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Associations between Metabolites and Obesity:
A Cross-Sectional Study in South African Adults with HIV

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Associations between Metabolites and Obesity: A Cross-Sectional Study in South African Adults with HIV

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2019

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Epidemiology
2023

Abstract

Associations between Metabolites and Obesity:
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By Richard A. Powers

Background: HIV infection puts individuals at increased risk for obesity and related chronic illnesses, and the link between specific metabolites and body mass index within this population, especially among those of African ancestry, is not well-defined. By using validated metabolites, this study aims to identify potential associations with BMI and develop targeted interventions to address health disparities in underrepresented populations.

Methods: This study conducted a cross-sectional study of 340 participants to investigate the association between metabolites and BMI. Linear regression was used to test the associations between validated metabolites and BMI while controlling for age, sex, race/ethnicity, and smoking status.

Results: Among the 154 validated metabolites, there were 20 with significant associations with BMI. 1-naphthylamine and tryptophan had the most statistically significant associations (*p-value* < 0.001), and phenylalanine, a replicated metabolite identified in prior studies, was also found to be highly significant (*p-value* $= 0.004$).

Discussion: The association between tryptophan and phenylalanine with BMI and obesity-related diseases may have significant public health implications as obesity is a major risk factor for a range of chronic illnesses. Future studies need to consider social determinants of health, such as food insecurity and SES, in addressing obesity-related health disparities. Further research is required to establish a concrete relationship and determine the precise association and validity of these metabolites with obesity-related outcomes.

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INTRODUCTION

Over the last 40 years, advances in antiretroviral therapy (ART) have greatly increased the longevity and quality of life for people with HIV (PWH). However, along with these great advances arose a new set of challenges to overcome. Age-related diseases, such as type 2 diabetes (T2D), cardiovascular disease (CVD), chronic kidney disease (CKD), and cancer, pose a much greater risk to PWH now than in the past (1,2). This trend is especially pronounced in many low- and middle-income countries (LMICs), causing an added strain to existing HIV care around the globe.

Obesity is a major risk factor for adverse health outcomes, such as T2D and CVD, and places a significant financial burden on the public health infrastructure in South Africa (3–5). The relationship between obesity and these chronic illnesses is exacerbated among PWH (1,2,6,7). According to the World Health Organization (WHO), over 1 billion people worldwide are considered obese, and this number is expected to increase in the coming years (4). South Africa has among the highest prevalence of obesity in sub-Saharan Africa, with 68% of women and 31% of men considered either overweight or obese (3,8). South Africa also has one of the highest prevalence of HIV infection in the world, with the province of KwaZulu-Natal (KZN) bearing the greatest burden (9). The findings of this study, which explores the associations between metabolites and obesity in PWH on ART, can help identify metabolic pathways and inform the development of targeted interventions to promote healthier lives for PWH. By identifying specific metabolites that are associated with obesity in this population, the analysis may contribute valuable evidence towards implementing targeted interventions that may prevent or treat obesity in PWH, ultimately leading to improved health outcomes and quality of life for this vulnerable population.

BACKGROUND

Metabolome-wide association studies (MWAS) are used to identify potential associations between metabolic variables and disease outcomes (10). This approach utilizes metabolic profiling via spectroscopic techniques (e.g., nuclear magnetic resonance spectroscopy and mass spectrometry) to measure thousands of metabolites within the body. Metabolites are small molecules that are produced as a result of various metabolic processes in the body (11). They can serve as indicators of biological functions and can provide insights into disease pathogenesis.

Several studies have utilized metabolomics to investigate the metabolic changes associated with obesity and to identify potential biomarkers for obesity-related diseases. Early MWAS research utilized an untargeted metabolomic approach to investigate metabolite changes and their possible association with obesity (12,13) This approach uses a top-down strategy, and its primary objective is hypothesis-generating (13). Technical advances in metabolomics led to more targeted approaches, which used identified and validated metabolites based on prior research. Some early studies using this strategy revealed specific metabolites associated with body fat distribution and risk factors for developing adiposity-associated co-morbidities in the general adult population, regardless of obesity status (14–17). In more recent studies, researchers have investigated the differences in metabolomic profiles of obese and normal-weight adults with obesity-related diseases (18–21). The top associated metabolites and metabolic pathways reported in the studies referenced above are presented in Table 1.

The identification of metabolites associated with body mass index (BMI) in PWH may help in the early detection of individuals at risk of becoming overweight or obese, which can lead to higher risk for developing non-communicable diseases (NCDs), and enable targeted interventions to prevent further weight gain (10). MWAS and similar research frameworks have

Table 1: Top associated metabolites and metabolic pathways from previous literature

Authors	Year	Metabolite/Metabolic Pathways
Hanhineva et al.	2013	Insulin, Cholesterol, VLDL, TG 54:1-3, TG 50:1-5, TG 55:1, PC 32:0
Kim et al.	2013	Betaine, Benzoic acid, Pyroglutamic acid, Pipecolic acid, N-phenylacetamide, Uric acid, L-aspartyl-L-phenylalanine, LysoPCs (C18:1, C18:2, C20:1, and C20:4), L-proline, Valine, L-leucine/isoleucine, Hypoxanthine, Glutamine, L-methionine, Phenylpyruvic acid, Carnitine derivatives, LysoPCs (C14:0, PC16:0, C15:0, C16:0, C17:1, C18:0, and C22:0)
Chen et al.	2015	L-kynurenine, Glycerophosphocholine (GPC), Glycerol 1-phosphate, Glycolic acid, Tagatose, Methyl palmitate, and Uric acid
Wang et al.	2016	2-Octenoylcarnitine, Eicosadienoic acid, 12-hydroperoxyeicosatetraenoic acid, 4-hydroxyestrone sulfate, LysoPE[18:1(11Z)/0:0], Thromboxane B2, Pyridinoline, Vitamin D3 glucosiduronate, 9,10-DHOME
Chashmniam et al.	2020	Glutamine, Asparagine, Alanine, L-Glutathione Reduced, 2-Aminobutyrate, Taurine, Betaine, Choline, D-Sphingosine, Glycine, Histidine, Isoleucine, L-Proline, Cholic Acid, Carnitine

Note: Only studies that explicitly reported the identified metabolites in their results were included above and, therefore, not all referenced studies are represented.

been primarily conducted in populations of European ancestry and from high-income countries. However, there is a need to validate these critical findings in populations of non-European ancestry and in LMICs (22).

Recently, Liu et al. (2022) performed a genome-wide association study (mGWAS) of multiple metabolites using a sample from a large cohort of PWH residing in South Africa. A major emphasis of the analysis was to sample individuals from diverse ancestries and geographic regions in order to assess gene-metabolite associations and identify associations across diverse populations; this could provide insights into disease risk at the molecular level and reduce health disparities among underrepresented populations (22). The mGWAS of 154 metabolites identified 22 significant genetic associations, at $p < 5 \times 10^{-8}$, and replicated several genetic associations of

metabolites in the general population. By analyzing the same population, our study utilizes the 154 metabolites previously identified to build more robust evidence of potential associations between these validated metabolites and BMI.

Our study uses BMI as an approximation for adiposity, providing a means of creating defined categories to determine weight status within the sample. Obesity has been identified as a significant risk factor for various chronic illnesses and metabolic dysfunction including T2D, CVD, and other NCDs (4,5). Huang et al. (2022) conducted a phenome-wide association study (PheWAS) of BMI and various adverse health outcomes (5). The investigators found BMI to be implicated as a genetically associated risk factor for CVD, chronic renal failure, respiratory failure, and musculoskeletal disorders among other disease outcomes. These chronic NCDs greatly increase disability-adjusted life years (DALYs) both globally and in South Africa (23). Between 2002 and 2016, the prevalence of overweight and obese women increased from 56% to 68% and rose similarly from 29% to 31% among men in South Africa (3,8). The province of KZN, where the study was conducted, has the highest rate of severe obesity among men and the second highest rate among women (8).

HIV/AIDS is the top contributor to both the national mortality rate and DALYs per 100,000 in South Africa (24). In 2012, the prevalence of HIV in South Africa was 12%, and by 2017, this number rose to 14% (or ~8 million people) despite increased funding and prevention efforts. The HIV incidence rate was 320,000 new infections per year between 2012 and 2017 (25). The highest prevalence of HIV in South Africa is in KZN with 18% of the population living with the illness (9). Together, obesity and HIV significantly predispose PWH for developing NCDs and living lower quality, shortened lifespans.

To date, few studies have investigated the relationship between metabolites and BMI among PWH and in LMICs such as South Africa (22). Furthermore, as has been emphasized, most related studies have performed these tests using samples from populations of mostly European ancestry (5,22,26–30). This study aims to address this gap in knowledge and provide valuable insights into the molecular mechanisms that underlie the association between obesity and related adverse health outcomes in PWH. Specific aims included (1) identifying key associations between BMI and validated metabolites, and (2) performing an ad hoc mediation analysis to identify potential mediators or effect modifiers (e.g., SES) between metabolites and BMI. The overarching goal of this study is to elucidate the complex relationship between obesity and chronic complications observed among PWH. The results of this research may assist in designing targeted interventions that promote healthier lifestyles for PWH. By pinpointing the particular metabolites linked to obesity in this group, the investigation can provide significant evidence for creating interventions that may avert or treat obesity in PWH. Ultimately, this could enhance the health outcomes and overall quality of life for this vulnerable population.

METHODS

Study Design and Participants

This study was a cross-sectional analysis of data from a sub-cohort of the KZN HIV AIDS Drug Resistance Surveillance Study (ADReSS) conducted within several clinics in KZN, South Africa. The study sample included 500 participants recruited from a state-funded hospital clinic, RK Khan (RKK), located in a peri-urban township, Chatsworth, near Durban, KZN from 2014 to 2016. The study included participants who were aged 18 years or older, qualified for

ART, and provided written informed consent. A total of 340 of these participants with metabolomics data available were included in the analysis.

Data Collection

Data were collected through questionnaires, physical examinations, and laboratory tests. The study collected demographic information, including age, sex, and race/ethnicity, as well as estimates of socioeconomic status, smoking status, and transportation methods used to attend clinic visits. Additionally, metabolic data were collected at baseline through laboratory testing. Ethics approval was obtained prior to the start of the study and approved by the institutional review boards at University of KwaZulu-Natal and Emory University.

Data Cleaning and Variable Imputation

All data cleaning was performed using R version 4.2.2. The following packages were used for data manipulation and analysis: tidyverse, rio, janitor, skimr, naniar, gtsummary, rstatix, sjPlot, mlbench, table1, nlme, ggplot2, qqman, flextable, knitr, and car. Data cleaning included merging datasets and removing duplicate entries.

Metabolomic data were quantified using high-resolution metabolic profiling with liquid chromatography and mass spectrometry to analyze plasma samples from participants prior to ART initiation. The metabolites were extracted using apLCMS and xMSanalyzer, and annotated using an in-house library. A total of 154 metabolites were successfully matched, with annotations allowing for small variations in mass and retention time. For a more detailed and comprehensive description of the metabolomic profiling process, refer to Liu et al., 2022. BMI was calculated using height and weight data collected closest to the blood draw used to calculate the

metabolomic data. BMI Status was categorized using CDC standard weight status categories (31). BMI has been criticized for its use to approximate adiposity; however, given the inability to gather relevant data using dual-energy x-ray absorptiometry (DXA), the gold standard, BMI has been found to be a suitable proxy variable among both general populations and PWH residing in East Africa (27,32). Covariates included in the model were age, sex, race, smoking status, and wealth indices. Age, sex, race, and smoking status were controlled for in the initial model. The decision to control for these variables was based on *a priori* evidence derived from similar studies (22,33–35).

Statistical Analysis

Data analyses were conducted using descriptive statistics and statistical tests, as appropriate. Linear regression was used to test the univariate associations of each validated metabolite as a predictor for BMI, controlling for race/ethnicity, sex, age, and smoking status. For this test, statistical significance was determined using $p < 0.05$. All analyses were conducted using R version 4.2.2 (URL Available online: https://www.R-project.org/). Summary tables were created using the gtsummary, table1, and flextable packages. Figures were created using the ggplot2 and qqman packages.

RESULTS

Characteristics of Study Participants

The cohort was comprised of 340 participants, all residing in South Africa and receiving care at the ART clinic at RKK Hospital in Chatsworth, KZN, South Africa. Among the study population, 59% of participants were female. The cohort had a median age at enrollment of 33

(±10) years with age staying relatively consistent across BMI status groups. The sample overwhelmingly self-identified as Black. The median BMI was 23 (±6.0) kg/m^2. For the overall cohort, 42% were considered either overweight or obese. Male participants had a lower median BMI compared to female participants in the study [21 (±5.0) vs. 25 (±6.3)] as well as had a lower percent who were considered overweight or obese. These statistics are consistent with the trends observed in the latest national statistics (4,8). Lastly, the majority of participants (58%) reported having less than a high school education. These data are presented in Table 2.

Collinearity was assessed among the significant metabolites by calculating variance inflation factor (VIF) values for each variable within each model. No variable had a VIF value greater than 5, indicating that collinearity is unlikely to be a concern among these variables.

Of the 154 targeted metabolites, the model yielded 20 metabolites associated with BMI with nominal p-values less than 0.05, after controlling for race/ethnicity, sex, age, and smoking status. These results, along with the metabolic feature, beta, standard error (SE), p-values, and false discovery rate (FDR) q-values are presented in Table 3, sorted by p-value.

Omics studies involve the analysis of a large number of variables, which increases the likelihood of obtaining false positive results; to address this issue, multiple testing correction methods are commonly employed (10,36). In this study, we used the FDR method as the correction approach. FDR controls for the expected proportion of false positive results among all tests deemed significant. This method is preferred over other statistical approaches, such as the Bonferroni correction, because it has greater power to detect significant results and is less likely to produce false negatives (36). By using FDR correction, we aimed to minimize the risk of reporting false positive findings and to increase the reliability of our results. All metabolites with a statistically significant p-value also had q-values less than 0.05 making them significant even

Table 2: Demographics and Summary Statistics

	Total (N=340)	Under-weight (N=30, 9%)	Normal Weight (N=168, 49%)	Over-weight (N=78, 23%)	Obese (N=64, 19%)
Sex					
Male	141 (41%)	19 (63%)	82 (49%)	27 (35%)	13 (20%)
Female	199 (59%)	11 (37%)	86 (51%)	51 (65%)	51 (80%)
Age at Enrollment, years					
Median (SD)	33 (±10)	34 (±10)	30 (±10)	35 (±9.3)	34 (±10)
Race/Ethnicity					
Black	322 (95%)	27 (90%)	162 (96%)	73 (94%)	60 (94%)
Colored	3 (1%)	0 (0%)	1 (1%)	0 (0%)	2 (3%)
Indian	15 (4%)	3 (10%)	5 (3%)	5 (6%)	2 (3%)
BMI, kg/m^2					
Median (SD)	23 (±6.0)	18 (±1.3)	21 (±1.7)	27 (±1.5)	34 (±4.6)
Smoking Status					
Smokes	21 (6%)	2 (7%)	15 (9%)	3 (4%)	1 (2%)
Does Not Smoke	319 (94%)	28 (93%)	153 (91%)	75 (96%)	63 (98%)
Education Level					
No Education	13 (4%)	1 (3%)	6 (4%)	5 (6%)	1 (2%)
Less than High School	196 (58%)	22 (73%)	97 (58%)	37 (47%)	40 (62%)
High School or Equivalent	52 (15%)	2 (7%)	26 (15%)	16 (21%)	8 (12%)
Certificate or Diploma	63 (19%)	5 (17%)	31 (18%)	14 (18%)	13 (20%)
College Degree	16 (5%)	0 (0%)	8 (5%)	6 (8%)	2 (3%)

Note: Percentages may not sum to 100% due to rounding.

after the multiple testing correction.

A quantile-quantile (QQ) plot was created to compare the distribution of observed p-values from the analysis to the expected log10(p-values) under the null hypothesis. Based on

Figure 1, the observed p-values appear to be mostly smaller than expected under the null hypothesis.

A volcano plot, presented in Figure 2, is another type of graphical tool commonly used in omics studies to display the results of a statistical test comparing two groups (37). It plots the negative logarithm of the p-value on the y-axis and the log2-fold change on the x-axis, with each data point representing one of the 154 metabolites tested. The plot helps to visualize which metabolites are significantly differentially expressed between two groups and provides insight into both the magnitude and direction of changes.

Figure 1: Quantile-quantile (QQ) Plot of p-values

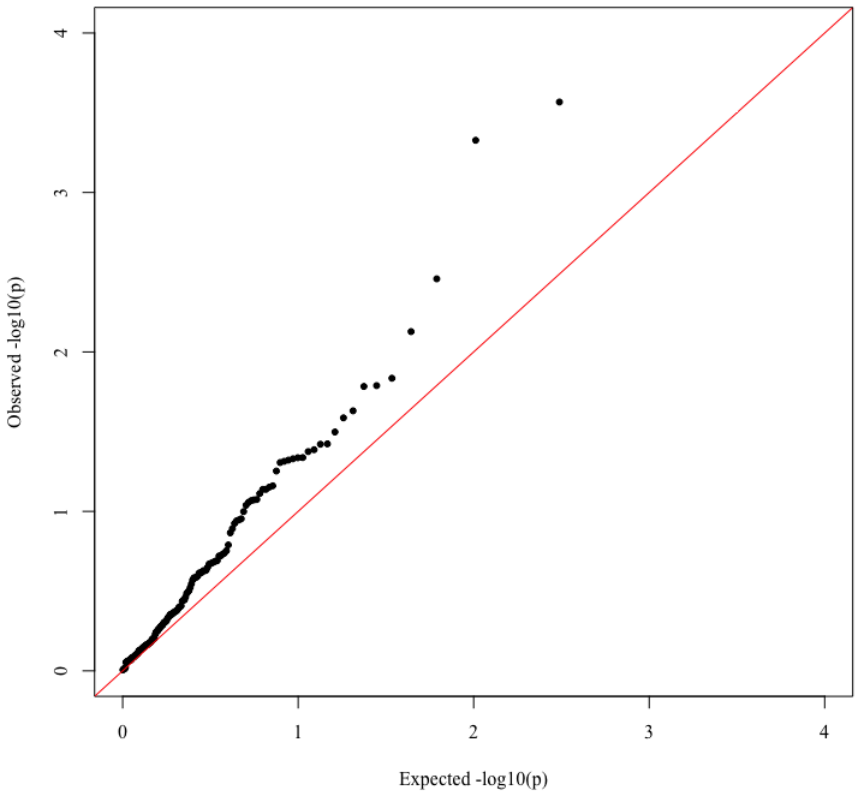

Figure 2: Volcano Plot

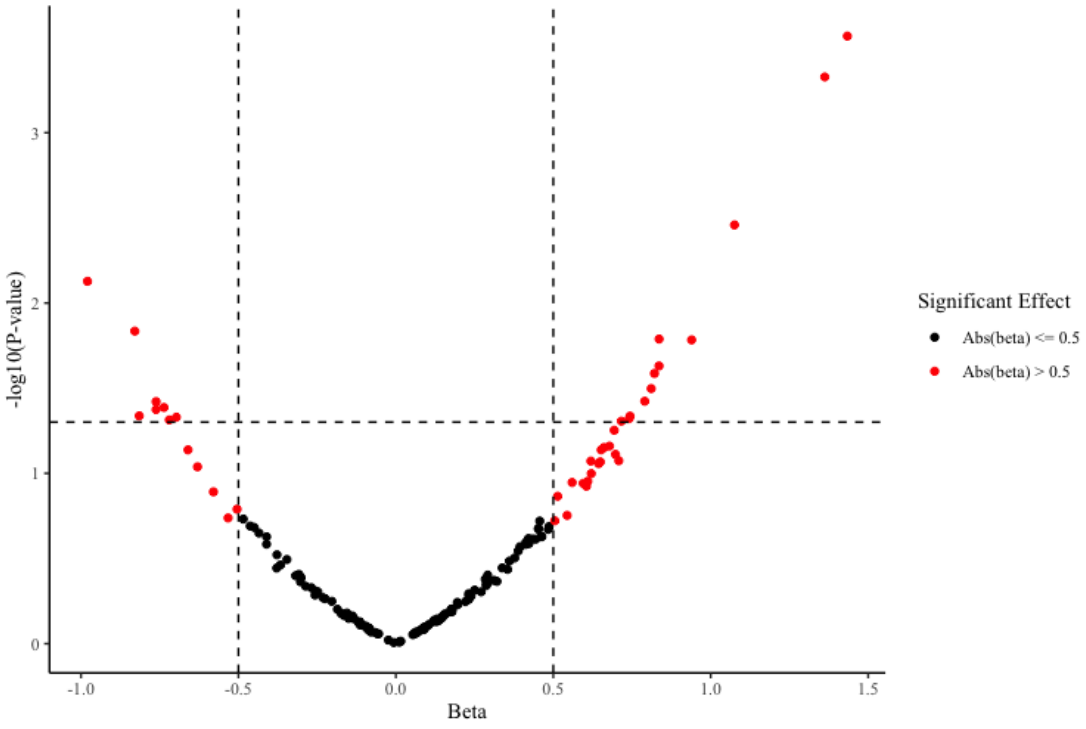

Table 3: Metabolites associated with body mass index.

Metabolite Name	Metabolic Feature	Beta	SE	P-value	Q-value
1-NAPHTHYLAMINE	mz144.0807_t46.2	1.43	0.39	< 0.001	0.005
TRYPTOPHAN	mz205.0972_t45.9	1.36	0.38	< 0.001	0.005
PHENYLALANINE	mz166.0862_t46.5	1.08	0.36	0.004	0.023
HOMOCYSTEINE	mz136.0428_t56.3	-0.98	0.36	0.008	0.038
URIDINE	mz245.0769_t45.3	-0.83	0.34	0.015	0.047
CYSTINE	mz239.017_t24.2	0.84	0.35	0.016	0.047
NORLEUCINE	mz130.0873_t23	0.94	0.39	0.017	0.047
CREATINE	mz132.0765_t72.5	0.84	0.37	0.023	0.049
BETA-ALANINE	mz90.0551_t71.3	0.82	0.37	0.026	0.049
3-METHYL-2-OXINDOLE	mz162.056_t60.4	0.81	0.38	0.032	0.049
SERINE	mz104.0351_t26.8	0.79	0.38	0.038	0.049
N-ACETYL-D-TRYPTOPHAN	mz247.1076_t34.1	-0.76	0.36	0.038	0.049
CITRULLINE	mz176.1029_t90.6	-0.74	0.36	0.041	0.049
3-HYDROXYBENZYL ALCOHOL	mz123.045_t27.4	-0.76	0.37	0.042	0.049
CITRAMALATE	mz147.0298_t23.1	-0.82	0.41	0.046	0.049
N-AMIDINO-L-ASPARTATE	mz176.0663_t49	0.74	0.37	0.046	0.049
CAFFEATE	mz179.035_t25.5	-0.70	0.35	0.047	0.049
D-PANTOTHENIC ACID	mz220.1182_t64.5	0.74	0.37	0.048	0.049
URACIL	mz113.0347_t39.3	-0.72	0.36	0.049	0.049
N-ACETYL-L-PHENYLALANINE	mz206.0825_t46.2	0.72	0.36	0.049	0.049

DISCUSSION

South Africa, similar to many other LMICs, faces significant public health challenges due to a high prevalence of infectious diseases and a growing burden of NCDs. Most studies investigating the relationships between metabolomics and adverse health outcomes have been conducted among populations of European ancestry and from high-income countries. These studies may not be relevant for individuals of non-European ancestry living in LMICs, as they may have different genetic ancestries and environmental exposures (22).

Metabolites of Interest

The association study between the 154 metabolites and BMI yielded 20 significant results (Table 3). 1-naphthylamine and tryptophan were the most statistically significant, and phenylalanine was a replicated BMI association, based on previous literature, with a high statistical significance.

1-naphthylamine is not a natural occurring metabolite but has a role as a human xenobiotic metabolite (38), and it was found to be highly significant in the analysis (p-value < 0.001). There was very little existing literature on the association of this metabolite with BMI or obesity. One study, involving animal models, found 1-naphthylamine as a significant obesity-associated gene expressed in the stomach (39). No other relevant evidence could be found in previous research, so this metabolic feature may be a potential novel association with BMI.

Tryptophan, an essential amino acid and endogenous compound involved in metabolic processes (11), showed the second highest significant association in the analysis. Numerous previous studies have linked tryptophan with BMI and obesity-related risk factors and diseases. For instance, a 2016 study of South African adults with HIV revealed that lower tryptophan

levels were associated with higher levels of inflammatory activity, likely caused by food insecurity (40). This underscores the potential role of SES as a mediator or effect modifier of tryptophan and obesity-related diseases. Additionally, a 2020 study reported that tryptophan, along with kynurenine, metabolic pathways were concomitantly altered in obese adults compared to non-obese controls (41). Another study in 2022 conducted global metabolomic profiling on a diverse cohort of women and found that elevated tryptophan and kynurenine levels were associated with decreased levels of microbial-derived metabolites which are critical for controlling inflammation and maintaining immune response in obese individuals (42). Altogether, these findings suggest that tryptophan is a promising metabolite to explore in the context of obesity-related diseases and that future research should consider the potential role of socioeconomic factors in mediating or modifying its effects.

Phenylalanine, a precursor for various neurotransmitters and an amino acid involved in protein synthesis (11), was the third most significant result (p-value = 0.004) in the analysis. This metabolite was identified as having a significant and relevant metabolic pathway between two groups: metabolic healthy obesity (i.e., obese but without hyperglycemia, hypertension and dyslipidemia) and metabolic abnormal obesity (i.e., obese with one or more abnormal metabolic index) (21). Additionally, phenylalanine has been found at higher levels in obese populations and those with metabolic dysfunction (e.g., insulin resistance, T2D, and high blood pressure) in general populations (26). With a significant association with BMI in our sample, phenylalanine might be a useful marker for early detection and intervention of obesity and metabolic dysfunction, however, further studies are necessary to determine the precise association and validity of this relationship.

The specific metabolites identified in this study may be potential biomarkers, etiological pathways, or therapeutic targets of BMI-related outcomes (e.g., obesity) among PWH. Although further research is required to establish a concrete relationship, the results of this study could be used to implement early interventions and provide better health outcomes.

SES was identified as a variable of interest to assess as a potential mediator or effect modifier of metabolic profiles and BMI due to its important effect on both metabolites and BMI. A recent multi-cohort analysis revealed a link between lower SES and unfavorable metabolic profiles (43). Meanwhile, extensive research has explored the impact of socioeconomic factors on BMI, finding significant effects at both individual and community levels, regardless of HIV status (44–46). However, the relationship between social strata and BMI may differ between high-income countries and LMICs, especially in the context of PWH residing in LMICs (45). Although ongoing research is examining these relationships more thoroughly through metabolomics, this study recommends further investigation of SES as a potential mediator or effect modifier between metabolic profiles and obesity to clarify the complex relationship.

Public Health Implications

The association between tryptophan and phenylalanine with BMI and obesity-related diseases may have significant public health implications. As far back as 1988, a study found evidence that altered plasma levels of these two amino acids and their response to carbohydrate intake may play a role in the regulation of food intake in obese individuals (47). Obesity is a major risk factor for a range of chronic diseases, including T2D, CVD, and some cancers (1,2,42). Identifying metabolites that are associated with BMI and obesity could help in the development of targeted interventions to prevent and manage obesity and these related diseases.

Furthermore, the potential mediating effect of SES on the relationship between metabolites and BMI highlights the need to consider social determinants of health in addressing obesity-related health disparities. Food insecurity, for example, can affect the levels of tryptophan and other metabolites that are associated with BMI, as shown in the 2016 study of South African adults with HIV (40) mentioned previously. Improving access to healthy food and addressing other social determinants of health may be essential to reducing the burden of obesity-related diseases, particularly among disadvantaged, underrepresented, and vulnerable populations.

In summary, the findings of this study may contribute to our understanding of the metabolic pathways that are associated with BMI and obesity-related diseases. Further research is necessary to confirm and extend these findings and to explore potential interventions to prevent and manage obesity and obesity-related diseases.

Limitations

The study was limited by a small sample size and inclusion of participants from a single hospital setting. Additionally, race and ethnicity can be important to consider more in depth in the model, but given that 95% of our participants identified as Black, our model was limited in its ability to further investigate the role of race in the associations.

CONCLUSION

In conclusion, this study investigated the association between 154 metabolites and BMI among PWH in South Africa. The results identified 20 unique metabolites with a significant association with BMI. Three metabolites of particular interest were 1-naphthylamine, tryptophan,

and phenylalanine, with tryptophan and phenylalanine having *a priori* associations with BMI and obesity-related diseases. The association between tryptophan and phenylalanine with BMI and obesity-related diseases may have significant public health implications as obesity is a major risk factor for a range of chronic diseases. The study also highlights the need to consider social determinants of health, such as food insecurity and SES, in addressing obesity-related health disparities. Further research is required to establish a concrete relationship and determine the precise association and validity of these metabolites with obesity-related outcomes.

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