Sex-Based Differences in Tuberculosis Immune Response: The Role of Leptin

**Authors**

* Guozheng Yang (ORCID: 0000-0001-7361-1847)

**Author affiliations**

1. College of Public Health, University of Georgia, Athens, GA, USA.

Corresponding author: guozheng0224@uga.edu

# 1. Abstract

## 1.1 Background:

## 1.2 Methods:

## 1.3 Results:

## 1.4 Conclusions:

# 2. Keywords:

*Write a summary of your project.*

# 3. Introduction

As a global threat, tuberculosis (TB) caused 10.8 million new cases in 2023 with Africa contributing 24% of these cases (1). Countries with high annual incidence rates are mostly in Africa, where TB incidence is significantly higher than in other regions. TB prevention and control strategies have shown progress in recent years. However, further efforts are needed to eliminate TB infection and transmission.

Males are more likely to develop TB disease than females, a difference that cannot be fully explained by socioeconomic status or access to healthcare (2,3). Research has shown that this sexual bias is linked to differences in immune systems, influenced by chromosome-encoded genes and hormones (4). Previous studies indicate that body fat mass could also affect immune responses (5,6). Since women generally have higher body fat storage, their immune systems may receive sufficient energy to support a stronger response to infections compared to men. In diseases like TB that often cause body wasting (7), differences in energy reserves may contribute to variations in immune response between men and women.

Leptin, a key link between metabolism and the immune system, plays an important role in activating and regulating immune responses (8,9). It functions as an energy indicator, signaling whether the body has enough energy to activate and sustain immune responses (10,11). For individuals with similar body fat mass, females generally have higher serum leptin levels than males (12). However, leptin levels may be lower in tuberculosis patients (13), suggesting a suppressed role in regulating metabolism and immune system stability. Since women typically have higher body fat stores and leptin levels, the impact on women may be less pronounced compared to men. However, there is a lack of evidence to demonstrate this sex difference.

This paper presents findings on sexual differences in metabolism and immune responses in individuals initially infected with TB. The primary hypothesis is that women have a stronger immune response to TB due to higher body fat storage. Additionally, leptin is hypothesized to mediate immune response based on body energy storage.

# 4. Methods

## 4.1 Study design and participants

From March to April 2017, a cross-sectional study was conducted in Kampala, Uganda. Sixty participants were recruited from Mulago Hospital, including 30 males and 30 females aged 15 years or older. All participants had a first episode of TB confirmed by culture or molecular methods. To prevent interference with metabolism and immune responses, individuals with co-morbidities such as asthma, kidney disease, liver disease, cancer, HIV, or diabetes were not enrolled. Those receiving hormonal or immune therapies were also excluded.

## 4.2 Body index measures

At enrollment, basic body indices, including age, sex, height (m), and weight (kg), were measured. Participants who provided informed consent underwent total body water (TBW, L) estimation using deuterium dilution. Specifically, participants received a precisely weighed oral dose of deuterium oxide (~25-30g , 99.8% purity) after an overnight fast. Participants will be asked to refrain from eating and to consume only the minimum amount of water needed to quench thirst during the 4-hour equilibration period. Saliva samples collected before and after a 4-hour equilibration period were analyzed using Fourier transform infrared spectrophotometry to determine deuterium concentration, and TBW was estimated by extrapolating deuterium dilution space, adjusted by a factor of 1.041 (14). Based on TBW estimates, lean body mass (LBM, kg) was further estimated assuming a hydration fraction of 0.732 (14). Body fat mass (FM, kg) was calculated by substituting LBM from body weight. The percentage of TBW, LBM, and FM were calculated based on body weight. Fat mass index (FMI) and Fat-free mass index (FFMI) were calculated using FMI = FM (kg) / height () and FFMI = LBM (kg) / height (). With consent, venous blood samples were collected to measure leptin levels (ng/ml). All measurements were conducted at the Institute of Infectious Diseases at Makerere University.

## 4.3 Immune response measures

To assess immune activation, flow cytometry was used to measure CD4+ and CD8+ T cell counts in venous blood samples (7 ml of plasma). The percentages of CD4+ and CD8+ cells were calculated by dividing their counts by the total lymphocyte count. Levels of C-reactive protein (CRP, mg/dl), interferon-gamma (INF-gamma, pg/ml), tumor necrosis factor-alpha (TNF-alpha, pg/ml), and interleukin-10 (IL-10, pg/ml) were also measured as indicators of immune response.

## 4.4 Statistical analysis

In the descriptive analysis, we compared the distribution of demographic characteristics (e.g., age, height, weight) and body composition indices (e.g., body fat, lean body mass, leptin level) between male and female participants using the median and interquartile range (IQR). Differences in immune responses were visualized with boxplots showing the distributions of available measures, including CD4+ cell counts, CRP levels, and INF-gamma levels. The association between log-transformed leptin levels and body fat, lean body mass, percentage of CD4+ cells, and percentage of CD8+ cells was assessed using scatterplots with Pearson correlation coefficients.

We examined the predictability of fat mass index to leptin level using a simple linear regression. A Poisson regression model was applied to CD4+ cell counts using a log link, with fat mass index and leptin level as predictors. All leptin levels in regression models used the logarithmic transformation. We estimated standardized regression coefficients and calculated the total, direct, and indirect effects of fat mass index on CD4+ cell counts (15). The mediation effect of leptin was quantified as the indirect effect divided by the total effect, expressed as the percentage of the total effect mediated by leptin (16). Confidence intervals (CI) at the 95% confidence level for all estimated effects were obtained using the bootstrap method with 500 replicates. The mediation analysis was first conducted for all participants. We then performed a subgroup analysis by applying the mediation analysis separately to male and female participants.

# 5. Results

## 5.1 Difference in body composition index by sex

The 30 male and 30 female participants had a similar age distribution ([Table 1](#tbl-body)). Males were taller (median = 168 cm, IQR: 158 cm-175 cm) and heavier (median = 55.2 kg, IQR: 50.0 kg-60.0 kg) than females, but BMI was similar between sexes (median: 19.48 vs. 19.20). Males had a higher body water proportion (median = 63.67%, IQR: 59.92%-68.09%) and lean body mass composition (median = 86.98%, IQR: 81.86%-93.01%), while females had a higher body fat composition (median = 21.37%, IQR: 14.74%-27.09%). After adjusting for height, males had a lower FMI (median: 2.46 vs. 4.41) but a higher FFMI (median: 16.80 vs. 15.19) than females. Leptin levels were higher in females than in males (median: 1.16 vs. 1.56).

|  |
| --- |
| Table 1: Body characteristics and composition by sex. |

## 5.2 Difference in immune response by sex

CRP, INF-gamma, TNF-alpha, and IL-10 levels showed no clear differences between males and females ([Figure 1](#fig-boxplot)). However, females had more compact distributions for CRP, TNF-alpha, and IL-10 levels. Both sexes had INF-gamma levels well above zero. Lymphocyte subsets varied between sexes. CD4+ levels were lower in males but showed less variation. The difference was not evident regarding CD8+ level. Among males, the median CD4+ count was around 300/uL, with a median percentage of about 20%, while females had a median count above 400/uL. Both sexes had CD4+ and CD8+ counts as low as nearly zero. The maximum CD4+ count reached 800/uL, whereas the maximum CD8+ count was over 400/uL in males and nearly 700/uL in females.

|  |
| --- |
| Figure 1: Comparison of immune response by sex. The boxplots denote immune response measures among male (blue) and female (orange) participants. |

## 5.3 Association between body composition and immune response

Leptin levels were strongly associated with body fat among females, with a positive correlation (Pearson correlation coefficient = 0.6, [Figure 2](#fig-sct)). In contrast, leptin levels showed a negative correlation with lean body mass in females (Pearson correlation coefficient = -0.4). Males had consistently lower leptin levels than females, with no evident correlation with body fat or lean body mass. No clear association was observed between leptin levels and lymphocyte subsets. The correlation between leptin levels and the percentage of CD4+ cells was weak (Pearson correlation coefficient = 0.09). Similarly, the correlation with the percentage of CD8+ cells was slightly negative but not significant (Pearson correlation coefficient = -0.13).

|  |
| --- |
| Figure 2: Association between log-transformed leptin level and body fat, lean body mass, percentage of CD4+ cells, and percentage of CD8+ cells. Male and female participants are denoted by blue and pink points, respectively. The size of point denotes body weight of each participant. R marks the Pearson correlation coefficients. |

## 5.4 Mediation effect of leptin in immune response activation

When fitting linear regression for all participants, leptin level was positively associated with FMI (=0.100, 95% CI: 0.064-0.135, [Table 2](#tbl-coef)). A similar positive association was observed among female participants (=0.113, 95% CI: 0.062-0.165), but not among males (=0.000, 95% CI: -0.003-0.002). When predicting CD4+ cell counts using FMI and leptin level for all participants, both predictors showed a positive association (=0.008, 95% CI: 0.000-0.017 for FMI; =0.364, 95% CI: 0.314-0.413 for leptin level). Among males, both FMI (=0.025, 95% CI: 0.011-0.040) and leptin level (=6.995, 95% CI: 4.489-9.500) were positively associated with CD4+ cell counts. However, among females, CD4+ cell counts were positively associated only with leptin level (=0.322, 95% CI: 0.262-0.381).

|  |
| --- |
| Table 2: Coefficient estimation (95% CI) from linear regression and Poisson regression. |

Among all participants, FMI had a notable effect on promoting CD4+ cell counts (total effect=0.25, 95% CI: -0.15-0.57, [Table 3](#tbl-ma)), with a large proportion (indirect effect=0.20, 95% CI: -0.14-0.55) mediated by leptin level. The mediation effect of leptin accounted for 82% of the total effect of FMI. A similar pattern was observed among female participants, where the effect of FMI (total effect=0.24, 95% CI: -0.35-0.67) was almost entirely mediated by leptin (indirect effect=0.24, 95% CI: -0.30-0.80). Among male participants, CD4+ cell counts were primarily influenced by the direct effect of FMI (total effect=0.09, 95% CI: -0.55-0.76; direct effect=0.10, 95% CI: -0.57-0.89).

|  |
| --- |
| Table 3: Mediation analysis with estimated effects and 95% CI. |

# 6. Discussion

## 6.1 Conclusions

# 7. References

1. Organization WH. Global tuberculosis report 2024. World Health Organization; 2024.

2. Horton KC, MacPherson P, Houben RM, et al. Sex differences in tuberculosis burden and notifications in low-and middle-income countries: A systematic review and meta-analysis. *PLoS medicine*. 2016;13(9):e1002119.

3. Ledesma JR, Ma J, Vongpradith A, et al. Global, regional, and national sex differences in the global burden of tuberculosis by HIV status, 1990–2019: Results from the global burden of disease study 2019. *The Lancet Infectious Diseases*. 2022;22(2):222–241.

4. Gupta M, Srikrishna G, Klein SL, et al. Genetic and hormonal mechanisms underlying sex-specific immune responses in tuberculosis. *Trends in immunology*. 2022;43(8):640–656.

5. Womack J, Tien PC, Feldman J, et al. Obesity and immune cell counts in women. *Metabolism*. 2007;56(7):998–1004.

6. Ilavská S, Horváthová M, Szabová M, et al. Association between the human immune response and body mass index. *Human immunology*. 2012;73(5):480–485.

7. Mupere E, Malone L, Zalwango S, et al. Wasting among uganda men with pulmonary tuberculosis is associated with linear regain in lean tissue mass during and after treatment in contrast to women with wasting who regain fat tissue mass: Prospective cohort study. *BMC infectious diseases*. 2014;14:1–10.

8. Naylor C, Petri WA. Leptin regulation of immune responses. *Trends in molecular medicine*. 2016;22(2):88–98.

9. Francisco V, Pino J, Campos-Cabaleiro V, et al. Obesity, fat mass and immune system: Role for leptin. *Frontiers in physiology*. 2018;9:640.

10. Lord G. Role of leptin in immunology. *Nutrition reviews*. 2002;60(suppl\_10):S35–S38.

11. Cava AL, Matarese G. The weight of leptin in immunity. *Nature Reviews Immunology*. 2004;4(5):371–379.

12. Hickey MS, Israel RG, Gardiner SN, et al. Gender differences in serum leptin levels in humans. *Biochemical and molecular medicine*. 1996;59(1):1–6.

13. Buyukoglan H, Gulmez I, Kelestimur F, et al. Leptin levels in various manifestations of pulmonary tuberculosis. *Mediators of inflammation*. 2007;2007(1):064859.

14. IAEA., IAEA. Introduction to body composition assessment using the deuterium dilution technique with analysis of saliva samples by fourier transform infrared spectrometry. International Atomic Energy Agency; 2011.

15. Sobel ME. Asymptotic confidence intervals for indirect effects in structural equation models. *Sociological methodology*. 1982;13:290–312.

16. Baron RM, Kenny DA. The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of personality and social psychology*. 1986;51(6):1173.