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

## 3.1 General Background Information

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 (m2) and FFMI = LBM (kg) / height (m2). With consent, venous blood samples were collected to measure leptin levels (ug/L). 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 (mg/dl), interferon-gamma (umol/ml), tumor necrosis factor-alpha (umol/ml), and interleukin-10 (ul/ml) were also measured as indicators of immune response.

*Sometimes you might want to show a schematic diagram/figure that was not created with code (if you can do it with code, do it).* [*Figure 1*](#fig-schematic) *is an example of some - completely random/unrelated - schematic that was generated with Biorender. We store those figures in the assets folder.*

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| Figure 1: A figure that is manually generated and shows some overview/schematic. This has nothing to do with the data, it’s just a random one from one of our projects I found and placed here. |

## 4.4 Statistical analysis

*This part is under construction:* I will firstly compare body indices and immune response measures between males and females. As all variables of interest are continuous, I will use Wilcoxon rank sum test. To measure leptin’s effect in modulating immune activation and to link body energy storage with immune response strength, my initial plan is to use regression analysis to examine leptin’s mediator effect, but I’m seeking a more appropriate method. Of note, the sample size is relatively small, which may influence the significance of hypothesis tests.

# 5. Results

## 5.1 Exploratory/Descriptive analysis

*Use a combination of text/tables/figures to explore and describe your data. Show the most important descriptive results here. Additional ones should go in the supplement. Even more can be in the R and Quarto files that are part of your project.*

[Table 1](#tbl-summarytable) shows a summary of the data.

Note the loading of the data providing a **relative** path using the ../../ notation. (Two dots means a folder up). You never want to specify an **absolute** path like C:\ahandel\myproject\results\ because if you share this with someone, it won’t work for them since they don’t have that path. You can also use the here R package to create paths. See examples of that below. I generally recommend the here package.

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| Table 1: Data summary table.   | skim\_type | skim\_variable | n\_missing | complete\_rate | factor.ordered | factor.n\_unique | factor.top\_counts | numeric.mean | numeric.sd | numeric.p0 | numeric.p25 | numeric.p50 | numeric.p75 | numeric.p100 | numeric.hist | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | factor | Gender | 0 | 1 | FALSE | 3 | M: 4, F: 3, O: 2 | NA | NA | NA | NA | NA | NA | NA | NA | | numeric | Height | 0 | 1 | NA | NA | NA | 165.66667 | 15.97655 | 133 | 156 | 166 | 178 | 183 | ▂▁▃▃▇ | | numeric | Weight | 0 | 1 | NA | NA | NA | 70.11111 | 21.24526 | 45 | 55 | 70 | 80 | 110 | ▇▂▃▂▂ | |

## 5.2 Basic statistical analysis

*To get some further insight into your data, if reasonable you could compute simple statistics (e.g. simple models with 1 predictor) to look for associations between your outcome(s) and each individual predictor variable. Though note that unless you pre-specified the outcome and main exposure, any “p<0.05 means statistical significance” interpretation is not valid.*

[Figure 2](#fig-result) shows a scatterplot figure produced by one of the R scripts.

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| Figure 2: Height and weight stratified by gender. |

## 5.3 Full analysis

*Use one or several suitable statistical/machine learning methods to analyze your data and to produce meaningful figures, tables, etc. This might again be code that is best placed in one or several separate R scripts that need to be well documented. You want the code to produce figures and data ready for display as tables, and save those. Then you load them here.*

Example [Table 2](#tbl-resulttable2) shows a summary of a linear model fit.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 2: Linear model fit table.   | term | estimate | std.error | statistic | p.value | | --- | --- | --- | --- | --- | | (Intercept) | 149.2726967 | 23.3823360 | 6.3839942 | 0.0013962 | | Weight | 0.2623972 | 0.3512436 | 0.7470519 | 0.4886517 | | GenderM | -2.1244913 | 15.5488953 | -0.1366329 | 0.8966520 | | GenderO | -4.7644739 | 19.0114155 | -0.2506112 | 0.8120871 | |

# 6. Discussion

## 6.1 Summary and Interpretation

*Summarize what you did, what you found and what it means.*

## 6.2 Strengths and Limitations

*Discuss what you perceive as strengths and limitations of your analysis.*

## 6.3 Conclusions

# 7. References

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