RESEARCH ARTICLE

Effective treatment of pulmonary tuberculosis restores plasma leptin levels

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To cite this article: Perna V, Pérez-Pérez A, Fernández-Riejos P, Polo-Padillo J, Batista N, Domínguez-Castellano A, Sánchez-Margalet V. Effective treatment of pulmonary tuberculosis restores plasma leptin levels. *Eur. Cytokine Netw.* 2013; 24(4): 157-61 doi:10.1684/ecn.2014.0346

ABSTRACT. An impaired immune response in tuberculosis patients seems to be related to weight loss that coexists with an immunoendocrine imbalance. Thus, wasting is well-recognised as a prominent feature of tuberculosis (TB), which may not be reversed even after six months of treatment. Adipokines may play a role in the immune response to *M. tuberculosis*, and TB may impair the expression of inflammatory adipokines, such as leptin. We aimed to study patients with pulmonary TB before and six months after treatment, by measuring plasma leptin, soluble leptin receptor and adiponectin, weight and body mass index. Nineteen patients with a diagnosis of pulmonary TB were included in the study. Blood samples were obtained before and six months after treatment, to measure plasma adipokine levels. We found an increase in plasma leptin levels after treatment ($p<0.05$). Even though BMI also increased, the extent was not enough to account for the changes in the leptin levels. On the other hand, plasma soluble leptin receptor and adiponectin levels did not change significantly after treatment.In conclusion, these results suggest that active TB infection may affect the expression of leptin, in addition to the wasting that may occur in these patients, and that effective TB treatment increases circulating leptin levels, probably restoring normal immunological competence.

Key words: leptin, adiponectin, tuberculosis

Tuberculosis (TB) remains the world's second leading cause of death by an infectious disease after the human immunodeficiency virus (HIV), with over 1 million deaths among HIV-negative subjects and 0.35 million deaths among HIV/TB co-infected patients [1]. In 2011, there were 8.7 million new cases of active TB worldwide (13% of which involved co-infection with HIV). Low income countries, and deprived areas within big cities in developed countries, present the highest TB incidences and TB mortality rates. These are settings where immigration, HIV infection and drug or alcohol abuse may coexist, and all of these factors are strongly associated with TB [2].

After close contact with an infected person, 30-50% of exposed, susceptible contacts acquire latent TB infection, and in the majority of cases, the initial infection remains clinically silent and microbiologically latent. Approximately 10% of the infected individuals will progress to active TB during their lifetime, due to reasons that are not completely understood [3]. Factors affecting host immunity such as malnutrition, alcoholism, advanced age, diabetes and immunosuppressive drug treatments, among others, may be related to this phenomenon [4, 5]. Pulmonary disease is the most common form of post-primary TB [1]. Symptoms such as, cough, expectoration, haemoptysis, fever, night sweats, anorexia and loss of body weight are frequently observed [6].

Proinflammatory cytokines are the prime candidates as the causative agents of the metabolic changes that eventually result in TB-associated weight loss [7]. At the same time, impaired immune responses in TB patients seem to be related to the weight loss that coexists with an immunoendocrine imbalance. Adipose tissue-derived hormones/cytokines, designated as adipokines, have been investigated extensively and proposed as markers of obesity and diabetes, especially leptin and adiponectin [8, 9]. Moreover, it is known that these adipokines represent a critical link between nutritional status, metabolism and immunity [10, 11]. Thus, according to its primary amino acid sequence that shows structural similarities to the long chain helical cytokine family, leptin has been classified as a pro-inflammatory cytokine [2, 6, 12], the leptin receptor (Ob-R) showing sequence homology to members of the class I cytokine receptor (gp130) superfamily [13, 14]. Consequently, a role for leptin in regulating immunity, inflammation and haematopoiesis has been proposed [15, 16]. In this regard, the role of leptin and leptin receptor in the activation of immune cells is now well established [17-20]. Leptin levels have been found to be suppressed in tuberculous patients and low leptin levels

doi: 10.1684/ecn.2014.0346

may contribute to increased susceptibility to infection, and recovery with sequelae lesions [21]. Plasma soluble leptin receptors (sOb-R) have not been previously measured in TB patients, but changes in sOb-R levels may change the availability of leptin to target tissues, and may suppress leptin action through inhibition of specific leptin-binding to membrane-bound receptors [22]. In addition, while increased sOb-R concentrations seem to block leptin action directly, reduced amounts of sOb-R may reflect decreased membrane expression of Ob-R as has been recently suggested [23].

Regarding circulating adiponectin levels, generally positive metabolic effects have been attributed to this adipokine [24], and it appears that an inverse relationship exists between adiponectin and inflammatory cytokines [25, 26]. Previous studies have found negative correlations for adiponectin levels and body mass index in TB, with increased adiponectin and decreased leptin levels, suggesting that the leptin-adiponectin ratio might be useful as a marker for the severity of the disease [27]. Nevertheless, even though adiponectin levels tended to be increased in the active TB group, it did not reach significant levels after considering multiple comparisons. On the other hand, other groups have found no changes in circulating adiponectin levels in TB [28].

In the present work, we aimed to study plasma leptin, sOb-R, adiponectin levels, and the leptin-ObR ratio in TB patients, before and after TB treatment. We hypothesised that plasma leptin and sObR, as well as adiponectin levels, would increase after treatment.

PATIENTS & METHODS

Study population

Nineteen patients, who had been diagnosed with active pulmonary TB and who had been attending the Infectious Diseases Medical Department of the Virgen Macarena University Hospital for one year, were included in this study. The study was approved by the local ethics committee (Comité Local de Etica e Investigación del Hospital Universitario Virgen Macarena), and informed written consent was obtained from all subjects. The investigations reported herein were carried out in accordance with the principles of the Declaration of Helsinki as revised in 2008. The diagnosis of active pulmonary TB was made clinically and radiologically, and was confirmed bacteriologically. Anti-TB treatment was established following the standard regimen, i.e. two months of ethambutol, isoniazid, rifampicin and pyrazinamide, followed by four months of isoniazid and rifampicin.

Plasma collection and cytokine assays

Blood samples were drawn from the forearm between 8:30 and 10:00 AM after a 12-hour, overnight fasting period. Two blood samples were obtained from each patient, before treatment and after completing treatment. Plasma was obtained from EDTA-treated blood. Samples were centrifuged at 3,500 rpm for 30 min at 4°C, and the plasma was stored at -80°C until the day of the analysis. Leptin (Quantikine, R&D Systems, detection limit 7.8 pg/mL), adiponectin (Quantikine, R&D Systems, detection limit 3.9 ng/mL), and sObR (Quantikine, R&D Systems, detection limit 0.128 ng/ml) plasma concentrations were determined using commercially available ELISA kits according to the manufacturers' instructions.

Statistical analysis

Differences between plasma levels before and after treatment were assessed by the paired-T test. Values were corrected using the weight gain for each patient. P values <0.05 were considered statistically significant. Statistical analysis was performed with SPSS v 15.0.

RESULTS

Characteristics of the study population

The demographic characteristics of the patients are shown in *table 1*. Weight and BMI were low in all of the patients but one. After treatment, BMI increased in all patients. The differences in the mean weight values was statistically significant ($p<0.05$), even though there was only a slight increase (10%) in BMI after six months of treatment.

Leptin, soluble ObR, and adiponectin levels in patients with active TB before and after anti-TB treatment

Figure 1 shows plasma values for leptin for each patient, before and after anti-TB treatment. There was an overall increase in leptin values after the treatment and this difference was statistically significant ($p<0.05$). Taken individually, plasma leptin increased in 16 out of 19 patients. The three patients that had lower leptin levels after treatment suffered from alcoholism.

Figure 2 shows plasma sOb-R levels in patients, before and after treatment. Mean plasma sObR levels seemed higher after treatment, but no significant differences were found between the two groups. We then calculated the leptin/sObR ratios: no significant differences were found between the two mean values, before and after anti-TB treatment.

Figure 3 shows plasma adiponectin levels in TB patients, before and after treatment. There were no significant differences in plasma adiponectin levels between the two groups (before and after treatment).

Table 1
TB patient characteristics

Age at presentation (years)	
Mean ± SD	44.5 ± 13.7
(Range)	(22-72)
Sex	
Female (%)	M - (89%)
Male (%)	F - (11%)
BMI	
Before treatment	19.5 ± 2.1
After treatment	21.8 ± 2.3
Co-morbidities	
Alcoholism	12 (63%)
Smoking	11 (58%)
Hepatitis C	3 (16%)
HIV	1 (5%)

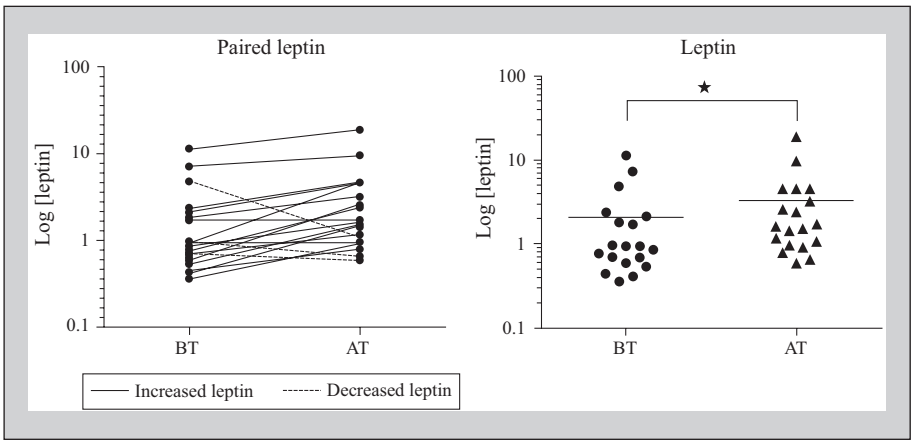

Figure 1

Plasma leptin levels in patients with active TB, before treatment (BT) and after treatment (AT). ★ $p<0.05$. The values are represented as the log of the values in ng/mL.

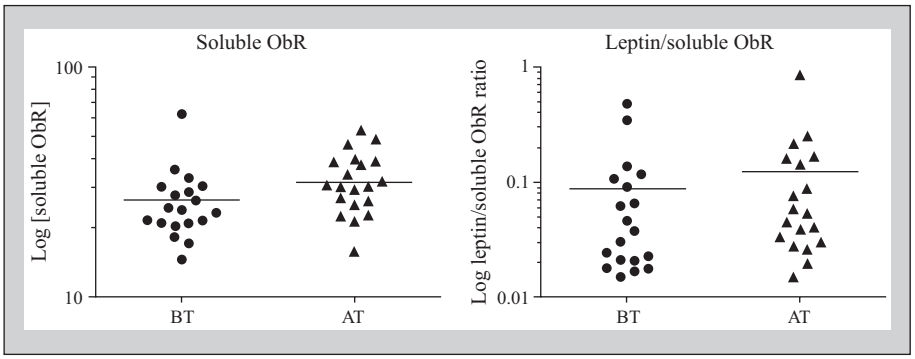

Figure 2

ObR plasma levels and the leptin/sObR ratio in patients with active TB, before treatment (BT) and after treatment (AT), represented as the log of the values in ng/ml (sObR).

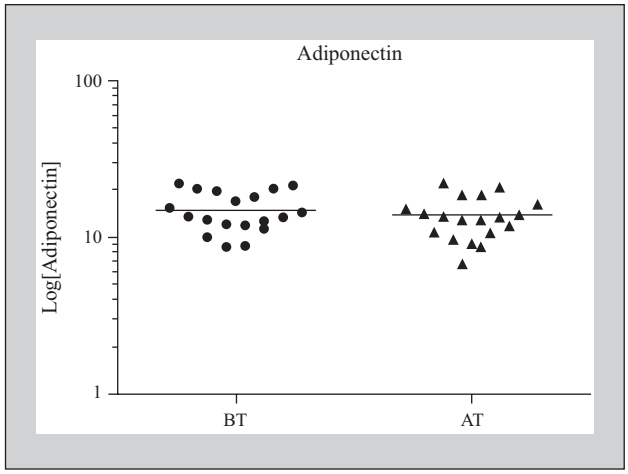

Figure 3

Adiponectin plasma levels in patients with active TB, before treatment (BT) and after treatment (AT), represented as the log of the values in μg/ml.

DISCUSSION

It is generally believed that malnourishment diminishes protective immunity against *M. tuberculosis* [6]. Moreover, it has been shown that the immunoendocrine imbalance in TB patients is associated with weight loss, which, in turn, correlates with the impairment of their specific, *in vitro*, cellular immune responses [4] Adipose tissue contributes to energy homeostasis by secreting cytokines termed adipokines. Leptin suppresses appetite and stimulates energy expenditure, while adiponectin increases insulin sensitivity. Additionally, adiponectin has an atheroprotective role, but in some chronic inflammatory/autoimmune diseases adiponectin may have pro-inflammatory effects. Leptin is a pro-inflammatory adipokine that activates T cells, inducing a Th1 phenotype [17, 19, 29], and helping the progression of autoimmune responses. Conversely, it has been shown that conditions of reduced leptin production are associated with increased susceptibility to infection [9]. Moreover, it has been shown that leptin-deficient mice are more susceptible to *M. tuberculosis* than wild-type mice, suggesting that leptin contributes to protection against TB [30]. In this regard, low plasma leptin levels have been previously reported in TB patients [31]. The leptin/BMI ratio was similarly reduced in TB patients [31], and therefore the immune response might have been further affected. On the other hand, other authors have found no differences in leptin levels in pulmonary TB [32], suggesting that leptin may not account for wasting in these patients. However, conversely, leptin has been suggested to be a component of a Th1 pattern of cytokines that is required for protective immunity against *M. tuberculosis* [33]. Therefore, lower leptin levels may contribute to the progression of pulmonary TB. It is for this reason that we wanted to check the possible changes in leptin levels

following TB treatment. We found a consistent increase in plasma leptin levels in TB patients following treatment, and therefore there was a significant increase in mean values for plasma leptin, even though there was only a slight increase in BMI (10%). These results suggest that TB infection might compromise the normal expression of leptin, which could contribute to the progression of the disease, and the treatment of these patients may therefore lead to the increase in plasma leptin levels. On the other hand, three patients were found to have decreased leptin levels after treatment. We have no explanation for this difference, although we may speculate that alcohol consumption might account for this discrepancy, since it has been previously reported that alcohol intake may affect leptin levels [34], and these three patients were alcoholics. Unfortunately, even though the recommendation of no alcohol consumption was included in the treatment, we have no objective assessment of the actual alcohol consumption by these three patients.

Plasma soluble leptin receptors have not been previously determined in TB patients, and Ob-R expression is known to be induced by leptin [35, 36]. Little is known about the regulation of sOb-R expression, but a metalloprotease seems to be involved [37]. Interventional studies in humans have shown that fasting increases plasma soluble leptin receptor levels, whereas leptin administration decreases leptin receptor levels [38]. We did not find any decrease in soluble leptin receptor levels in response to TB treatment, even though we found an increase in leptin levels. Conversely, we found some increase in the mean sOb-R levels, although this increase was not statistically significant. In addition, the increases in sOb-R levels yielded no significant differences in the leptin/sOb-R ratio following TB treatment.

Adiponectin levels have been found to be increased in certain TB patients in some studies [31], but not significantly increased in others [31]. We found plasma adiponectin levels similar to those previously observed [31], but we found no significant differences in adiponectin levels after TB treatment, suggesting that this adipokine may not be so important in the TB process.

In conclusion, TB treatment produces an increase in plasma leptin levels, with no changes in sOb-R or adiponectin levels, suggesting that leptin may play a role in the immune response to pulmonary TB, the disease possibly further suppressing leptin production, as suggested by other authors [28], whereas TB treatment seems to restore leptin levels.

Disclosure. *Financial support: none. Conflict of interest: none.*

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