The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/47491

Please be advised that this information was generated on 2020-02-27 and may be subject to change.
Interleukin-6 and Human Immunodeficiency Virus Load, But Not Plasma Leptin Concentration, Predict Anorexia and Wasting in Adults with Pulmonary Tuberculosis in Malawi

Monique van Lettow, Jos W. M. van der Meer, Clive E. West, Reinout van Crevel, and Richard D. Semba

Johns Hopkins University School of Medicine (M.v.L., R.D.S.), Baltimore, Maryland 21205; Radboud University Nijmegen Medical Centre (J.W.M.v.d.M., R.v.C.), 6500 HC, Nijmegen, The Netherlands; and Division of Human Nutrition and Epidemiology (C.E.W.), Wageningen Agricultural University, 6700 AH Wageningen, The Netherlands

Background: Wasting is a prominent feature of tuberculosis and may be more severe among individuals with HIV coinfection. It is likely that several biological mechanisms, including the anorexia of infection, are contributing to wasting.

Objective: The purpose of this study was to determine whether leptin concentrations, in relation to the inflammatory cytokine response and level of HIV infection, are contributing to loss of appetite and wasting in adults with pulmonary tuberculosis and HIV infection.

Design: We characterized plasma leptin concentrations in relationship with self-reported loss of appetite, body mass index, fat mass (FM), IL-6, and HIV load in a cross-sectional study of 500 adults who presented with pulmonary tuberculosis in Zomba, Malawi.

Results: Plasma leptin concentrations, associated with FM, significantly decreased by increasing tertile of plasma HIV load (P = 0.0001). Leptin concentrations were inversely associated with plasma IL-6 concentrations after adjusting for sex, age, FM, and HIV load. Plasma leptin concentrations were associated with neither loss of appetite nor wasting. Inflammation, reflected by increased IL-6 concentrations, was associated with loss of appetite (odds ratio, 3.41; 95% confidence interval, 1.91–6.09), when adjusted for sex, age, FM, leptin concentrations, and HIV load. A high plasma HIV load was associated with severe wasting, defined as body mass index less than 16.0 kg/m² (odds ratio, 2.14; 95% confidence interval, 1.09–4.19) when adjusted for sex, age, IL-6, FM, and leptin concentrations.

Conclusion: This study suggests that the anorexia and wasting seem primarily determined by the level of inflammation and the level of HIV infection in patients with tuberculosis and HIV coinfection.

First Published Online May 31, 2005

Abbreviations: BIA, Bioelectrical impedance analysis; BMI, body mass index; CI, confidence interval; FM, fat mass; OR, odds ratio.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.
in HIV-infected and uninfected adults with pulmonary tuberculosis in relation to nutritional status, level of inflammation, and level of HIV infection. Moreover, we aimed to determine whether leptin concentrations, in relation to inflammation and level of HIV infection, are contributing to loss of appetite and wasting.

To address these objectives, we characterized plasma leptin concentrations in relationship with self-reported loss of appetite, body mass index (BMI), FM derived from bioelectrical impedance analysis (BIA), plasma IL-6, and plasma HIV load in a cross-sectional study of 500 adults who presented with pulmonary tuberculosis in Zomba, Malawi.

**Subjects and Methods**

The study population consisted of 500 adults who presented with new sputum-positive pulmonary tuberculosis in Zomba Central Hospital between July 1999 and September 2001. This cross-sectional sample was drawn from a micronutrient supplementation study. Subjects were offered HIV testing and screened for HIV antibodies after written informed consent. All subjects were given appropriate pre- and posttest HIV counseling. Subjects received standard short course chemotherapy for tuberculosis as per guidelines of the Malawi National Tuberculosis Program (13). Adults with a previous history of treated pulmonary tuberculosis were excluded. Three sputum samples from each subject were examined with Auramine-O dark fluorescent staining method. Sputum-positive pulmonary tuberculosis was considered proven when at least one of three sputum stains showed acid-fast bacilli. HIV infection was diagnosed on the basis of a positive rapid test (Determine 1/2 Rapid test; Abbott Laboratories, Johannesburg, South Africa) and confirmed by a positive ELISA for HIV-1 antibodies (Wellcozyme; Wellcome Diagnostics, Dartford, Kent, UK).

Body weight was determined to the nearest 0.1 kg using an adult balance (Seca 700 balance, Seca Corp., Hanover, MD), and standing height was determined to the nearest centimeter. Wasting was defined as BMI (weight/height²) less than 16.0 kg/m², in accord with World Health Organization strata for BMI grading for severity of malnutrition (14).

Single-frequency BIA was performed at 50 kHz and 800 μA (RIL Systems, Inc., Detroit, MI) with standard tetrapolar lead placement. BIA measurements were performed in triplicate for each subject. The reproducibility of repeated BIA measurements was greater than 99%. To calculate FM and body cell mass, equations that were validated in a sample of adults with and without HIV infection were used (15, 16). A standard questionnaire, with closed questions, was used to determine loss of weight and loss of appetite. Loss of appetite was considered positive when the subject gave loss of appetite as a reason for the observed loss of weight in the last month.

Blood samples were obtained by venipuncture (Sarstedt Monovette, Newton, NC) at initial diagnosis of tuberculosis. Subjects were not asked about prior food intake. Aliquots of plasma were made in trace element-free cryovials, and samples were stored in liquid nitrogen. Plasma samples were kept in liquid nitrogen or at −70°C until the time of laboratory analyses. Plasma HIV load was measured using quantitative HIV-1 RNA PCR (Amplicon monitor, version 1.5; Roche, Branchburg, NJ) with a sensitivity limit of 400 HIV RNA copies per milliliter.

Plasma leptin concentrations were measured by ELISA using human leptin quantikine colorimetric sandwich ELISA kit (R&D Systems, Inc., Minneapolis, MN). Plasma IL-6 concentrations were measured by ELISA (human IL-6; R&D Systems). Quality control was assessed by repeated analysis of pooled human plasma controls run at the beginning and end of each analysis. Standard curves were run periodically using standard reference material 986C (National Institute of Standards and Technology, Gaithersburg, MD). Throughout all analyses, the plasma samples were run in a masked fashion. Due to the unavailability of some sample aliquots, plasma IL-6 and HIV load could not be measured in 1 and 16 samples, respectively.

Comparisons of categorical data were made using χ² tests. Comparisons between continuous variables were made using t tests. Appropriate variable transformations were made to reduce the skewness of the data, such as log₁₀ transformation for leptin, IL-6, and HIV load. Univariate ANOVA was used to test for linear trends of plasma leptin concentrations across categories of plasma HIV load. Linear regression models were used to explore the relationships between plasma leptin concentrations and FM, IL-6, and HIV load. Univariate and multivariate logistic regression models were used to evaluate determinants of self-reported loss of appetite and associations with severe wasting.

A significance level of P < 0.05 was used in this study. Statistical analyses were conducted using software packages SAS 8.01 (SAS Institute, Cary, NC) and SPSS 9.0 (SPSS, Inc., Chicago, IL). The protocol was approved by the institutional review boards of the Johns Hopkins School of Medicine (Baltimore, MD) and the College of Medicine, University of Malawi (Blantyre, Malawi), with final approval by the Office for Protection from Research Risk of the National Institutes of Health (Bethesda, MD).

**Results**

The study population consisted of 370 HIV-positive and 130 HIV-negative adults with sputum-positive pulmonary tuberculosis. Of all participants, 69% (156 of 227) of men and 78% (214 of 273) of women were HIV positive. Table 1 shows characteristics of study participants by sex and HIV status, such as age, body composition, IL-6, HIV load, and plasma leptin concentrations.

When comparing men and women (data not shown), men were older and had lower FM (P = 0.0001), log₁₀ leptin concentrations (P = 0.0001) and higher body cell mass (P = 0.0001).

**TABLE 1. Characteristics of adults with and without HIV infection presenting with pulmonary tuberculosis in Zomba, Malawi**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV negative (n = 71)</td>
<td>HIV positive (n = 156)</td>
<td>P</td>
<td>HIV negative (n = 59)</td>
<td>HIV positive (n = 214)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>33.1 ± 11.6</td>
<td>36.0 ± 7.9</td>
<td>0.03</td>
<td>30.5 ± 11.2</td>
<td>32.2 ± 8.6</td>
</tr>
<tr>
<td>Loss of appetite (%) reported</td>
<td>22.5</td>
<td>39.7</td>
<td>0.01</td>
<td>28.8</td>
<td>41.1</td>
</tr>
<tr>
<td>Loss of weight (%) reported</td>
<td>74.6</td>
<td>81.4</td>
<td>0.30</td>
<td>81.4</td>
<td>86.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.7 ± 3.1</td>
<td>18.5 ± 2.6</td>
<td>0.58</td>
<td>18.6 ± 2.7</td>
<td>18.3 ± 2.8</td>
</tr>
<tr>
<td>Wasting, BMI &lt;18.5 (%)</td>
<td>54.9</td>
<td>54.5</td>
<td>0.95</td>
<td>54.2</td>
<td>58.4</td>
</tr>
<tr>
<td>Severe wasting, BMI &lt;16.0 (%)</td>
<td>14.7</td>
<td>21.0</td>
<td>0.84</td>
<td>19.9</td>
<td>19.2</td>
</tr>
<tr>
<td>Body cell mass (% of weight)</td>
<td>41.0 ± 5.8</td>
<td>39.4 ± 3.1</td>
<td>0.001</td>
<td>33.6 ± 3.2</td>
<td>33.2 ± 3.2</td>
</tr>
<tr>
<td>FM (% of weight)</td>
<td>7.2 ± 4.8</td>
<td>7.4 ± 5.2</td>
<td>0.81</td>
<td>1.36 ± 0.5</td>
<td>1.33 ± 0.5</td>
</tr>
<tr>
<td>Log₁₀ IL-6 (pg/ml)</td>
<td>1.28 ± 0.6</td>
<td>1.32 ± 0.5</td>
<td>0.54</td>
<td>1.36 ± 0.5</td>
<td>1.33 ± 0.5</td>
</tr>
<tr>
<td>Log₁₀ HIV load</td>
<td>6.52 ± 0.6</td>
<td>5.32 ± 0.5</td>
<td>0.54</td>
<td>1.36 ± 0.5</td>
<td>1.33 ± 0.5</td>
</tr>
<tr>
<td>Leptin, actual range (pg/ml)</td>
<td>2.45 ± 0.5</td>
<td>2.33 ± 0.6</td>
<td>0.17</td>
<td>3.12 ± 0.6</td>
<td>3.02 ± 0.5</td>
</tr>
</tbody>
</table>

a Mean ± SD for continuous variables.

b P values assessed by t tests for continuous variables, χ² tests for categorical data.
with log10 IL-6 concentrations (\(\text{correlated with log10 leptin concentrations. In multivariate linear regression that adjusted for sex, age, and all other variables in the model, log10 leptin concentrations remained independently associated with FM (\(P = 0.0001\)) and inversely with log10 IL-6 concentrations (\(P = 0.02\)). The adjusted regression coefficient \(J\) for a linear association with log10 leptin concentrations was 4.18 (95% CI, 3.57–4.80) for FM and -0.10 (95% CI, -0.18 to -0.01), for log10 IL-6 concentrations. The association between log10 HIV load and log10 leptin concentrations did not reach significance in multivariate analysis (\(P = 0.06\)).

Figure 1 shows plasma leptin concentration increases proportionally with percentage of body fat. In univariate analysis, the fitted regression lines for men and women were log10 leptin = 1.86 + 0.07 + FM and log10 leptin = 2.26 + 0.04 + FM, respectively, with a difference in the slope of the regression lines between log10 plasma leptin and FM among men and women (\(P = 0.001\)).

### Determinants of appetite and wasting

The relationships between self-reported loss of appetite and plasma leptin concentrations, IL-6, and HIV load are shown in Table 3. To determine which factors contribute to loss of appetite, we examined the relationships in multivariate logistic regression models that adjusted for sex, age, FM, and all other variables in the model. This table shows that plasma leptin concentrations were not associated with loss of appetite. In a univariate logistic regression analysis, log10 IL-6 (\(P = 0.0001\)) and log10 HIV load (\(P = 0.03\)) were associated with loss of appetite. In multivariate logistic regression analysis, only higher log10 IL-6 (\(P = 0.0001\)) remained associated with loss of appetite. The adjusted odds ratio (OR) was higher for higher log10 IL-6 concentrations.

### TABLE 2. Factors associated with plasma leptin concentrations in adults with pulmonary tuberculosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient, (\beta) univariate(^a)</th>
<th>95% CI</th>
<th>(P)</th>
<th>Regression coefficient, (\beta) multivariate(^a)</th>
<th>95% CI</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM (% of weight)</td>
<td>5.00</td>
<td>4.57 to 5.43</td>
<td>0.0001</td>
<td>4.18</td>
<td>3.57 to 4.80</td>
<td>0.0001</td>
</tr>
<tr>
<td>Log(_{10}) IL-6 (pg/ml)</td>
<td>-0.34</td>
<td>-0.45 to -0.23</td>
<td>0.0001</td>
<td>-0.10</td>
<td>-0.18 to -0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Log(_{10}) HIV load</td>
<td>-0.28</td>
<td>-0.39 to 0.17</td>
<td>0.0001</td>
<td>-0.08</td>
<td>-0.17 to 0.01</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\(^a\) Univariate and multivariate linear regression; adjusted for sex, age, and all other variables in the model.
for an independent association of log10 IL-6 with loss of appetite was 3.41 (95% CI, 1.91–6.09).

The relationships between severe wasting and plasma leptin concentrations, IL-6, and HIV load are shown in Table 4. To determine which factors contribute to severe wasting, we examined the relationships in multivariate logistic regression models with severe wasting, defined as BMI less than 16 kg/m², as the outcome variable. In univariate analyses, log10 leptin (P = 0.0001), log10 IL-6 (P = 0.0001), and log10 HIV load (P = 0.0006) were associated with severe wasting. In multivariate analysis that adjusted for sex, age, the interaction between leptin and FM, and all other variables in the model, only higher HIV load (P = 0.03) remained associated with severe wasting. The adjusted OR for an independent association of log10 HIV load with severe wasting was 2.14 (95% CI, 1.09–4.19).

Discussion

This study demonstrates that plasma leptin concentrations are associated with FM and the inflammatory cytokine (IL-6) response. Leptin reflected the percentage of FM and decreased with the increase of HIV load. Inflammation, characterized by IL-6 concentrations, was associated with loss of appetite, and level of HIV-replication, characterized by plasma HIV load, was associated with severe wasting. This study suggests that leptin does not seem to account for the anorexia and weight loss in tuberculosis. The anorexia and wasting in patients with tuberculosis and HIV coinfection seem primarily determined by the level of inflammation and the level of HIV infection.

The role of leptin in tuberculosis has been limited to adults without tuberculosis and HIV coinfection (10–12, 17). In 30 patients with tuberculosis in Turkey, higher leptin concentrations were described among those with active tuberculosis, compared with controls (10). Similar results were reported in another study from Turkey involving 25 patients with tuberculosis (11). However, these differences were significant only among women but not among men in the first study (10) and only among men but not among women in the second study (11) from Turkey.

One study from Indonesia found lower leptin concentrations in 60 HIV-negative patients with active tuberculosis, compared with 30 healthy controls (12). And in effect, leptin concentrations in the untreated tuberculosis patients from Indonesia were similarly low as those in our study. In the present study, plasma leptin concentrations were not different between HIV-negative subjects and HIV-positive subjects in the lowest tertile of HIV load. However, plasma leptin concentrations significantly decreased by increasing tertile of plasma HIV load. The lower leptin concentrations in tuberculosis patients with high plasma HIV load may simply be attributed to further deprived nutritional status because body fat is the most important determinant of plasma leptin concentrations. This is consistent with the observation that the nutritional status in patients with pulmonary tuberculosis in Malawi significantly decreases by increasing HIV load (18).

We demonstrated that leptin concentrations reflected the percentage of FM, but although leptin concentrations were higher among female than among male patients, the regres-

TABLE 3. Factors associated with self-reported loss of appetite in adults with pulmonary tuberculosis

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Univariate OR (95% CI)</th>
<th>P</th>
<th>Multivariate OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log10 leptin (pg/ml)</td>
<td>0.82 (0.62–1.09)</td>
<td>0.17</td>
<td>1.06 (0.65–1.72)</td>
<td>0.81</td>
</tr>
<tr>
<td>Log10 IL-6</td>
<td>2.60 (1.73–3.91)</td>
<td>0.0001</td>
<td>3.41 (1.91–6.09)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Log10 HIV load</td>
<td>1.52 (1.04–2.33)</td>
<td>0.03</td>
<td>1.38 (0.90–2.12)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

a Univariate and multivariate logistic analysis; adjusted for sex, age, FM, and all other variables in the model.
sion lines for the two genders intersect, with a steeper slope for men than women. Similar data in human populations and experimental animals generally show regression lines that remain parallel across a wide range of body fat. This is to our knowledge the first study that demonstrates this unusual feature, which may be specific for chronic disease, but further research is needed to gain insight into this characteristic.

The study from Indonesia reported a negative association between C-reactive protein and leptin, whereas our study demonstrates a negative association between the inflammatory cytokine (IL-6) response and leptin concentrations in adults with pulmonary tuberculosis (with and without HIV infection). Experimental and animal studies have shown that inflammatory mediators are able to increase leptin production leptin (4, 10, 19, 20). However, the acute inflammatory response is different from the more chronic inflammatory response in tuberculosis. The results from this study support the hypothesis of van Crevel et al. (12) that the prolonged inflammatory response in tuberculosis may deplete or exhaust leptin production.

Theoretically, decreased leptin concentrations could impair protective cellular immunity to Mycobacterium tuberculosis (12) because leptin is necessary for an effective cell-mediated immune response (21). For instance, CD4 T lymphocyte activities are suboptimal in the absence of leptin (7, 21). The present study suggests that the inflammatory response may deplete leptin production, which theoretically suppresses immunity. Low leptin levels, as a result of poor nutritional status and chronic inflammatory response, may be a contributing factor in suppressing the immune function and worsening the outcome of tuberculosis. Further studies are needed to clarify whether this hypothesis is true.

Conversely, in theory, low plasma leptin concentrations should increase appetite and decrease energy expenditure. However, the present study shows that leptin was not associated with loss of appetite, after adjusting for other factors, and this finding reinforces the conclusion of Schwenk et al. (17) that leptin does not account for the weight loss and anorexia in tuberculosis.

We demonstrated that leptin concentrations were not, but IL-6 was, associated with loss of appetite. Experimental studies showed that parenteral administration of cytokines (including IL-6) reduce food intake, suggesting a role in the anorexia during infection (4, 5). Earlier studies also suggested a role of cytokines (including IL-6) in the development of wasting in the pre-era of highly active antiretroviral therapy (22, 23). Further research is needed into the role of cytokines in the physiological control of eating and energy balance during acute and chronic infection.

The present study suggests a correlation between plasma leptin concentrations and HIV load, for which two hypothetical mechanisms can be drawn. First, HIV infection per se (through nonleptin- and non-IL-6-mediated mechanism) results in wasting and loss of body fat. Furthermore, decreased leptin concentrations as a result of loss of body fat occur mainly with higher concentrations of HIV load (perhaps suggesting that these changes happen only with more severe or longer duration of HIV infection, which may be of relevance regarding the pathophysiology lipodystrophy syndrome in HIV-infected patients on HAART). Second, increased IL-6 concentrations (either directly or through other mechanisms of chronic inflammation) results in blunting of appetite, which aggravates weight loss, fat loss, and subsequently a decrease in leptin production.

A limitation of this study is that blood samples were not uniformly collected in a fasted state, which would have increased the confidence of our conclusions. This study suggests that leptin does not play a role in the anorexia and wasting in tuberculosis but that it may play a role as a mediator between nutritional status and host defense, which could explain thin people’s susceptibility to tuberculosis or the link between malnutrition and disease outcome. In addition, IL-6 production was associated with loss of appetite. Current knowledge (4) on the different mechanisms involved in the anorexia of infection suggests some therapeutic options for treatment, including substances that antagonize cytokine action in combination with nutritional support.

Acknowledgments

We thank Dana Totin Moncrief, Barbara Dancheck, Amanda Ray, and Michelle Ricks for their contributions to laboratory and data analyses.

Received December 27, 2004. Accepted May 20, 2005.

Address all correspondence to: Monique van Lettow, M.P.H., 550 North Broadway, Suite 700, Baltimore, Maryland 21205. E-mail: mv lettow@mailbox.com.

Reprints will not be available from the author.

This work was supported in part by the National Institutes of Health (Grant AI41956); the Fogarty International Center, the Wageningen Agricultural University, and the Radboud University Nijmegen Medical Centre.

References

6. van Gaal LF, Wauters MA, Mertens IL, Considine RV, Leeuw de IH 1999
18. van Lettow M, Harries AD, Kumwenda JJ, Ziljstra EE, Clark TD, Taha TE, Semba RD 2004 Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. BMC Infect Dis 4:61

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.