001	<0.001	<0.001	0.99
22:5n−6 (%nmol)	0.32 ± 0.15	0.05 ± 0.09	0.04 ± 0.07	<0.001	<0.001	<0.001	0.99
22:5n−3 (%nmol)	0.7 ± 0.4	0.1 ± 0.1	0.2 ± 0.1	<0.001	<0.001	<0.001	0.40
22:6n−3 (%nmol)	2.5 ± 0.9	1.7 ± 0.9	1.6 ± 1.0	0.002	0.0021	0.015	0.99
Total 20:5n−3 + 22:6n−3 (%nmol)	3.3 ± 1.2	1.9 ± 1.0	1.8 ± 1.2	<0.001	<0.001	<0.001	0.99
Total SFAs (%nmol)	44.8 ± 3.1	46.8 ± 2.2	47.1 ± 2.6	0.010	0.012	0.03	0.99
Total MUFAs (%nmol)	13.4 ± 2.7	13.9 ± 1.7	13.9 ± 2.6	0.65	—	—	—
Total PUFAs (%nmol)	41.8 ± 2.6	39.3 ± 3.0	39.0 ± 2.7	<0.001	0.003	0.018	0.99
n−6 (%nmol)	37.4 ± 2.4	37.3 ± 3.0	37.0 ± 3.2	0.92	—	—	—
n−3 (%nmol)	4.2 ± 1.4	2.1 ± 1.1	2.0 ± 1.3	<0.001	<0.001	<0.001	0.99
n−6/n−3 Ratio	9.8 ± 3.1	20.7 ± 11.8	25.5 ± 21.0	<0.001	0.006	0.003	0.99
20:3n−9/20:4n−6 Ratio	0.03 ± 0.1	0.00 ± 0.00	0.00 ± 0.01	<0.001	<0.001	<0.001	0.54
Total PLs (nmol/mL)	5583 ± 1420	2729 ± 615	3072 ± 843	<0.001	<0.001	<0.001	0.54
Total TGs (nmol/mL)	8669 ± 5716	2726 ± 905	4186 ± 2066	<0.001	<0.001	<0.001	0.027

[1] SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; PLs, phospholipids; TGs, triglycerides.
[2] Non-HIV unhealthy controls had elevated serum triglycerides (>150 mg/dL) and/or insulin resistance.
[3] Overall P values are from type 3 score statistics for continuous variables and chi-square test for categorical variables.
[4] Mean ± SD (all such values).

and triglyceride fatty acids, which was not evident in the non-HIV unhealthy controls with elevated serum triglycerides, who had a 27% lower phospholipid fraction than triglyceride fraction. This was further exacerbated in the HIV subjects, who had a 36% lower phospholipid fraction relative to their triglyceride fraction compared with the non-HIV healthy controls.

DISCUSSION

The percentage of subjects with the metabolic syndrome was higher in the HIV group (46%) than in the non-HIV unhealthy control group with elevated serum triglycerides and/or abnormal insulin sensitivity (33%) or the non-HIV healthy control group (0%). Compared with non-HIV unhealthy controls with elevated triglycerides, serum triglycerides were significantly worse and HDL concentrations were lower in the HIV patients, which suggested that the lipid abnormalities were greater and may have been attributable, at least in part, to HIV disease or HAART medication use.

Serum phospholipid fatty acid profiles reflect differences in both dietary intakes and in metabolism. De novo lipogenesis is characterized by increases in SFAs, such as 16:0 and 18:0, and their desaturase products 16:1 and 18:1. Higher concentrations of these fatty acids were seen in the HIV subjects and in the non-HIV unhealthy control group. Because there were no noted differences in dietary intake of SFAs between the 3 groups, the higher con-

centrations of these fatty acids (16:1 and 18:0) suggest an increase in de novo lipogenesis in both of these groups. Thus, the increase in lipogenesis reported with the metabolic syndrome seen in subjects with HIV receiving HAART and in non-HIV patients with developing metabolic syndrome is supported by these data.

Comparison of the phospholipid fatty acid percentages (%nmol) between the HIV subjects and the non-HIV control groups indicated higher percentages of the long-chain n–3 fatty acids 20:5n–3, DPA, and DHA and higher concentrations of the long-chain n–6 fatty acids 20:3n–6, 22:4n–6, and 22:5n–6. A higher total percentage of the n–3 fatty acids was seen in the subjects with HIV with no significant difference in the percentage of the total n–6 fatty acids between the subjects with HIV and either of the non-HIV control groups. These data suggest an overall increase in the elongation and desaturation of both n–6 and n–3 fatty acids of ≥20 carbons in the subjects with HIV, which, importantly did not extend to 20:4n–6. This was not due to the differences in dietary intakes of n–3 fatty acids shown in Table 2, because control for this in the multivariate analysis did not change the results. This suggests that there was a preferential selection for n–3 fatty acids in the synthesis of the phospholipids, and the pattern of n–3 and n–6 fatty acids indicates increased fatty acid metabolism by elongation and desaturation of both the n–6 and n–3 series of very-long-chain PUFAs (>18 carbons). One study conducted in children with HIV, who were taking medications

including protease inhibitors, showed a stimulatory effect on Δ^6-desaturase and a decrease in Δ^5-desaturase (27). Our data suggest an increase in both the Δ^6- and Δ^5-desaturases in the HIV subjects.

It is important to note that the percentage of 20:4n–6 in the phospholipid fraction remained similar in all 3 groups. This suggests a priority in metabolism to maintain concentrations of this fatty acid despite different disease conditions. This is critical, because this fatty acid is the principal precursor of eicosanoids, particularly the proinflammatory eicosanoids of the E2 series (28), and changes in its availability could influence production of this inflammatory factor (29), which could further affect the disease process of the metabolic syndrome, other chronic diseases, and HIV itself. The higher concentrations of the n–3 series of fatty acids also provide increased substrate for the formation of less inflammatory eicosanoids of the E3 series, which could in fact suppress the inflammatory responses (30). The usual effect of an increased dietary intake of EPA is to increase concentrations of this fatty acid in phospholipids and to reduce 20:4n–6 concentrations by displacement (31). The significantly higher amounts of dietary EPA and higher concentrations of EPA in the serum phospholipids of the subjects with HIV than in either of the non-HIV control groups was not associated with lower concentrations of 20:4n–6, however. There is no explanation for this observation. This suggests that there was a relative increase in 20:4n–6 production in the HIV patients, because the usual decrease in 20:4n–6 in the presence of higher EPA concentrations was not seen. The lower n–6/n–3 ratio in the phospholipid fatty acid fraction in subjects with HIV appears to have been principally due to changes in metabolism, because correction for the dietary intake in the multivariate analysis did not change the results.

Vessby (13) reported that the typical fatty acid pattern seen in individuals with the metabolic syndrome was characterized by an increased proportion of SFAs, especially palmitic acid, and a reduced proportion of linoleic acid. Our data show higher concentrations of stearic acid than of palmitic acid in those at risk of the metabolic syndrome, with or without HIV, and a lower concentration of linoleic acid observed only in the HIV subjects at risk of the metabolic syndrome and not in either of the non-HIV control groups. Vessby (13) proposed that the metabolic syndrome manifests an increased activity of Δ^9- and Δ^6-desaturase but a low activity of Δ^5-desaturase. We did see a statistically significant higher concentration of palmitoleic acid (16:1n–7) in subjects at risk of the metabolic syndrome, with or without HIV, which indicates an increase in Δ^9-desaturase. Higher concentrations of DGLA, which indicate an increase in Δ^6-desaturase, were seen only in subjects with HIV and not in the non-HIV unhealthy controls. Both 22:4n–6 and 22:5n–6 were higher in subjects with HIV, which is consistent with increased distal desaturase activity and elongase activity and supports an increase in distal desaturase activity in HIV with HAART. Whether the changes we observed were due to HIV or to the medications could not be determined on the basis of our data.

Increases in 16:1n–7 and in DGLA have been associated with insulin resistance (13, 32–34). These fatty acids are generally very low in the diet (34); therefore, increased levels would reflect changes in endogenous metabolism. However, whereas both the HIV patients and the non-HIV unhealthy controls had elevated concentrations of 16:1n–7 in the phospholipid fraction, only the HIV subjects had an elevated DGLA concentration,

which perhaps suggests the potential for a more rapid progression of the metabolic syndrome as a consequence of increased desaturase activity.

The ratio of triglycerides to phospholipids appears to change with the development of the metabolic syndrome, with less phospholipids present to balance the increase in serum triglycerides. This became more severe in the HIV patients than in the non-HIV unhealthy controls and suggests a greater difficulty in clearing lipids from the blood in HIV subjects taking medication.

In summary, the phospholipid fatty acid patterns observed suggest an increase in the de novo lipogenesis in the HIV subjects and in the non-HIV unhealthy controls, which reflects the development of the metabolic syndrome. Higher percentages of the n–3 fatty acids were seen in the phospholipids fraction of HIV subjects, which persisted even after the control for differences in the dietary intakes of these fatty acids. The significantly higher concentrations of fatty acid products of distal elongation and desaturation observed in the subjects with HIV suggests an increase in both Δ^6- and Δ^5-desaturation in this adult population with HIV. Whether this was due to HIV or to medication use, especially protease inhibitors, was not ascertained. However, the percentage of 20:4n–6 remained the same in all 3 groups. Our data support the value of assessing both dietary fat intakes to correct for differences in intakes and assessing serum fatty acid changes to interpret metabolic alterations and the risk of cardiovascular disease and type 2 diabetes in subjects with HIV taking medications.

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