De novo lipogenesis predicts short-term body-composition response by bioelectrical impedance analysis to oral nutritional supplements in HIV-associated wasting1–4

Rebecca Hoh, Andrew Pelfini, Richard A Neese, Mary Chan, John P Cello, Frederick O Cope, Bonnie C Abbruzese, Ernie W Richards, Kecia Courtney, and Marc K Hellerstein

ABSTRACT We studied the effects of enteral supplements on protein and energy intakes, body composition, energy expenditure, and gastrointestinal histology in 49 subjects with human immunodeficiency virus–associated weight loss (12.7 ± 0.9% of body wt). We also determined whether a stable–isotope mass spectrometric measurement at baseline might predict the short-term response of fat-free mass (FFM) measured by bioelectrical impedance analysis. Thirty-nine subjects completed the study after being randomly assigned to receive either a whole–protein–based (n = 22) or a peptide–based (n = 17) formula. A nonsupplemented, nonrandomly assigned group (n = 13) was followed concurrently. Both formulas were well tolerated. Voluntary intakes of energy and protein from nonsupplement sources decreased significantly during supplementation [by 819–1638 kJ (196–382 kcal)/d and 5.6–14.4 g protein/d, respectively; P < 0.01] but to a lesser extent than the intake from the supplement [2300–2510 kJ(550–600 kcal)/d and 19–28 g protein/d, respectively], so that net increases in intakes of protein and energy (P < 0.03), as well as of several vitamins and trace elements were increased. Nevertheless, the mean FFM did not increase for the group as a whole, although there was considerable interindividual heterogeneity. Changes in FFM at 6 wk were significantly inversely correlated (r = 0.65, P < 0.01) with baseline synthesis of fat (de novo hepatic lipogenesis), but not with other potential measures of energy intake (insulin-like growth factor 1 or its binding protein) or inflammation (soluble tumor necrosis factor receptors I or II). The prospective identification of FFM response by measurement of de novo hepatic lipogenesis supported the hypothesis that the subset of wasting patients whose FFM is unresponsive to nutrient supplementation have altered nutrient metabolism. Am J Clin Nutr 1998;68:154–63.

KEY WORDS AIDS wasting, fat-free mass, enteral supplementation, gastrointestinal symptoms, de novo lipogenesis, mass spectrometry, peptide formula, humans

INTRODUCTION The wasting syndrome associated with human immunodeficiency virus (HIV) infection is a significant worldwide public health problem (1–6). Weight loss is the AIDS-defining diagnosis in 10–20% of cases and is essentially universal in advanced AIDS. Fatigue, weakness, inability to carry out the usual activities of daily life, and reduced participation in shared activities (including meals) also have a profound effect on quality of life in HIV-infected patients. Moreover, several workers (3, 4) have shown a correlation between survival and lean body mass (LBM) or other markers of nutritional state in AIDS.

Attempts to maintain or replenish LBM in AIDS patients by provision of nutrients have generally been disappointing, however (2, 7–10). Home total parenteral nutrition given to AIDS patients with weight loss failed to increase LBM, measured as total body potassium, despite increases in body fat stores (8). Administration of the appetite-stimulating, progestational agonist hormone megestrol acetate to AIDS patients with weight loss (5, 11, 12) increases food intake by 20–25% with an average weight gain of ~0.4 kg/wk (1 lb/wk), but 70–100% of the weight gained has been fat (7, 9, 10). These results in AIDS patients are consistent with previous reports that aggressive nutritional support of cancer patients (11, 12) or of septic intensive-care-unit patients (13) tends mostly to increase body fat, even as LBM decreases. Interindividual variability observed for LBM response to nutritional therapies in AIDS (8) has led to the suggestion that pathogenic subsets may exist within the AIDS-wasting population (ie, patients with starvation and patients with ongoing infection). No techniques for prospective identification of patients in each subset have been available, however.

Of potential relevance here is the documentation of metabolic abnormalities in HIV infection. These include hypertriglyc—

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eridemia (14–16), increased resting energy expenditure (17–20), less efficient preservation of lean tissue relative to fat compared with simple starvation (5), and paradoxically increased rates of de novo hepatic lipogenesis (DNL) despite prior weight loss (15–20). Whether the presence of metabolic abnormalities in AIDS patients can predict the LBM response to nutrients has not been determined, however.

The high prevalence of diarrhea and gastrointestinal infections in AIDS (21) suggests disadvantages as well as possible advantages for the use of oral nutrient supplementation. On the one hand, it is possible that liquid supplements might worsen bowel symptoms. Moreover, if a patient with the AIDS wasting syndrome has a primary appetite disturbance, provision of oral nutrients as a supplement to food may result in a proportional reduction in voluntary food intake. If a compensatory reduction in food protein and energy intakes cancels out the increase as a result of the supplement itself, the net benefit of oral supplementation would be questionable as well. On the other hand, oral feeding has lower associated costs and risks and represents a more physiologic route than does parenteral feeding. Enteral nutrients can also preserve gut function and prevent the atrophy characteristically observed during parenteral nutrition; partial hydrolysates of protein, containing oligopeptides, may be particularly effective in this regard (22).

We report here on the effects of oral nutritional supplements in subjects with weight loss and subjective gastrointestinal symptoms associated with HIV infection. Our focus was to determine the response of fat-free mass (FFM) to nutrient supplementation and whether this response can be predicted by baseline measurements. We address the following specific questions: 1) Are oral supplements well tolerated in patients with HIV-associated weight loss and subjective gastrointestinal symptoms? 2) Is voluntary food intake proportionately reduced when supplements are given, thereby canceling out any net beneficial effect of nutrients administered? 3) Does a peptide-based formula differ from a standard whole-protein–based formula with regard to clinical outcomes? 4) Is FFM as measured by bioelectrical impedance analysis (BIA) consistently repleted by oral supplementation and, if not, can FFM-responsive and unresponsive individuals be prospectively identified?

SUBJECTS AND METHODS

Study design

The study was designed as a clinical trial comparing a standard whole-casein-protein (WP)–based oral supplement (Ensure; Ross Laboratories, Columbus, OH) with an enzymatic digest of a soy, peptide-based (PEP) supplement (Advera; Ross Laboratories) in AIDS patients with weight loss. Data relating to energy intake, body composition, and nutritional status and their metabolic correlates are presented. Subjects (n = 49) were randomly assigned to receive either the WP or the PEP supplements. At the end of the 6-wk study period, 39 persons had completed the study and were evaluable, based on compliance with testing and supplement intake. Because we were unable to enroll HIV-infected patients in the San Francisco area to undergo metabolic ward studies if there were a possibility of their being randomly assigned into a “no-dietary-intervention study” arm (ie, a nonsupplemented study arm), a group (n = 13) of nonsupplemented subjects was enrolled independently of the supplemented group and was followed concur-
TABLE 2
Baseline clinical characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>WP group (n = 22 M, 0 F)</th>
<th>PEP group (n = 15 M, 2 F)</th>
<th>Unsupplemented group (n = 12 M, 1 F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>38 ± 12</td>
<td>40 ± 2</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>151.9 ± 10</td>
<td>168.5 ± 6.2</td>
<td>177 ± 2</td>
</tr>
<tr>
<td>Karnofsky score</td>
<td>80 ± 3</td>
<td>82 ± 4 [16]</td>
<td>78 ± 4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.3 ± 1.4</td>
<td>66.8 ± 2.2</td>
<td>64.7 ± 2.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5 ± 0.4</td>
<td>21.6 ± 0.6</td>
<td>20.6 ± 0.6</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>15</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>HIV-symptomatic</td>
<td>7</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Prior weight loss (kg)</td>
<td>−10.7 ± 0.9</td>
<td>−8.8 ± 0.8</td>
<td>−9.6 ± 2.7</td>
</tr>
<tr>
<td>(% usual body wt)</td>
<td>−13.7 ± 0.9</td>
<td>−11.4 ± 0.8</td>
<td>−12.6 ± 1.8</td>
</tr>
<tr>
<td>CD4+ T cell count (× 10⁹/L)</td>
<td>178 ± 49[20]</td>
<td>211 ± 53</td>
<td>124 ± 40</td>
</tr>
</tbody>
</table>

1There were no significant differences. WP: whole-protein–based formula; PEP: peptide-based formula. n in brackets.
2 ± SEM.
3 On the basis of CD4+ T cell classification or Centers for Disease Control and Prevention (Atlanta)-defined symptoms.

Patient population

Subjects were recruited by advertisement and by word-of-mouth. Entrance criteria were as follows: 1) documented HIV seropositivity or AIDS-defining diagnosis; 2) aged 18–60 y; 3) documented involuntary weight loss > 5% of usual body weight over the preceding 2 y; 4) not <70% of ideal body weight as determined by Metropolitan Life Insurance Tables (24); 5) subjective complaint of ≥ 2 loose stools/d or a change from usual bowel habits; 6) Karnofsky score > 65 (scale 0–100); 7) no history of metabolic disorders or other diseases unrelated to AIDS; 8) not taking medications known to have metabolic or nutritional effects (megestrol acetate, corticosteroids, cannabinoid derivatives, theophylline, lithium, and β-blockers); 9) no acute catabolic illness in the preceding 60 d; 10) on a stable medication regimen for > 1 mo; 11) prior testing for gastrointestinal pathogens by the patient’s personal physician, including stool cultures; 12) willingness to maintain a record of activity and diet; 13) no history of allergy to soy products, casein protein, or other dietary components of the formulas; 14) not pregnant; and 15) ability to give informed consent.

Clinical characteristics of the subjects at the time of entrance into the study are shown in Table 2. Twenty-six of 39 supplemented subjects had a diagnosis of AIDS at the time of enrollment (20 on the basis of prior opportunistic infection, 6 on the basis of < 200 CD4+ lymphocytes × 10⁹/L). Two subjects developed an AIDS-defining condition during the supplementation period (1 in each group). Five subjects in both the PEP and WP groups developed a new secondary opportunistic infection or wasting. Characteristics of the nonsupplemented group were not significantly different from either supplemented group (Table 2). Subjects gave written, informed consent and all procedures were approved by the University of California at San Francisco Committee on Human Research.

Clinical measures

Physical examination and Karnofsky scores were recorded by the same physician at each visit. Body composition was measured by BIA. Bioelectrical resistance and reaction were determined by using a current of 800 µA at 50 kHz with a Xitron 400 B instrument (Xitron Technologies Inc, San Diego). Regression equations for single-frequency measurements were used to calculate fat and FFM. The CV for repeated BIA measurements in weight-stable subjects with AIDS is 1.8% of FFM and 1.4% for fat (n = 23 subjects, repeated measurements within 10 d) in our laboratory (unpublished observations, 1995). Food intake was determined from 7-d weighed food records by using the NUTRITIONIST III computer program (N-Squared Computing, Salem, OR) for analysis of food content. The SD of 7-d weighed food records in our laboratory for AIDS patients is ± 4% or ≈420 kJ(100 kcal)/d (7). Resting energy expenditure was determined after an overnight fast and 20–30 min of quiet resting, by using indirect calorimetry (DeltaTrac Metabolic Cart; SensorMedics, Yorba Linda, CA). Serum lipids, complete blood counts, triiodothyronine, and 20–30 min of quiet resting, by using indirect calorimetry (DeltaTrac Metabolic Cart; SensorMedics, Yorba Linda, CA). Serum lipids, complete blood counts, triiodothyronine, and vitamin concentrations were determined by MetWest Laboratories (San Francisco). Daily energy requirements were calculated with the Harris-Benedict equation (25) by using an activity factor of 1.4. The daily protein requirement was calculated as 1.3 g·kg body wt⁻¹·d⁻¹. Soluble tumor necrosis factor receptors (sTNFRs) I and II were measured in the laboratory of Hans Sauerwein (University of Amsterdam) as described elsewhere (26). Insulin-like growth factor (IGF-I) and its binding protein 3 (IGF-I BP-3) were measured by the laboratory of Michelle Oster (Genentech, South San Francisco) as described previously (27).

Metabolic ward (stable-isotope) infusion studies

DNL was measured by a stable-isotope mass spectrometric method that we described in detail elsewhere (15, 28–31). In brief, subjects were admitted to the General Clinical Research Center of the San Francisco General Hospital for 36 h. Diet was ad libitum until 2200 of day 1, after which time food was withheld overnight. A constant intravenous infusion of sodium [1-¹³C]acetate (400 mg/h) in 0.9% saline was begun at 0200. The infusion was continued for 15 h, until 1700. Subjects fasted until 0900, after which ad libitum intake was allowed (breakfast at 0900, lunch at 1200, supper at 1700 on day 2). Blood samples were drawn before the infusion of [¹³C]acetate (baseline) and then at 0830, 0900, 1100, 1300, 1500, and 1700. Fed-state DNL values were used for correlations on the basis of previous studies showing fed-state values to be most affected by HIV infection (15) and most responsive to recombinant cytokine administration in animal models (32).

Isolation of metabolites and mass spectrometric analyses

VLDL was isolated from serum by ultracentrifugation and transmethylated to fatty acid methyl esters (FAMEs) as
TABLE 3
Intake of nutrient before and during supplementation

<table>
<thead>
<tr>
<th></th>
<th>WP group (n = 22)</th>
<th>PEP group (n = 17)</th>
<th>Unsupplemented (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 6 wk</td>
<td>Baseline 6 wk</td>
<td>Baseline 6 wk</td>
</tr>
<tr>
<td>Energy (% of needs/d)</td>
<td>104 ± 6 119 ± 6²</td>
<td>98 ± 8 105 ± 8²</td>
<td>100 ± 7 90 ± 7</td>
</tr>
<tr>
<td>Protein (% of needs/d)</td>
<td>114 ± 8 129 ± 6²</td>
<td>105 ± 10 122 ± 10²</td>
<td>115 ± 12 98 ± 14</td>
</tr>
<tr>
<td>Vitamins and trace elements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A (IU/d × 10⁻¹)</td>
<td>11.1 ± 2.3 9.2 ± 1.2</td>
<td>9.1 ± 1.8 7.9 ± 1.0</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>173 ± 18 215 ± 19</td>
<td>133 ± 18 277 ± 25²</td>
<td>-</td>
</tr>
<tr>
<td>Folate (g/d)</td>
<td>3.16 ± 33 453 ± 36²</td>
<td>276 ± 30 449 ± 21²</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B-12 (g/d)</td>
<td>8.2 ± 2 8.1 ± 1</td>
<td>11.8 ± 3 26.8 ± 2²</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B-6 (mg/d)</td>
<td>2.2 ± 0.2 3.0 ± 0.1</td>
<td>1.9 ± 0.2 3.1 ± 0.2²</td>
<td>-</td>
</tr>
<tr>
<td>Magnesium (mg/d)</td>
<td>319 ± 28 348 ± 18</td>
<td>240 ± 26 359 ± 24²</td>
<td>-</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>20 ± 1 24 ± 2</td>
<td>18 ± 2 25 ± 3</td>
<td>-</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>12 ± 1 17 ± 2²</td>
<td>11 ± 2 1.5 ± 1²</td>
<td>-</td>
</tr>
<tr>
<td>Copper (mg/d)</td>
<td>1.6 ± 0.1 1.6 ± 0.1</td>
<td>1.2 ± 0.2 2.1 ± 0.1²</td>
<td>-</td>
</tr>
<tr>
<td>Manganese (mg/d)</td>
<td>3.1 ± 0.4 3.7 ± 0.2</td>
<td>2.3 ± 0.2 5.0 ± 0.4²</td>
<td>-</td>
</tr>
</tbody>
</table>

¹± SEM, WP, whole-protein-based formula; PEP, peptide-based formula.
²Significantly different from baseline, P < 0.05 (matched paired t test).
³Significantly different from WP group, P < 0.05 (two-sample t test).

ORAL SUPPLEMENTS AND HIV-ASSOCIATED WASTING

described elsewhere (15, 30). FAMEs were analyzed by using an HP model 5970 gas chromatograph-mass spectrometer (Hewlett-Packard Co, Palo Alto, CA) with a 20-m fused silica isothermal column at 200°C. Ions at a mass-to-charge ratio (m/z) of 270–272, representing M₂ to M₄ of VLDL palmitate were quantified by selected ion monitoring. Enrichment of the precursor acetyl-CoA units entering VLDL palmitate was calculated by mass-isotopomer-distribution analysis (28–31). The pattern of fractional abundances of different mass isotopomers in a polymer is a function of the proportion of precursor subunits that are isotopically labeled, according to the binomial or multinomial expansion. For palmitate, the ratio of excess M₂ to M₄ enrichment reveals the isotopic enrichment of the acetyl-CoA precursor pool (29, 31). Fractional hepatic DNL is then calculated based on the precursor-product relation, by using equations described previously for the mass-isotopomer-distribution analysis technique (29, 31).

Statistical analyses
Changes from baseline measurements were evaluated for statistical significant with a matched paired t test. Differences in response between the 2 treatment groups were evaluated with a two-sample t test (Δ₂ - Δ₁). Mathematically, the two-sample t test comparing changes in each group is equivalent to a test for interaction between group and time by repeated-measures analysis of variance (ANOVA). If data were nonnormal, the Shapiro-Wilks test was performed on ranked data. Correlation coefficients were determined by least-squares linear regression analysis. STATVIEW (Abacus Concepts, Inc, Berkeley, CA) was used for the analyses.

RESULTS
There were no significant differences observed between the 2 supplemented groups for any outcome variables. Data for the 2 groups are therefore presented combined, except for nutrient intake data, which are presented separately. Energy intake was close to 100% of predicted needs at baseline in all groups (Table 3). At 6 wk of supplementation (Figure 1, A and B), total energy intake increased significantly in both supplemented groups by 10–15% (Table 3). Protein intakes showed a similar increase (Table 3). Nonsupplement food energy intake decreased significantly (Figure 1A) from baseline, by 819 kJ(196 kcal)/d and 5.6 g protein/d in the WP group and by 1834 kJ(432 kcal)/d and 14.4 g protein/d in the PEP group (P < 0.01 for both groups and both indexes), but not enough to compensate for the roughly 2.3–2.5 MJ(550–600 kcal)/d and 17–28 g protein provided in the supplement (Figure 1, A and B). In the nonsupplemented group, energy and protein intakes at follow-up were not significantly different from baseline but were significantly lower than in either supplemented group (P < 0.05). Intakes of other nutrients are shown in Table 3. Significant increases from baseline were observed for several vitamins and trace elements in the supplemented groups.

Resting energy expenditure
Baseline resting energy expenditure was 108 ± 3% of that predicted for the group as a whole, with no significant differences between supplemented and nonsupplemented groups. Resting energy expenditure remained > 100% of that predicted for all groups at 6 wk without any significant changes from baseline values.

Gastrointestinal symptoms
Although one of the entrance criteria was a perception by the subject of altered bowel habits or loose stools, the average number of bowel movements at baseline was within normal limits (2.8 ± 0.4 movements/d), and subjects had no objective evidence of malabsorption. The 2 oral supplements were well tolerated. Stool frequency (by history) decreased nonsignificantly in the WP group and nearly significantly (P = 0.06) in the PEP group. When data from the 2 supplemented groups were combined to assess the effects of oral supplementation per se, the reduction in stool frequency was significant, though modest (to 2.3 ± 0.3 movements/d, P = 0.01).

Functional and laboratory indexes
Karnofsky scores were not significantly altered during the supplementation period at 6 wk: 81 ± 3 to 84 ± 3 (n = 38 sup-
FIGURE 1. Effect of 6 wk of oral supplementation on mean (± SEM) energy and protein intakes from food and the liquid supplement. *Significantly different from baseline, $P<0.05$. **Significantly different from supplemented (ie, whole-protein–based and peptide-based formula groups combined), $P<0.05$. 
plemented subjects) compared with 78 ± 4 to 75 ± 3 (n = 13 unsupplemented subjects). CD4+ T cell counts did not change significantly.

Body weight and body composition

Body weight had not changed significantly at 6 wk (Table 4). Mean values for percentage body fat or FFM also did not change significantly in either group, although there was substantial interindividual variability (see below).

Relation between hepatic synthesis of fat and change in FFM at 6 wk of supplementation

There was a wide range in values for fed-state DNL among individuals studied (Figure 2A), as we observed previously in HIV-infected humans (15, 33). The group mean was significantly elevated compared with values in control subjects (Figure 2A). When baseline DNL was plotted against the change in FFM observed during 6 wk of supplementation (Figure 2B), a highly significant inverse correlation was observed (r = −0.65, P < 0.001). Moreover, when baseline values for fed-state DNL were divided into tertiles, there was a significant difference in FFM gain (Figure 2C) during supplementation. Those in the highest DNL tertile (DNL > 10.0%) lost an average of 1.2 kg FFM; those in the lowest DNL tertile (DNL < 4.6%) gained 0.9 kg FFM, and those in

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**TABLE 4**

Effects of oral supplementation on body composition

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Postsupplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>66 ± 7.5</td>
<td>65.9 ± 7.7</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>55.9 ± 4.7</td>
<td>56.2 ± 4.7</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>10.2 ± 5.7</td>
<td>9.7 ± 5.7</td>
</tr>
</tbody>
</table>

* x ± SD; n = 36. There were no significant differences from baseline. Data for both formula groups (whole-protein–based and peptide-based) were combined.

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**FIGURE 2.** A: Scatter diagram of de novo hepatic lipogenesis (DNL) measurements (fed-state) in healthy control subjects and in AIDS patients with wasting syndrome. DNL data in control subjects are from previously reported studies (7, 19), whereas data for the AIDS patients are from the present study. B: Correlation between baseline metabolic status (measured prospectively as hepatic DNL (see text) and change in fat-free mass (FFM) in response to 6 wk of oral supplementation. The correlation coefficient (r²) between basal DNL and change in FFM was −0.42 (P < 0.01). C: Baseline DNL divided into tertiles in relation to change in FFM. x ± SEM. Significantly different from other tertiles, P < 0.01.
the middle DNL tertile (between 4.6% and 9.8%) increased FFM by 0.3 kg; values for those in the highest DNL tertile were significantly different from values for those in the lowest tertile ($P < 0.05$).

**Relation between DNL and other measures of inflammation or dietary adequacy**

Measurement of sTNFR-I and -II in the circulation may be useful as an index of TNF secretory rate and inflammatory cytokine action in vivo (26). No correlation was observed between FFM response and either sTNFR-I or sTNFR-II and the inverse correlations between TNFRs and DNL were not significant (Figure 3). The baseline concentration of sTNFR-I ($n = 11$, $\bar{x} \pm SD$) was $1.5 \pm 1.0 \mu g/L$ (normal range: $0.3–2.9 \mu g/L$) and of sTNFR-II ($n = 11$) was $8.3 \pm 6.0 \mu g/L$ (normal range: $1.9–8.5 \mu g/L$). Another approach is to test whether indexes that reflect dietary protein-energy adequacy might predict the clinical FFM response to nutrient supplementation or correlate inversely with DNL. Serum IGF-I and IGF-I BP-3 concentrations reflect dietary adequacy and may respond to changes in nutrient intake (27). Serum IGF-I concentrations were normal (normal range: $105 \pm 40 \mu g/L$) in the subjects ($n = 13$; IGF-I $= 80 \pm 6 \mu g/L$). There was no significant correlation between either IGF-I or IGF-I BP-3 concentrations and DNL (fed-state; Figure 4) or clinical FFM response (data not shown). Thus, the biosynthetic measure appeared to be a more sensitive indicator of underlying metabolic milieu than were sTNFR-I or -II or IGF-I.

**DISCUSSION**

Several questions relating to the use of enteral supplements for the management of HIV-associated weight loss were addressed in this study. Whether the addition of an oral supplement results in a net benefit in FFM by 0.3 kg; values for those in the highest DNL tertile were significantly different from values for those in the lowest tertile ($P < 0.05$).

Relation between DNL and other measures of inflammation or dietary adequacy

Measurement of sTNFR-I and -II in the circulation may be useful as an index of TNF secretory rate and inflammatory cytokine action in vivo (26). No correlation was observed between FFM response and either sTNFR-I or sTNFR-II and the inverse correlations between TNFRs and DNL were not significant (Figure 3). The baseline concentration of sTNFR-I ($n = 11$, $\bar{x} \pm SD$) was $1.5 \pm 1.0 \mu g/L$ (normal range: $0.3–2.9 \mu g/L$) and of sTNFR-II ($n = 11$) was $8.3 \pm 6.0 \mu g/L$ (normal range: $1.9–8.5 \mu g/L$). Another approach is to test whether indexes that reflect dietary protein-energy adequacy might predict the clinical FFM response to nutrient supplementation or correlate inversely with DNL. Serum IGF-I and IGF-I BP-3 concentrations reflect dietary adequacy and may respond to changes in nutrient intake (27). Serum IGF-I concentrations were normal (normal range: $105 \pm 40 \mu g/L$) in the subjects ($n = 13$; IGF-I $= 80 \pm 6 \mu g/L$). There was no significant correlation between either IGF-I or IGF-I BP-3 concentrations and DNL (fed-state; Figure 4) or clinical FFM response (data not shown). Thus, the biosynthetic measure appeared to be a more sensitive indicator of underlying metabolic milieu than were sTNFR-I or -II or IGF-I.

**DISCUSSION**

Several questions relating to the use of enteral supplements for the management of HIV-associated weight loss were addressed in this study. Whether the addition of an oral supplement results in a net benefit in nutrient intake in free-living HIV-infected subjects had not been answered previously. Voluntary food intake might diminish in proportion to the energy and protein provided by the supplement, thereby canceling out the benefit of supplementation. In fact, however, energy and protein from foods did decrease significantly but this did not cancel out the net dietary benefit of the oral supplements (Figure 1). We believe that this is the first documentation in ambulatory AIDS patients of a net increase in dietary energy and protein intake by oral supplementation. Both formulas were well tolerated. Although these subjects had perceived alterations in bowel habits, they had normal numbers of bowel movements at baseline, with no objective evidence of altered gastrointestinal function. No significant differences were observed between the PEP-supplemented and WP-supplemented groups for any outcome measure.

The effects of oral nutrient supplementation on body composition were particularly interesting in the context of previous studies of nutritional therapies for HIV-associated weight loss (7–10, 15, 20). On the one hand, no significant change in FFM was observed for either supplemented group (Figure 2), although substantial interindividual variation was observed. The inability to increase FFM reliably with enteral supplementation is consistent with results of previous studies in which total parenteral nutrition (7) and megestrol acetate were used in AIDS patients (8–10, 12). Kotler et al (7) suggested that a subset of FFM responders to total parenteral nutrition exists, characterized clinically by gastrointestinal disorders resulting in poor food intake, whereas nonresponders were characterized by ongoing opportunistic infections. There was no way to prospectively identify which group a patient was in, however (7). Our study group differed from the group studied by Kotler et al (7) in that their subjects appeared to have had more active infectious complications. Nevertheless, our finding that measurement of a metabolic abnormality by mass spectrometry—namely, increased synthesis of fat (DNL) at baseline—prospectively identified individuals who were less likely to have an increase in FFM despite receiving the same extent of nutrient supplementation (Figure 2). B and C) supports the hypothesis (7, 8) that this subset of FFM nonresponders is biologically real and that an abnormal metabolic milieu may contribute to this lack of response. Differences in baseline DNL accounted for a high proportion of the variability in FFM response to nutrient supplementation (Figure 2B).

A possible criticism of this conclusion relates to the precision and accuracy of BIA as an index of FFM. Currently, there is no consensus regarding a gold standard for measuring FFM in humans, particularly in patients with underlying disease. The question of precision (reproducibility) and accuracy (in relation

**FIGURE 3.** Relation between measurements of soluble tumor necrosis factor receptor (sTNFR-I or sTNFR-II) and de novo lipogenesis (DNL). There was nonsignificant inverse correlation for both measures compared with DNL ($r^2 = 0.16$, and $r = –0.40$ for sTNFR-II ($P = 0.25$) and $r^2 = 0.31$ and $r = –0.56$ for sTNFR-I ($P = 0.075$).
to actual FFM) of BIA needs to be considered separately. We achieve within-subject reproducibility of 1.4–1.8% in weight-stable AIDS patients with a diagnosis of prior wasting when repeat measurements are performed within 7–10 d (see above). The changes in FFM observed here (Figure 2B) were therefore unlikely to be attributable to measurement error. The larger question of what BIA actually represents in terms of body compartments was not answered definitively here. Because the same uncertainties apply to dual-energy X-ray absorptiometry, total body potassium, and other available techniques (34), concurrent measurements with these techniques would not have provided a final resolution of the uncertainty, although they may have provided indirect support through correlation. The subjects studied here had no known disorders of water or electrolyte metabolism, had stable serum electrolyte values, and had no evidence of edema. Nevertheless, it is recognized that BIA measurements are unavoidably uncertain and may represent the weakest link in the hypothesis tested here.

Why might DNL be useful as predictive of nutrient responsivity? We and others have studied DNL in humans and experimental animals under a variety of conditions (15, 20, 28–30, 32, 33, 35–46). The main factors that have been shown to be associated with stimulated DNL to date (reviewed in references 28 and 46) as follows are: inflammation or large amounts of cytokines administered exogenously (15, 20, 32, 33, 35, 44), obesity with insulin resistance (37), short-term massive carbohydrate overfeeding (39, 40, 42), very-low-fat (10–15%) liquid diets (43), ingestion of fructose (36, 37) or ethanol (45), and the follicular menstrual phase in young women (38). Because the AIDS patients with wasting reported here were not obese and had low rather than high serum insulin concentrations (18; MK Hellerstein, A Strawford, unpublished observations, 1997), were wasted rather than overfed, did not receive fructose or ethanol during the infusion study, and were all men, most of these factors were excluded as the cause of increased DNL in the present study. Thus, increased DNL in HIV infection presumably reflects the actions of cytokines (32, 33, 35, 44) on the liver or the effect of unknown lipogenic stimulators. DNL may serve as a more sensitive index of subclinical cytokine presence in tissues in patients with wasting than is the circulating measure of cytokines, which are insensitive and have not given consistent results (44). The redirection of nutrients away from lean and into fat stores that is observed when such patients are provided nutritional support (8–13) may relate to the metabolic actions of cytokines (2).

FFM-unresponsive patients, if identifiable in advance, could be selected for the generally more expensive alternate anabolic agents (eg, recombinant growth hormone, androgens, and anti-cytokine agents) in addition to or in place of nutrient supplementation. Soluble TNF receptor concentrations in serum may reflect HIV disease progression (47) but did not in our study reflect underlying metabolic state at the time of supplementation, supporting the report of Godfried et al (26) that sTNFR-I and -II did not correlate with resting energy expenditure or serum triglyceride concentrations in HIV-infected men. We cannot exclude the possibility that soluble TNF receptors have a significant nonlinear (ie, logarithmic) relation to DNL, however, (Figure 3). These results suggest that prospective identification of the subgroup of AIDS patients with a wasting syndrome who are FFM unresponsive or fat-tissue hyperresponsive to nutrient provision (8–10) is possible, although it requires a kinetic measurement. In other clinical settings, kinetic measures have been reported that stratify metabolic response to dietary interventions: for example, the observation (48) that a reduced clearance rate of LDL is the best predictor of a hypolipidemic response to diets low in saturated fats. Although stable-isotope mass spectrometric kinetic techniques are not traditionally thought of as clinical tests, there is no reason in principle or practice why they could not be used for this purpose if costs were reasonable.

The lack of change in body weight for the group as a whole despite an average increase in energy intake of 6.3–8.4 MJ(1500–2000 kcal)/wk over 6 wk, is of interest. Resting energy expenditure did not change during supplementation but total energy expenditure was not measured. It was reported previously (49) that total energy expenditure is not elevated in patients with AIDS-associated wasting despite high resting energy expenditure, leading to the suggestion (50) that adaptive reductions in voluntary activity might be present in this population and serve to reduce

![Figure 4](ORAL_SUPPLEMENTS_AND_HIV-ASSOCIATED_WASTING_161.png)

**FIGURE 4.** Relation between insulin growth factor I (IGF-I) or IGF-I binding protein 3 (BP-3) concentrations and de novo lipogenesis (DNL). The correlation was not significant for either measure compared with DNL (IGF-I: \( r^2 = 0.001, P > 0.50 \); IGF-I BP-3: \( r = 0.16, P > 0.25 \).)
total energy needs. The increased energy intake in our subjects may therefore have resulted in greater activity-related expenditure (ie, increased exercise) rather than in increases in stored energy. This explanation remains speculative, however, in the absence of measurements of total energy expenditure or activity, but would have important functional implications if it were confirmed.

In summary, the questions that we raised initially can be answered as follows: oral supplements were well tolerated in the patients with HIV-associated weight loss studied; there were no significant differences in response to the PEP and WP formulas; and voluntary intake of food energy and protein decreased during supplementation, but the net effect was a significant (10–15%) increase in total energy and protein intakes. Nevertheless, FFM was not consistently repleted by oral supplementation. It was, however, possible to prospectively identify FFM nonresponders as compared with FFM responders (ie, subjects able to increase FFM with nutrient supplementation alone) by baseline measurement of DNL, supporting the hypothesis that the subset of FFM-unresponsive patients is characterized by an abnormal metabolic milieu.

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