0021-972X/92/7405-1045$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 74, No. 5
Printed in U.S.A.

# Lipids, Lipoproteins, Triglyceride Clearance, and Cytokines in Human Immunodeficiency Virus Infection and the Acquired Immunodeficiency Syndrome*

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**ABSTRACT.** Infection causes disturbances in lipid metabolism that may be mediated by cytokines. Therefore we studied plasma lipids, lipoproteins, triglyceride (TG) metabolism, and serum cytokines in three groups: patients with the acquired immunodeficiency syndrome (AIDS) without active secondary infection, patients with evidence of human immunodeficiency virus infection but without clinical AIDS (HIV+), and controls.

Plasma TGs and FFA were increased in AIDS, while plasma cholesterol, high density lipoprotein (HDL) cholesterol, apolipoprotein-A-1 (Apo-A-1), low density lipoprotein (LDL) cholesterol, and Apo-B-100 levels were decreased. Increased TG levels in AIDS were primarily due to increases in very low density lipoprotein of normal composition; in addition, LDL and HDL were TG enriched. In HIV+, TGs and FFA were not increased, but total cholesterol, HDL cholesterol, Apo-A-1, and Apo-B-100 were significantly decreased.

Interferon-$\alpha$ (IFN$\alpha$) and C-reactive protein levels were increased in AIDS, but tumor necrosis factor and haptoglobin levels were not. There was a significant correlation between plasma TGs and IFN$\alpha$ levels ($r = 0.477$; $P < 0.01$), but not between TGs and tumor necrosis factor, C-reactive protein, haptoglobin, or P-24 antigen. In addition, there was no relationship between circulating IFN$\alpha$ levels and plasma cholesterol, HDL cholesterol, Apo-A-1, LDL cholesterol, Apo-B-100, or FFA. TG clearance time and postheparin lipase were significantly decreased in AIDS and HIV+. There was a strong correlation between serum IFN$\alpha$ levels and TG clearance time in AIDS and HIV+ ($r = 0.783$; $P < 0.001$).

In summary, decreases in cholesterol and cholesterol containing lipoproteins (including HDL) in both AIDS and HIV+ precede the appearance of hypertriglyceridemia and are not related to IFN$\alpha$ or TG levels. Our data raise the possibility that with development of AIDS, subsequent increases in IFN$\alpha$ may contribute to increases in plasma TG levels in part by decreasing the clearance of TG. (*J Clin Endocrinol Metab* **74:** 1045–1052, 1992)

HYPERTRIGLYCERIDEMIA due to increased very low density lipoproteins (VLDL) has been reported in bacterial, parasitic, and viral infections (1–7). Decreased plasma cholesterol levels have also been reported during infection (4–8). Infection can increase plasma triglyceride (TG) levels by decreasing the clearance of circulating lipoproteins, a process thought to be the result of reduced lipoprotein lipase (LPL), or by stimulating hepatic lipid synthesis through increases in either hepatic fatty acid synthesis or reesterification of fatty acids derived from lipolysis (reviewed in Ref. 8). These alterations in TG metabolism are thought to be produced by the cytokines that mediate the immune response, including tumor necrosis factor (TNF), interleukin-1, and the interferons (IFNs) (reviewed in Ref. 8).

In a previous pilot study of out-patients, we found that the acquired immunodeficiency syndrome (AIDS) was frequently accompanied by increased serum TG levels (9). In that group of subjects there was also a trend toward decreased serum cholesterol levels (9). Preliminary data indicate that the increase in serum TGs correlates with circulating levels of IFN$\alpha$ (10), a cytokine whose levels are well recognized to be elevated in AIDS (reviewed in Ref. 10). There is controversy as to whether TNF levels are elevated in AIDS (10–14); however, TNF is rapidly cleared from the circulation, and longer term markers of TNF action have not been analyzed. The mechanisms by which hypertriglyceridemia develops in AIDS are not yet known.

In this paper we study a new cohort of subjects with AIDS and human immunodeficiency virus (HIV) infection as in-patients under metabolic ward conditions. Neither TNF levels nor acute phase response markers of TNF action correlate with changes in plasma TG or

Received April 17, 1991.
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* This work was supported by grants from the Department of Veterans Affairs, the Universitywide AIDS Research Program of the University of California, and the NIH (DK-40990).
† Recipient of a Clinical Investigator Award from the Department of Veterans Affairs.

lipoprotein levels. Rather, our data suggest that circulating IFN$\alpha$ induces hypertriglyceridemia in part by decreasing lipase activity and slowing the clearance of TG, but that changes in plasma cholesterol levels are mediated by a separate mechanism.

## Subjects and Methods

### *Patients*

This study was approved by the Committee on Human Research, University of California, San Francisco. Fifteen subjects with AIDS, as defined by the revised Centers for Disease Control criteria (15), were studied. None had signs of active secondary opportunistic infections at the time of study. Subjects were excluded for dyspnea, hypoxia, sputum production, urinary tract symptoms, positive urinalysis, pathogenic sputum culture, positive urine or blood culture, or acute pathology on chest or sinus films. Fourteen subjects with HIV infection, as determined by presence of antibodies against HIV measured by enzyme-linked immunoabsorbent assay and confirmed by Western blot (HIV+), were studied. HIV+ patients had neither a history of opportunistic infection or malignancy nor symptoms or signs of infection, as described above. Sixteen controls who did not have HIV antibodies were also studied. The three groups were age matched; all were male. None had diabetes, kidney failure, nephrotic syndrome, active hepatitis, or cirrhosis. No patient was taking drugs known to affect lipid metabolism at the time of study.

Subjects were admitted to a metabolic ward, the Special Diagnostic and Treatment Unit, at the San Francisco V.A. Medical Center for all tests. AIDS and HIV+ subjects had body weights and body mass indices that were not significantly different from controls.[1] All subjects were allowed to eat an *ad libitum* diet consisting of 15% protein, 45% carbohydrate, and 40% fat. AIDS and HIV+ subjects were not anorexic and had caloric intakes that were not significantly different from those of the controls (see Footnote 1). All plasma samples were obtained in the morning after an observed 14-h overnight fast. On one morning, subjects had TG clearance measured as described below. On another morning, subjects received heparin (50 U/kg), and a sample was taken for postheparin lipase, as described below. Samples for lipoprotein analysis and TG clearance were collected in chilled EDTA tubes; the plasma was rapidly separated, then stored at 4 C until analysis (within 3 h for clearance) or ultracentrifugation (within 48 h for lipoproteins). All other samples were rapidly frozen and stored at −70 C.

### *Analytical methods*

TG and phospholipid were assayed enzymatically (Wako Pure Chemical Industries, Ltd., Richmond, VA). Cholesterol was measured using a kit from Sigma (St. Louis, MO). Protein concentrations in lipoproteins were assayed by the Triton method (16). Apolipoprotein-A-1 (Apo-A-1), Apo-B-100, and C-reactive protein were measured by radial immunodiffusion (Tago, Inc., Burlingame, CA). Haptoglobin was measured by immunoprecipitation (Beckman Instruments, Inc., Brea, CA). FFA were analyzed as previously described (17).

High density lipoprotein (HDL) cholesterol as well as $HDL_2$ and $HDL_3$ subfractions were measured using the sequential manganese-heparin/dextran sulfate method (18), except that 12,000 mol wt dextran sulfate (Spectrum, Gardena, CA) was used at a final concentration of 0.25 mg/dL for the precipitation of $HDL_2$, and EDTA was added to 4 mM for the cholesterol oxidase step. Low density lipoprotein (LDL) and VLDL cholesterol were calculated by the method of Friedewald *et al.* (19). P24 antigen levels were measured by the UCSF Immunology Reference Laboratory.

Serum IFN levels were measured by a bioassay that assesses the protection of A549 cells from challenge with encephalomyocarditis virus, as described in detail previously (10), and expressed in international reference units. The limit of detectability is 3 IU/mL. Antibody against IFN$\alpha$ (but not IFN$\beta$ or IFN$\gamma$) neutralized all of the circulating IFN in these subjects. TNF levels were determined using an enzyme-linked immunosorbent assay from T Cell Sciences (Cambridge, MA). The sensitivity was 10 pg/mL, as defined by a coefficient of variance of less than 10%. In previous use of this assay, values in the range of 3–10 pg/mL have been reported (12).

### *Lipoprotein preparation*

Three milliliters of plasma collected in EDTA were overlaid with 1 mL density 1.006 saline and centrifuged for $1.8 \times 10^6$ $g \cdot$ min; the chylomicron fraction was removed by tube slicing. To isolate VLDL, the infranatant was overlaid with density 1.006 saline and centrifuged again at 200,000 × $g$ for 22 h (20). TGs were then measured to determine the relative contribution of chylomicrons and VLDL to the increase in plasma TGs. Apo lipoproteins were analyzed in these fractions, using 3–10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (21). LDL and HDL were prepared by a gradient centrifugation technique, as previously described (9). For composition studies, lipoproteins were centrifuged again at 200,000 × $g$ for 22 h using density 1.006 for TG-rich lipoproteins, density 1.063 for LDL, and density 1.21 for HDL (20).

### *TG clearance*

The clearance of TG-rich particles was determined by the iv fat tolerance test, as described previously (22–24), using 20% Soyacal iv fat emulsion (Alpha Therapeutic Corp., Los Angeles, CA) at 0.1 g/kg BW. Seven plasma samples were taken over 50 min, and clearance was measured by nephelometry (22). Regression of a semilog plot of clearance was linear for each patient, and the $t_{1/2}$ of clearance was determined. The iv fat tolerance test correlates highly with the fractional turnover rate of endogenous TGs and clearance of chylomicrons and VLDL (23, 24). This clearance is not merely a reflection of TG levels, as clearance is not slowed in patients with disorders of hypertriglyceridemia that are due to increased VLDL production (25, 26). However, in fasting controls, serum TG levels are proportional to clearance (22–24). The ability to use a single

[1] Grunfeld, C., M. Pang, L. Shimizu, J. K. Shigenaga, P. Jensen and K. R. Feingold, Am. J. Clin. Nutr (In Press).

TABLE 1. Plasma lipid and Apo lipoprotein levels

| | Control | HIV+ | AIDS | AIDS *vs.* control | HIV+ *vs.* control | AIDS *vs.* HIV+ |
|---|---|---|---|---|---|---|
| TG (mmol/L) | 1.15 ± 0.122 | 1.24 ± 0.167 | 2.29 ± 0.281 | <0.0005 | NS | <0.001 |
| Cholesterol (mmol/L) | 4.73 ± 0.172 | 3.88 ± 0.203 | 3.90 ± 0.295 | <0.02 | <0.01 | NS |
| HDL cholesterol (mmol/L) | 1.27 ± 0.066 | 0.809 ± 0.043 | 0.804 ± 0.049 | <0.0001 | <0.0001 | NS |
| $HDL_3$ cholesterol (mmol/L) | 0.978 ± 0.030 | 0.667 ± 0.031 | 0.621 ± 0.040 | <0.0001 | <0.0001 | NS |
| $HDL_2$ cholesterol (mmol/L) | 0.295 ± 0.046 | 0.143 ± 0.021 | 0.183 ± 0.027 | <0.025 | <0.005 | NS |
| Apo-A-1 (mg/dL) | 124 ± 8.24 | 90.4 ± 4.50 | 86.8 ± 5.40 | 0.0002 | <0.001 | NS |
| LDL cholesterol (calculated; mmol/L) | 3.13 ± 0.169 | 2.64 ± 0.190 | 2.18 ± 0.266 | 0.0025 | NS | NS |
| Apo-B-100 (mg/dL) | 74.5 ± 4.28 | 58.0 ± 3.38 | 59.5 ± 6.40 | <0.05 | <0.025 | NS |
| VLDL cholesterol (calculated; mmol/L) | 0.530 ± 0.056 | 0.566 ± 0.076 | 1.050 ± 0.129 | <0.0002 | NS | <0.001 |
| FFA (nmol/mL) | 1193 ± 84 | 1248 ± 96 | 1480 ± 77 | <0.05 | NS | <0.1 |

n = 16 for controls, n = 14 for HIV+, and n = 15 for AIDS, except for FFA, where n = 14 for HIV+ and n = 13 for AIDS.

source of TG-rich particles eliminates the problems of comparing clearance between patients who might produce chylomicrons or VLDL of varying compositions. The iv administration of lipid also avoids differences due to malabsorption. Finally, a commercially available lipid particle (Soyacal) avoids the need to process large amounts of plasma from HIV-infected patients.

*Postheparin lipase*

Lipase activity was determined in postheparin plasma collected 15 min after the injection of 50 U heparin/kg BW. Plasma was stored at −70 C until analyzed. Total lipase activity was assayed by the method of Krauss *et al.* (27). Hepatic lipase was measured after preincubation with protamine sulfate (27). LPL activity represents the difference between total lipase and hepatic lipase (27). Subjects with platelet counts less than 100,000 did not receive heparin administration for determination of lipase levels (two each for AIDS and HIV+ patients).

*Statistics*

Data are presented as the mean ± SE. Means were compared using analysis of variance. Correlations were performed by linear regression analysis. Variance was calculated by univariate analysis.

## Results

*Plasma lipid and Apo lipoprotein levels*

In this new cohort of subjects with AIDS, plasma TG levels were again found to be increased to twice the control value (Table 1). Eight of 15 subjects (53%) with AIDS had hypertriglyceridemia (TG, >190 mg/dL). In these HIV+ subjects, mean plasma TG levels were not significantly elevated above control values, and only 1 had hypertriglyceridemia *vs.* none of the controls. Using sequential ultracentrifugation, 97 ± 1.1% of the d < 1.006 TG was in VLDL in controls *vs.* 95 ± 1.3% in AIDS patients ($P$ = NS). The very small amounts of lipoproteins in the chylomicron fraction were found to contain more B-100 than B-48, indicating that even the chylomicron fraction contained significant amounts of large VLDL. Thus, nearly all of the increased TG at density less than 1.006 is from VLDL.

A trend toward decreased serum cholesterol levels was previously seen in AIDS and HIV+ subjects, but did not quite reach statistical significance (9). In the present patient population, serum cholesterol was significantly decreased in AIDS and HIV+ (Table 1).[2] Therefore, the lipoprotein distribution of cholesterol and Apo lipoproteins was analyzed. There was a highly significant decrease (37%) in serum HDL levels in AIDS and HIV+ patients. Significant decreases were seen in both $HDL_3$ (AIDS, 32%; HIV+, 37% decrease) and $HDL_2$ (AIDS, 51%; HIV+, 38% decrease) cholesterol. In parallel, there were significant decreases in serum Apo-A-1 levels, the major Apo lipoprotein of HDL. A significant reduction (30%) was seen in calculated LDL cholesterol in AIDS patients. In HIV+ subjects, there was a trend toward reduced LDL cholesterol, but this was not statistically significant. Apo B-100 levels were significantly decreased in both AIDS and HIV+, reflecting the decrease in LDL levels. Calculated VLDL cholesterol was increased in AIDS patients compared to controls. As described below, the composition of VLDL is unaltered in AIDS; hence, the use of these calculations is appropriate.

There was a small increase (24%) in FFA levels in patients with AIDS (Table 1). FFA levels in HIV+ fell between the levels in AIDS and control subjects, but were not significantly different from either.

*Lipoprotein composition*

VLDL composition in AIDS did not differ from that in controls (Fig. 1, *left panel*). LDL particles were rela-

[2] The mean levels of cholesterol reported here are quite similar to those reported previously (9, 10). In a previous study, cholesterol was 190 ± 16 in controls and 155 ± 7.9 mg/dL in AIDS ($t$ = 1.961; a $t$ = 2.000 would give $P$ = 0.05). The SEs are smaller here; hence, this decrease reached significance.

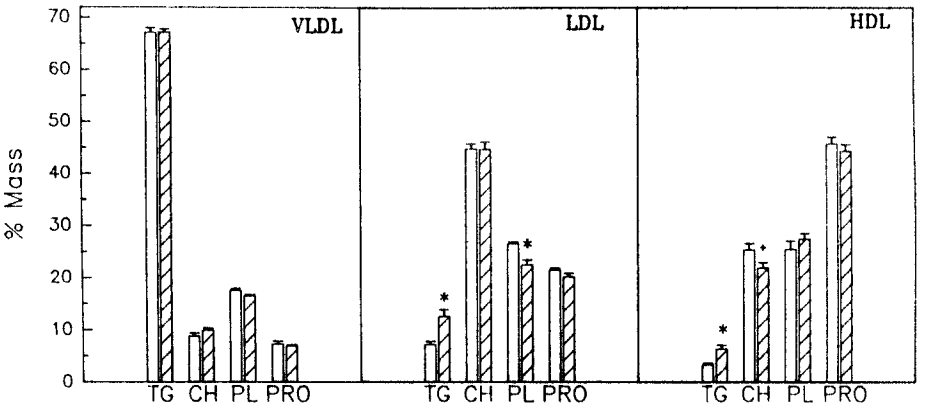


FIG. 1. Lipoprotein composition. Lipoproteins were prepared from plasma of control (□) and AIDS (▨) subjects, as described in *Materials and Methods*. Lipoproteins were analyzed for TG, cholesterol (CH), phospholipid (PL), and protein (PRO), as described in the text. *Left panel*, VLDL; *middle panel*, LDL; *right panel*, HDL. *, $P < 0.001$; +, $P < 0.05$ (by analysis of variance).

tively TG rich in patients with AIDS (74% above control), with a corresponding decrease in phospholipid content (Fig. 1, *middle panel*). However, the LDL in AIDS remained equally cholesterol rich and had similar protein content. HDL in AIDS was also relatively TG rich (89% increased), with a small decrease in cholesterol levels (Fig. 1, *right panel*). However, the protein concentration of HDL was unchanged. In HIV+, the increases in TG in LDL and HDL did not reach significance (data not shown). When lipoprotein compositions and Apo levels are examined, it can be estimated that most of the increase in serum TGs was due to VLDL, with little contribution of LDL and HDL to TGs.

### *Circulating cytokines, acute phase response proteins, and P24 antigen*

IFN$\alpha$ is consistently increased in AIDS (reviewed in Ref. 10), but there is disagreement as to whether TNF is elevated (10–14). Previous data suggested that elevations in IFN$\alpha$ correlate with serum TG levels in AIDS (10). In this new group of patients with AIDS, we also found that 80% of patients with AIDS had detectable circulating IFN$\alpha$ compared to only 21% of HIV+; no control had detectable IFN$\alpha$ (Table 2).

TNF levels were not significantly elevated in AIDS, nor was there an increased frequency of detectable TNF levels (Table 2). Because TNF is released in a pulsatile fashion and is cleared rapidly from the circulation, we also measured C-reactive protein and haptoglobin; the production of these two acute phase response proteins is stimulated by TNF (28, 29). There was a significant increase in C-reactive protein levels in AIDS patients, although only 40% had detectable levels (Table 2). Haptoglobin levels stayed elevated for longer periods during the acute phase response, but there was no difference in haptoglobin levels in AIDS or HIV+ patients compared to controls (Table 2).

P24 antigen may be found in the circulation during HIV infection. One third of AIDS and one half of HIV+ patients had detectable P24 levels compared to none of the controls (Table 2). The range of P24 levels was quite broad, so that these numbers were not significantly different from control values.

### *Correlation between plasma TGs and cytokines, acute phase response proteins, or P24 antigen levels*

There was a significant correlation between serum TG levels and IFN$\alpha$ (Fig. 2). The correlation in this group of patients ($r = 0.477$; $P < 0.01$) was similar to that found previously (10) in subjects studied as out-patients ($r = 0.446$; $P < 0.002$). One patient with AIDS in the present study had a relatively high plasma TG level (marked by an *asterisk* in Fig. 2). He was not previously known to have hyperlipidemia, but he most likely has familial dyslipoproteinemia, as his mother has hyperlipidemia and his father died of premature coronary artery disease. When the data from this patient are excluded, the r value increases to 0.593, with a significance of $P < 0.001$.

There was no significant correlation between plasma TG levels and circulating TNF ($r = 0.146$; $P$ = NS), C-reactive protein ($r = 0.013$; $P$ = NS), haptoglobin ($r = -0.031$; $P$ = NS), or P24 antigen ($r = 0.099$; $P$ = NS). There was also no difference in the levels of TGs in AIDS and HIV+ subjects with or without detectable levels of TNF (181 ± 49 *vs.* 153 ± 18 mg/dL), C-reactive protein (166 ± 34 *vs.* 155 ± 20 mg/dL), or P24 antigen (152 ± 24 *vs.* 162 ± 24 mg/dL).

In contrast to the correlation of IFN$\alpha$ with plasma TGs, there was no significant correlation between circulating serum IFN$\alpha$ and levels of cholesterol ($r = 0.100$; $P$ = NS), HDL cholesterol ($r = -0.011$; $P$ = NS), $HDL_3$ cholesterol ($r = -0.054$; $P$ = NS), $HDL_2$ cholesterol ($r = 0.056$; $P$ = NS), Apo-A-1 ($r = 0.110$; $P$ = NS), LDL cholesterol ($r = -0.127$; $P$ = NS), Apo-B-100 ($r = -0.190$; $P$ = NS), or FFA ($r = 0.143$; $P$ = NS). These data suggest that the correlation between IFN$\alpha$ and plasma TGs was specific for defects in TG metabolism. In addition, increased levels of circulating TNF or acute phase response

TABLE 2. Circulating cytokine, acute phase response proteins, and P24 antigen levels

| | Control (n = 16) | HIV+ (n = 14) | AIDS (n = 15) | AIDS *vs.* control | HIV+ *vs.* control | AIDS *vs.* HIV+ |
|---|---|---|---|---|---|---|
| IFNα | | | | | | |
| U/mL | 0 | 2.26 ± 1.55 | 17.4 ± 4.61 | 0.0001 | NS | <0.001 |
| No. positive (%) | 0 (0) | 3 (21) | 12 (80) | | | |
| TNF | | | | | | |
| pg/mL | 2.06 ± 1.53 | 0.42 ± 0.42 | 1.46 ± 0.68 | NS | NS | NS |
| No. positive (%) | 2 (12.5) | 1 (7) | 4 (27) | NS | NS | NS |
| C-Reactive protein | | | | | | |
| mg/dL | 0 | 0.02 ± 0.02 | 0.32 ± 0.17 | <0.025 | NS | <0.05 |
| No. positive (%) | 0 (0) | 1 (7) | 6 (40) | | | |
| Haptoglobin (g/L) | 0.961 ± 0.139 | 0.88 ± 0.142 | 0.797 ± 0.194 | NS | NS | NS |
| P24 antigen | | | | | | |
| pg/mL | 0 | 33.9 ± 12.0 | 30.1 ± 25.2 | NS | NS | NS |
| No. positive (%) | 0 (0) | 7 (50) | 5 (33) | | | |

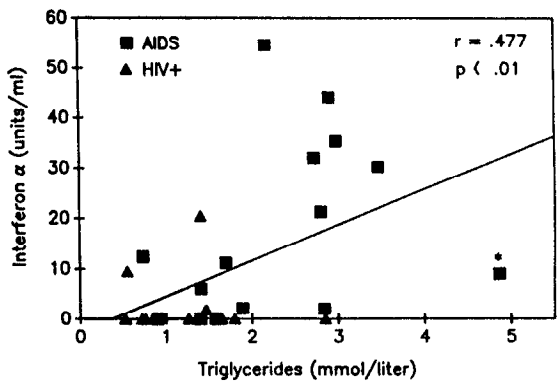


FIG. 2. Correlation between IFNα and TG in AIDS and HIV+. Serum from patients with AIDS (■) and HIV+ subjects (▲) was analyzed for IFNα and TG, as described in *Materials and Methods*. Linear regression analysis was used to determine the line, r value, and *P* value. The AIDS patient marked with an *asterisk* has familial dyslipoproteinemia, as described in the text.

proteins do not account for the decreases in cholesterol, HDL cholesterol, Apo-A-1, LDL cholesterol, or Apo-B-100 levels in AIDS and HIV+ (data not shown).

### *TG clearance*

The clearance of TG-rich particles was significantly slower in AIDS patients, with a half-life that was 2.7-fold longer than that in controls (Table 3). The clearance of TGs in HIV+ patients fell in between those in AIDS and control subjects. The enzymes involved in TG clearance were also decreased; total lipase, hepatic lipase, and LPL were each decreased by 27% in AIDS patients compared to controls (Table 3). Values for lipases in HIV+ subjects fell in between, but were not significantly different from, those in AIDS patients or controls.

There was a highly significant correlation between circulating levels of IFNα and TG clearance time ($t_{1/2}$) in AIDS and HIV+ patients (Fig. 3; $r = 0.783$; $P < 0.001$). The patient with AIDS who had a familial history of dyslipoproteinemia (marked with an *asterisk* in Fig. 3) had the highest plasma TG levels despite having relatively lower levels of IFNα. However, he had a TG clearance time that was only slightly prolonged, commensurate with his circulating IFNα level. The findings in this patient reaffirm the high correlation between IFNα levels and TG clearance time and emphasize that TG clearance is not solely dependent on TG levels. IFNα accounts for 61% ($r^2 = 0.613$) of the variance in TG clearance in AIDS and HIV+ patients. The correlation between TG clearance time and IFNα was similar when analyzed only for AIDS ($r = 0.767$; $P < 0.001$).

We also found a highly significant correlation between TG clearance time and TG levels in AIDS and HIV+ subjects (Fig. 4). TG clearance could account for approximately 33% of the variance in TG levels in AIDS and HIV+ ($r^2 = 0.335$). The patient with familial dyslipoproteinemia is again marked with an *asterisk* in Fig. 4; as

TABLE 3. TG clearance and postheparin lipase activity

| | Control | HIV+ | AIDS | AIDS *vs.* control | HIV+ *vs.* control | HIV+ *vs.* AIDS |
|---|---|---|---|---|---|---|
| TG clearance time ($t_{1/2}$, min) | 15.2 ± 1.37 | 25.0 ± 2.50 | 41.5 ± 5.16 | <0.0001 | <0.05 | <0.002 |
| Total lipase | 15.1 ± 0.93 | 12.4 ± 1.40 | 11.0 ± 1.01 | <0.02 | <0.1 | NS |
| Hepatic lipase | 11.2 ± 0.95 | 9.20 ± 1.30 | 8.16 ± 0.90 | <0.05 | NS | NS |
| LPL | 3.86 ± 0.28 | 3.23 ± 0.28 | 2.83 ± 0.19 | <0.01 | <0.1 | NS |

All lipases are expressed as nanomoles of FFA per mL plasma/h, 13 for AIDS and 12 for HIV+.

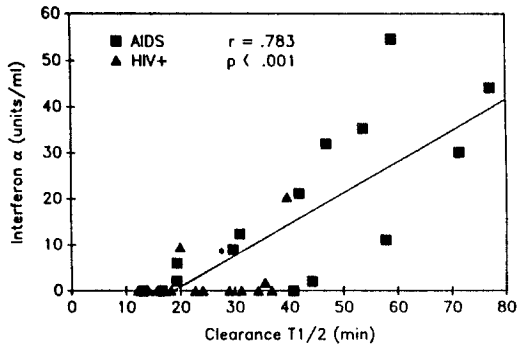


FIG. 3. Correlation between IFN$\alpha$ and TG clearnce time ($t_{1/2}$) in AIDS and HIV+. Patients with AIDS (■) and HIV+ subjects (▲) had serum analyzed for IFN$\alpha$ and TG clearance, as described in *Materials and Methods*. The line, r value, and *P* value represent linear regression analysis. The AIDS patient marked by an *asterisk* is the same patient with familial dyslipoproteinemia marked in Fig. 2.

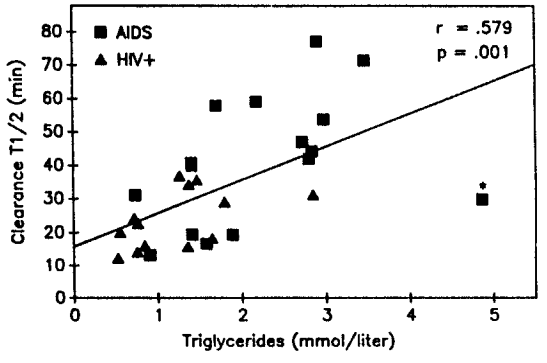


FIG. 4. Correlation between TG clearance time ($t_{1/2}$) and TG levels in AIDS and HIV+. Serum TG levels and TG clearance were measured in AIDS (■) and HIV+ (▲) patients, as described in *Materials and Methods*. The line, r value, and *P* value represent linear regression analysis. The AIDS patient marked by an *asterisk* is the same patient with familial dyslipoproteinemia marked in Figs. 2 and 3.

TABLE 4. Correlation between IFN$\alpha$ levels and measures of TG metabolism in AIDS and HIV+ subjects

| | r | *P* |
|---|---|---|
| IFN$\alpha$ *vs.* TG clearance | 0.783 | <0.001 |
| IFN$\alpha$ *vs.* total postheparin lipase | −0.355 | NS |
| IFN$\alpha$ *vs.* hepatic lipase | −0.328 | NS |
| IFN$\alpha$ *vs.* LPL | −0.276 | NS |

n = 29 for triglyceride clearance (AIDS = 15; HIV+ = 14). n = 25 for lipase measurements (AIDS = 13; HIV+ = 12).

expected from Figs. 2 and 3, his TG levels were relatively higher than his clearance alone would predict.

Correlations between IFN$\alpha$ and lipases were weaker and did not reach significance (Table 4), but the number of lipase determinations was smaller (low platelet counts prevented heparin administration); the correlation between lipase and IFN$\alpha$ might have been significant with a larger number of subjects. IFN treatment of humans (30, 32) and cultured fat cells (8) decreases LPL activity. There was no correlation between TNF levels and TG clearance or lipases (data not shown).

## Discussion

Previous studies of plasma lipids during human infection examined self-limited infections during active or convalescent phases (1, 4–7). Here we analyzed a syndrome characterized by chronic viral infection. A previous study indicated that TG levels are relatively stable in these patients (9). Unlike acute viral infection, IFN$\alpha$ levels are also sustained in AIDS (10). None of the subjects had acute secondary infection.

In this study plasma TG levels were significantly elevated in subjects with AIDS. FFA levels were also elevated. The increase in plasma TG levels was due to increased VLDL of normal composition; LDL and HDL were relatively TG rich, but contributed little to the increase in plasma TGs. In this new cohort, subjects who were HIV+ did not have significantly elevated TG levels. These subjects underwent an observed 14-h fast on a metabolic ward. In contrast, subjects were studied as outpatients previously (9, 10). In addition, all but one of these subjects were homosexual men, whereas a high percentage of iv drug users were studied previously (9, 10).

Total cholesterol, HDL cholesterol (including $HDL_2$ and $HDL_3$), Apo-A-1, and Apo-B-100 were decreased to a similar degree in both AIDS and HIV+. LDL cholesterol was significantly decreased in AIDS, while LDL cholesterol in HIV+ patients fell in between values in AIDS and control subjects. The striking decreases in cholesterol, Apo lipoprotein, and, especially, HDL cholesterol levels in HIV+ subjects who have not yet developed hypertriglyceridemia imply that disturbances in cholesterol metabolism, including those in HDL cholesterol, precede development of frank elevations in serum TGs during HIV infection.

Many different infections have been shown to disturb lipid metabolism (1–7). Some infections increase TG levels during the acute febrile phase (1, 2, 7), whereas in others, TG levels increase during the immediate convalescent phase (4–6). One study found that decreases in LDL and HDL began in the acute phase of illness before increases were seen in TG levels (5). Decreases in cholesterol levels persisted during the convalescent phase when TG levels finally rose. In experimental viral infection, cholesterol also falls before TG levels rise (4). Other studies have shown both decreases in cholesterol and increases in TG during infection in humans (reviewed in Ref. 7). Changes in diet during acute infection may influence plasma TG levels. The data in other infections, therefore, also support the concept that changes in cholesterol metabolism are not merely related to changes in

plasma TG levels *per se* and may be induced by different stimuli.

Cytokines are thought to mediate the metabolic disturbances of infection (8), and previous studies have suggested that IFNα and possibly TNF are elevated in AIDS (10–14). We again found that IFNα was elevated in most subjects with AIDS (80%). In this new cohort we once again failed to find a significant increase in TNF levels; thus, five of six studies of patients with AIDS do not find elevations in circulating TNF (10–14)[3]. The single report that found elevated TNF levels in AIDS primarily studied patients during acute opportunistic infections (11). In this study we excluded subjects with obvious secondary infections. Hypertriglyceridemia in AIDS persists in the absence of secondary infection (9). It is possible that increases in TNF levels could have been missed, as TNF is secreted in pulsatile form and is rapidly cleared from the circulation. Therefore, because TNF has been shown to induce the acute phase response (28, 29), we also measured two markers for the acute phase response, C-reactive protein and haptoglobin. Increased C-reactive protein was seen in AIDS, but only 40% of the subjects were positive. There was no signficant increase in serum haptoglobin levels in AIDS or HIV+.

As seen previously (10), plasma TG levels show a significant correlation with IFNα levels in AIDS. In contrast, it was not possible to show any relationship between levels of TNF, acute phase response proteins, or P24 antigen and plasma TG levels. Our studies, therefore, suggest that IFNα can influence plasma TG levels. The relationship between IFNα and lipid metabolism appears to be limited to plasma TGs. There is no correlation between IFNα and plasma cholesterol, HDL cholesterol, Apo-A-1, LDL cholesterol, Apo-B-100, or FFA in AIDS and HIV+ subjects. These data suggest that the disturbances in cholesterol metabolism are independent of IFNα and reinforce other data which show that changes in cholesterol metabolism occur before those in TG metabolism during infection. IFN therapy has been variably associated with increases in TG and decreases in total and HDL cholesterol (30–33). It is possible that humans are more sensitive to the cholesterol-lowering effects than to the TG-raising effects of IFN and that conventional IFN assays are too insensitive to measure levels in HIV+ subjects. IFNα appears at about the time of development of symptomatic AIDS (reviewed in Ref. 10). Disturbances in TG metabolism (but not cholesterol metabolism) appear in parallel with IFNα. However, the finding of decreased cholesterol early in the course of HIV infection suggests an earlier host response to HIV.

Another important new finding is that TG clearance is markedly prolonged in AIDS and even slowed in HIV+. We found a highly significant correlation between IFNα levels and TG clearance time. The subject with AIDS who had the highest TG level had a familial history of dyslipoproteinemia, and his TG levels are strikingly elevated compared to his low level of IFNα, but his TG clearance was only slightly prolonged, commensurate with his slightly elevated level of IFNα. The data from this subject reemphasize that TG clearance by this method is not merely a reflection of plasma TG levels and provide further evidence in support of the hypothesis that IFNα increases serum TG levels in part by slowing TG clearance. Despite variations in basal VLDL production and clearance, the influence of prior dietary intake and the effect of genetic differences in TG metabolism that are known to account for variations seen in normal subjects, we still found a highly significant correlation between circulating IFNα levels and both plasma TG and TG clearance in AIDS and HIV infection. Total postheparin, lipase, hepatic lipase, and LPL activities were all decreased in AIDS. Our subjects were studied after an observed 14-h fast, when VLDL production and clearance are in equilibrium. Therefore, given the small decreases in lipase, it is not surprising that their VLDL was of normal composition and not TG rich. In a previous study of acute infections in humans, fasting VLDL composition was also normal despite decreases in postheparin lipase (5). Fasting VLDL composition is also normal in subjects who are heterozygous for LPL deficiency (34). However, it is likely that TG-enriched lipoproteins will appear in the density less than 1.006 fraction under the influence of a marked TG load, such as during meals.

There was an excellent correlation between TG clearance and serum TG in AIDS and HIV+. Slowed clearance of TG could contribute to approximately one third of the variance in plasma TG levels ($r^2 = 0.335$) in AIDS and HIV+ subjects. Multiple factors have been shown to influence plasma TG levels during the response to infection, including both decreases in TG clearance and LPL activity and increases in *de novo* hepatic lipogenesis and VLDL production (reviewed in Ref. 8). Cytokines, including IFNα, have been implicated in each of these changes by studies *in vivo* or *in vitro* (8). It is possible that other aspects of TG metabolism are disturbed in AIDS and HIV infection. In preliminary data we found that *de novo* lipogenesis is increased in such patients and that the increase correlates with circulating IFNα levels.[3] Many patients with AIDS show stable weight (9) despite the profound disturbances in lipid metabolism shown here. Cytokine-induced hyperlipidemia is not inevitably linked to wasting (35). We do not yet know what additional factors are required to produce cachexia.

In summary, our data raise the possibility that circu-

[3] Hellerstein, M., C. Grunfeld, K. Wu., M.. Christiansen, S. Kaempfer, C. Kletke and C. H. L. Shackleton, in preparation.

lating levels of IFN$\alpha$, which are increased in AIDS, influence plasma TG levels and TG metabolism. However, the decreases in plasma cholesterol levels and the major cholesterol-carrying Apo lipoproteins appear to precede the increase in serum TGs and do not correlate with IFN$\alpha$. As similar metabolic defects are described, it may be possible to learn how the body compensates for some of these metabolic disturbances and which of them cause harmful consequences.

## Acknowledgments

We thank M. Cho for excellent editorial assistance. The cooperation and support of our patients and the staff of the Infectious Disease Clinic and the Special Diagnostic and Treatment Unit are greatly appreciated.

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