Metabolic hormones and ethnic aspects in obesity

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CHAPTER 4

YOUNG AND LEAN SOUTH ASIANS HAVE HIGHER LEPTIN AND LOWER GHRELIN LEVELS BEFORE AND DURING A MIXED MEAL TOLERANCE TEST COMPARED TO EUROPIDS

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ABSTRACT

Objectives

In South Asians, an unfavorable metabolic phenotype characterized by central obesity, insulin resistance, and dyslipidemia is more common than in Europids. Since various hunger and satiety hormones play a crucial role in managing energy balance, we aimed to investigate excursions of peptide YY (PYY), ghrelin, and leptin in response to a mixed meal tolerance test (MMTT) in young and lean South Asians and Europids.

Method

PYY, ghrelin, and leptin were measured in plasma obtained during an extended MMTT (up to 240 min) in young and lean South Asian (n=24) and Europid (n=25) males and females.

Results

At baseline and throughout the MMTT, no differences in PYY levels were observed between South Asian and Europid males and females. South Asian males had lower ghrelin levels before and throughout the MMTT compared to Europid males. Both South Asian males and females exhibited higher leptin levels at baseline and throughout the MMTT, resulting in a higher total area under the curve ($tAUC_{0\text{-}240}$) of leptin in South Asians compared to Europids. In addition, baseline leptin levels strongly positively correlated with fat mass and fat percentage in South Asian males and females, but generally not in Europids.

Conclusion

South Asian males and females exhibit higher leptin, while only South Asian males showed lower ghrelin levels at baseline and throughout an MMTT compared to Europids. The potential contribution of high leptin levels to the disadvantageous metabolic phenotype in South Asians remains to be determined.

INTRODUCTION

In 2022, 1 in 8 people were living with obesity (1, 2), a disease resulting from a long-term positive energy balance leading to excess storage of nutrients in adipose tissue (3). Obesity is associated with impaired health and an increased risk of developing various diseases including type 2 diabetes mellitus (T2DM), cardiovascular diseases, and certain types of cancers (4). In most individuals, obesity results from a combination of genetic predisposition, psychosocial factors, and exposure to obesogenic environments (4). Moreover, individuals from certain ethnic backgrounds, such as those of South Asian descent, have an increased risk of developing obesity and obesity-related diseases (5).

In South Asia, and particularly in Pakistan and India, the prevalence of obesity markedly increased since the last decades and is expected to increase further in the coming years (2, 6, 7). Moreover, South Asians have a metabolic phenotype that makes them more susceptible to health impairments arising from obesity, such as insulin resistance and cardiovascular disease (8). This increased risk for obesity-related diseases is not only seen in people currently living in South Asia but is even more pronounced among people of South Asian descent residing in Western countries (9). The disadvantageous metabolic phenotype of South Asians is characterized by a low lean mass, high fat mass, insulin resistance, and dyslipidemia (10). In addition, we (11) and others (12) reported a lower resting energy expenditure in South Asians, which predisposes them to a positive energy balance. This phenotype is hypothesized to be partly an adaptation to repeated periods of severe famine in the 19th and 20th centuries (13). Further contributing to this positive energy balance could be the previously found higher dietary energy intake in South Asians compared to Europids (14). Although numerous other factors contributing to this phenotype have been studied in recent years, many underlying factors remain unknown. For example, it is still unknown whether impaired satiety signaling could play a role in increasing their susceptibility to maintaining a positive energy balance.

Obesity is often associated with disruption of various hunger and satiety hormones, such as ghrelin, leptin, and peptide YY (PYY) (15-19). The hunger hormone ghrelin is released from the stomach and small intestine to prepare the body for a meal. Ghrelin stimulates appetite directly via receptors located in the hypothalamus and indirectly via ghrelin receptors on the vagal nerve (20, 21). In addition to regulating hunger, ghrelin promotes gluconeogenesis in the liver, stimulates gastric secretion and motility, and promotes lipid storage in adipocytes (22). On the other hand, the satiety hormone leptin, which is produced by adipocytes, signals through leptin receptors in the hypothalamus and brainstem, where it promotes satiety and increases energy expenditure (23, 24). Similarly, the satiety-inducing hormone PYY is released postprandially from the distal gut and binds

to its receptors (i.e., Y1 and Y2 receptors) in the area postrema and dorsal vagal complex located in the hindbrain, where it induces satiety and increases energy expenditure (19).

Understanding baseline levels and postprandial excursions of these hormones in South Asians versus Europids could provide valuable insights into underlying mechanisms that contribute to energy balance and the unfavorable metabolic phenotype of South Asians. Therefore, we aimed to investigate baseline levels and excursions of ghrelin, leptin, and PYY in young and lean South Asians compared to Europids during an extended (up to 240 min) mixed meal tolerance test (MMTT).

METHODS

Study Design

This study used samples obtained from the CAMI study (Elucidating the high cardiovascular disease risk in South Asians: focus on monocyte phenotype and incretin hormones), an observational study conducted at the Leiden University Medical Center (LUMC) between June and October 2023. The study was approved by the Medical Ethics Committee of the LUMC and undertaken in accordance with the principles of the revised Declaration of Helsinki (25). Written informed consent was obtained from all participants prior to inclusion. The clinical trial is registered at ClinicalTrial.gov (no. NCT05829018). The primary objective of the CAMI study was to compare immune cell composition between lean adolescent Dutch South Asians (hereinafter: 'South Asians') and BMI- and age-matched Dutch Europids (hereinafter: 'Europids'). In this manuscript, we report on one of the secondary objectives.

Participants

A total of forty-nine lean and healthy participants were included in the study, namely South Asian males (n=12) and females (n=12), and Europid males (n=13) and females (n=12). Additional inclusion criteria were a body mass index (BMI) of 18.0-25.0 kg/m^2 and age of 18-30 years. We included an additional (13th) Europid male as we encountered a technical problem during the collection of the samples for the primary endpoint of this study from one Europid male.

Participants were recruited especially through social media advertisements and by recalling participants from previous studies. Eligibility to participate in the study was tested primarily during a telephonic screening that consisted of questions about their heritage, body weight, height, and medical history. South Asian ethnicity was defined as having all four grandparents from Surinam, Bangladesh, India, Nepal, Pakistan, Afghanistan, Bhutan, or Sri Lanka. Europid ethnicity was defined by having

four grandparents originating from Europe. Exclusion criteria were the presence of an (auto-)immune disease, genetic lipid-associated disorders, chronic renal or hepatic disease, use of medication known to influence glucose and/or lipid metabolism, abuse of alcohol or other substances, smoking, vigorous exercise (more than 3 times per week), and milk or soy allergy.

Screening procedure

Participants refrained from vigorous exercise for 48 hours and from alcohol or caffeinated beverages for 24 hours prior to the study day. They consumed a standardized meal the evening before, which consisted of either a prepared supermarket meal with comparable ratio of carbohydrates and lipids (ranging from 450–600 kcal) or a similar meal at home and drank only water. After an overnight fast, participants arrived at the LUMC at 08:00 am, where they completed questionnaires about their medical history and current health. Subsequently, body weight and body composition were measured using bioelectrical impedance analysis (BIA) (InBody720, InBody CO., Ltd., CA, USA). In addition, height, waist, and hip circumference were measured with a measuring tape. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). To complete the screening procedure, a blood sample was collected by inserting a catheter into the antecubital vein for venous blood sampling. This was done using Vacutainer SST II Advance Gel and EDTA tubes to measure full blood count, glucose, insulin, and parameters related to kidney function, liver function, and lipid metabolism. If participants met the inclusion criteria based on the questionnaires, body composition measurements, and screening blood sample data, the MMTT was started directly after.

Mixed meal tolerance test

Upon inclusion, a baseline sample was taken using Vacutainer SST II Advance Gel tubes, a BD™ P800 collection tube, and an EDTA tube. Around 9.00 am, within 5 minutes, participants consumed a standardized liquid meal (200 mL, 300 kcal, 36.8 g carbohydrates, 12.0 g protein, and 11.6 g fat; Nutridrink strawberry flavor, Nutricia, The Netherlands). Blood samples were drawn at 7 time points (0 or baseline, 30, 60, 90, 120, 180, and 240 minutes). Blood collected in the BD™ P800 collection tubes and EDTA tubes was immediately stored on ice, while the Vacutainer SST II Advance Gel tube was clotted for at least 30 minutes at room temperature. The samples were then centrifuged to separate the plasma from the serum and were subsequently stored at -80°C until batch-wise analyses. Plasma levels of ghrelin, leptin, and PYY were measured from blood samples collected in the BD™ P800 collection tubes using a U-Plex Assay Platform (Meso-Scale Diagnostics, Gaithersburg, MD, USA). Commercial kits were used to measure serum total cholesterol (Roche Diagnostics, Woerden, The Netherlands), plasma glucose (Instruchemie, Delfzijl, The Netherlands), and serum insulin (Crystal Chem, Elk Grove Village, IL, USA).

Statistical analysis

Data are presented as means ± standard deviation. The normality of data was evaluated using the Shapiro-Wilk test, along with visual histograms, and Q-Q plots. For the baseline characteristics, waist-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Lean mass was calculated by subtracting the fat mass from the total body weight, both measured by BIA. Body fat percentage was calculated by dividing fat mass by body weight and multiplying by 100. HOMA-IR was calculated by multiplying fasting insulin levels (mU/L) with fasting glucose levels (mmol/L) and dividing by 22.5. Baseline characteristics were compared between ethnicities within the same sex using an independent t-test for normally distributed data (age, weight, length, BMI, hip circumference, fat mass, lean mass, and fasting glucose). Data that were not normally distributed data were log10 transformed to achieve normality (i.e., total cholesterol) and then analyzed using an independent t-test. For data that remained not normally distributed after log10 transformation (i.e., waist circumference, waist-hip ratio, body fat percentage, fasting insulin, HOMA-IR, and total triglycerides), a non-parametric test was used.

To compare the excursion of the hunger and satiety hormones during an MMTT, the total area under the curve (tAUC) was calculated using the trapezoid rule (26). To determine the incremental AUC (iAUC), which represents the area under the curve starting from the baseline, the area below the baseline value was subtracted from the tAUC. The Mann-Whitney U test, a non-parametric test, was used to compare tAUC and iAUC between the two ethnicities, as not all data followed a normal distribution. In addition, a two-way repeated measures ANOVA was applied, with 'time' as the within-subject factor and 'ethnicity' as the between-subject factor, to compare the hormone excursion during an MMTT between ethnicities. The general linear model's estimated marginal means comparison, corrected using the Bonferroni method to account for multiple testing, was used to compare the means at each time point between ethnicities. The leptin/ghrelin ratio was calculated by dividing baseline leptin levels (ng/mL) by baseline plasma ghrelin levels (ng/mL) and comparing the results between ethnicities by sex with the non-parametric Mann-Whitney U test, as the data were not normally distributed. Correlations between variables were assessed using Spearman's correlations due to the non-normal distribution of some data.

All statistical analyses were performed using SPSS v.29.0.1.0. Armonk, NY: IBM Corp. All graphs were created with GraphPad Prism software version 9.3.1 for Windows (GraphPad Software, San Diego, California, USA). The threshold for significance was set at $P < 0.05$.

RESULTS

Baseline characteristics

As described elsewhere (27), South Asian males were shorter than Europid males (1.79 ± 0.06 m *vs.* 1.86 ± 0.07 m; P = 0.015), while body weight was similar (74.9 ± 7.2 kg *vs.* 73.6 ± 6.0 kg), resulting in a higher BMI among South Asian compared to Europid males (23.3 ± 1.5 kg/m^2 *vs.* 21.3 ± 1.5 kg/m^2; P = 0.004). In addition, South Asian males had more fat mass (13.5 ± 5.5 kg *vs.* 7.3 ± 2.0 kg; P = 0.002) and a higher body fat percentage (18.0 ± 7.2% *vs.* 9.8 ± 2.3%; P < 0.001) than Europid males. Furthermore, South Asian males had higher serum total cholesterol levels than Europid males (3.6 ± 0.5 mmol/L *vs.* 3.1 ± 0.4 mmol/L; P = 0.027).

Similarly, South Asian females were shorter than Europid females (1.63 ± 0.06 m *vs.* 1.74 ± 0.08 m; P = 0.003), and in addition had a lower body weight (60.3 ± 5.7 kg *vs.* 68.1 ± 9.1 kg; P = 0.020), resulting in a similar BMI compared to Europid females (22.6 ± 1.8 kg/m^2 *vs.* 22.5 ± 1.2 kg/m^2). South Asian females had a lower lean mass than Europid females (41.2 ± 3.6 kg *vs.* 51.5 ± 6.6 kg; P < 0.001).

No significant differences were observed in fasting glucose, insulin, or HOMA-IR between South Asian versus Europid males and between South Asian and Europid females.

South Asian males and females have similar plasma peptide YY levels at baseline and throughout an MMTT compared to Europid males and females

Generally, plasma levels of the satiety-inducing hormone PYY remained largely stable during the MMTT in males and females. In male South Asians *vs.* Europids, plasma PYY levels did not differ at baseline (1.08 ± 0.47 ng/mL *vs.* 1.43 ± 0.57 ng/mL; P = 0.109) or at other time points during the MMTT (**Fig. 1A**). In addition, plasma PYY did not change over time between ethnicities ($P_{Interaction}$ = 0.708, **Suppl. Table 1**) with no difference in tAUC (P = 0.168; **Fig. 1B**).

In female South Asians *vs.* Europids, plasma PYY levels also did not differ at baseline (1.51 ± 0.75 ng/mL *vs.* 1.15 ± 0.31 ng/mL; P = 0.233) or at other time points during the MMTT except at 240 minutes, being higher in South Asians compared to Europids (**Fig. 1C**). However, this did not result in a difference over time ($P_{Interaction}$ = 0.490; **Suppl. Table 2**) or a difference in tAUC (P = 0.310) between both ethnicities (**Fig. 1D**).

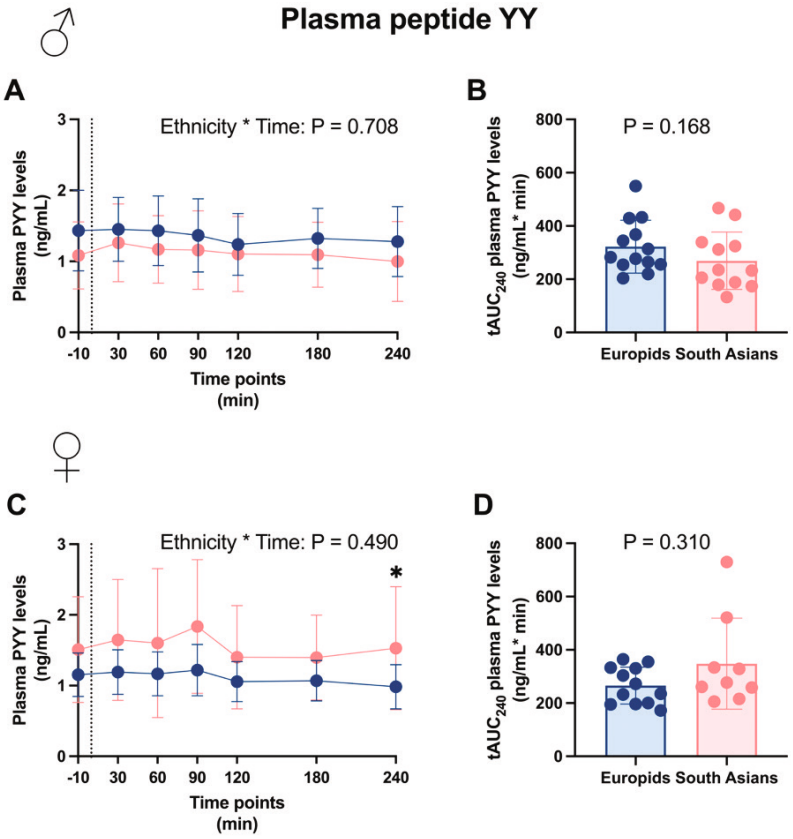

Figure 1. Plasma peptide YY levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graph showing plasma peptide YY (PYY) levels before and during a mixed meal tolerance test (MMTT) in South Asian males (n=12) compared to Europid males (n=13) **(A)**. Box plots showing the total area under the curve (tAUC) **(B)** in South Asian and Europid males. Similarly, a line graph showing the plasma PYY levels during an MMTT in South Asian females (n=9) compared to Europid females (n=12) **(C)** and box plots showing the tAUC **(D)** for South Asian and Europid females. Circles represent means in **A** and **C**, and individuals' values in **B** and **D** and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. The dotted lines in **A** and **C** indicate the time of the ingestion of the liquid meal. We were unable to retrieve a blood sample of three South Asian females at one time point.

South Asian males have lower plasma ghrelin levels at baseline and throughout an MMTT compared to Europid males

Generally, plasma levels of the hunger hormone ghrelin first decreased during the MMTT, and from 120 min on increased. In male South Asians *vs.* Europids, plasma ghrelin levels were lower at baseline (2.25 ± 1.42 ng/mL *vs.* 3.74 ± 2.08 ng/mL; P = 0.049). In addition, during the MMTT plasma ghrelin levels remained lower at 30, 60, 90, 120, and 240 minutes (all P < 0.05, **Fig. 2A**), resulting in a tendency towards a lower tAUC in South Asian compared with Europid males (P = 0.060; **Fig. 2B**). No significant difference in the excursion of plasma ghrelin levels was observed over time between ethnicities ($P_{Interaction}$ = 0.484; **Suppl. Table 1**).

In females, baseline plasma ghrelin levels did not differ between South Asians and Europids (2.88 ± 1.58 ng/mL *vs.* 3.47 ± 1.95 ng/mL; P = 0.651) nor during the MMTT (**Fig. 2C**). As in males, excursion of plasma ghrelin levels did not differ between ethnicities ($P_{Interaction}$ = 0.295; **Suppl. Table 2**), and tAUC was not different between ethnicities (**Fig. 2D**).

South Asian males and females have higher plasma leptin levels at baseline and throughout an MMTT compared to Europid males and females

Plasma levels of the satiety-inducing hormone leptin initially decreased, and from 120 min on increased. In South Asian males, plasma leptin levels were higher at baseline (82.9 ± 53.5 ng/mL *vs.* 21.7 ± 21.5 ng/mL; P < 0.001) and during all other time points during the MMTT (all P < 0.01) compared to Europid males (**Fig. 3A**). In addition, excursion of plasma leptin levels differed between ethnicities ($P_{Interaction}$ = 0.015; **Suppl. Table 1**) and South Asians had a higher tAUC (P < 0.001; **Fig. 3B**) compared to Europids. Compared to males, leptin levels were markedly higher in females of both ethnicities, which is in line with their higher fat mass. In South Asian females, circulating plasma leptin levels were significantly higher at baseline (410 ± 236 ng/mL *vs.* 183 ± 129 ng/mL; P = 0.030) and at 180 minutes during the MMTT (P = 0.043) (**Fig. 3C**) compared to Europid females. Again, excursion of plasma leptin levels differed between ethnicities ($P_{Interaction}$ = 0.047; **Suppl. Table 2**) and South Asians had a higher tAUC (P = 0.041) compared to Europids (**Fig. 3D**).

South Asian males and females have higher leptin/ghrelin ratio before and throughout the MMTT compared to Europid males and females

Given the reciprocal function of leptin (satiety-inducing) and ghrelin (hunger-inducing), the leptin/ghrelin ratio is a measure for hunger suppression (28, 29). The ghrelin/leptin ratio was overall higher at baseline and during the MMTT in South Asian *vs.* Europid males (**Fig. 4A**) and females (**Fig. 4B**).

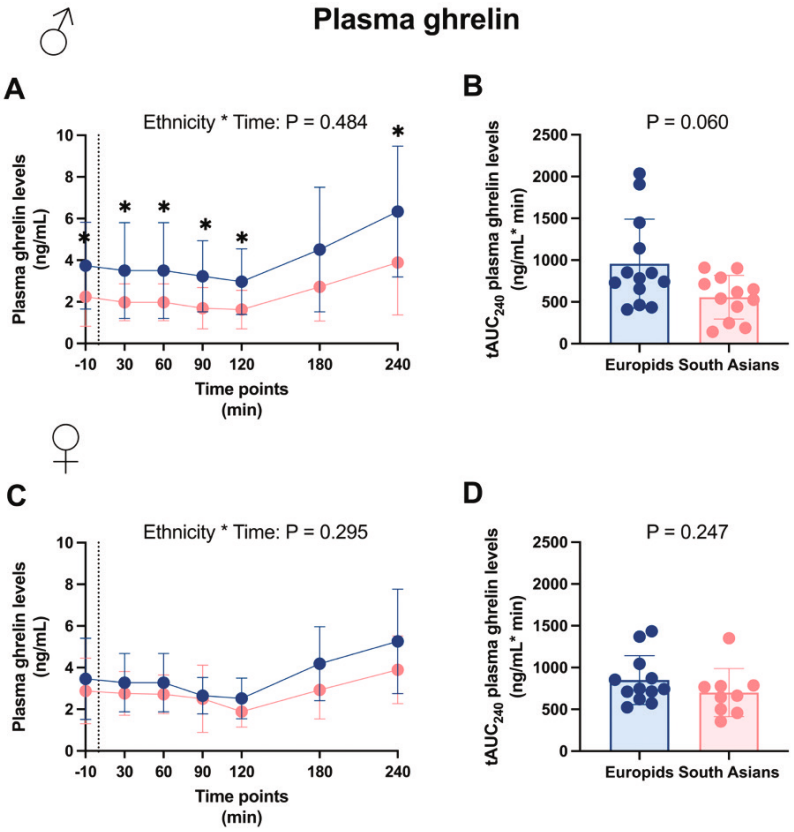

Figure 2. Plasma ghrelin levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graph showing plasma ghrelin levels before and during a mixed meal tolerance test (MMTT) in South Asian males (n=12) compared to Europid males (n=13) **(A)**. Box plots showing the total area under the curve (tAUC) **(B)** in South Asian and Europid males. Similarly, a line graph showing the plasma ghrelin levels during an MMTT in South Asian females (n=9) compared to Europid females (n=12) **(C)** and box plots showing the tAUC **(D)** for South Asian and Europid females Circles represent means in **A** and **C**, and individuals' values in **B** and **D**, and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. The dotted lines in **A** and **C** indicate the time of the ingestion of the liquid meal. We were unable to retrieve a blood sample of three South Asian females at one time point.

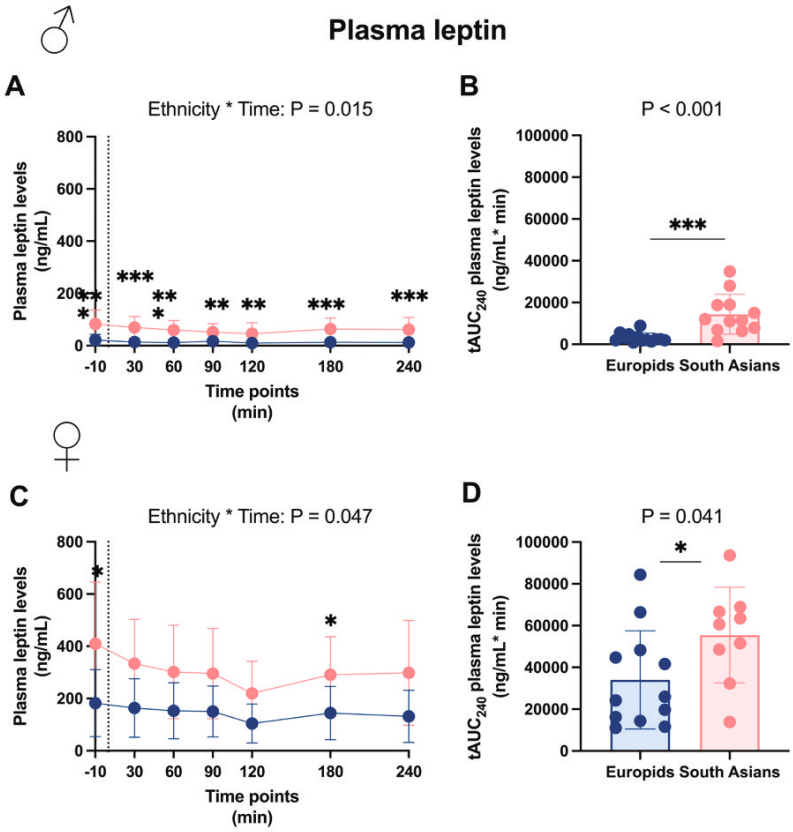

Figure 3. Plasma leptin levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graph showing plasma leptin levels before and during a mixed meal tolerance test (MMTT) in South Asian males (n=12) compared to Europid males (n=13) **(A)**. Box plots showing the total area under the curve (tAUC) **(B)** in South Asian and Europid males. Similarly, a line graph showing the plasma leptin levels during an MMTT in South Asian females (n=9) compared to Europid females (n=12) **(C)** and box plots showing the tAUC **(D)** for South Asian and to Europid females. Circles represent means in **A** and **C**, and individuals' values in **B** and **D**, and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. The dotted lines in **A** and **C** indicate the time of the ingestion of the liquid meal. We were unable to retrieve a blood sample of three South Asian females at one time point.

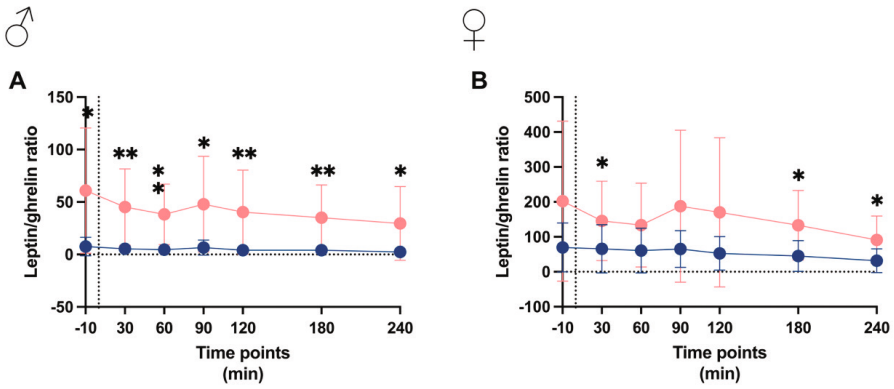

Figure 4. Plasma leptin/ghrelin ratio before and during a mixed meal tolerance test in South Asian compared to Europid males and females

Line graphs showing the plasma leptin/ghrelin ratio at baseline in South Asian males (n=12) compared to Europid males (n=13) **(A)** and in South Asian females (n=12) and Europid females (n=12) **(B)**. Circles represent means and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. The dotted lines indicate the time of the ingestion of the liquid meal.

Since previous studies showed a relationship between a higher leptin/ghrelin ratio and increased BMI, as well as reduced insulin sensitivity (29, 30), we correlated the baseline leptin/ghrelin ratio with both BMI and HOMA-IR in both ethnicities. In South Asian males, no correlation was observed between leptin/ghrelin ratio and BMI (rho = 0.266, P = 0.404; **Suppl. Fig. 2A**) or HOMA-IR (rho = 0.455, P = 0.138; **Suppl. Fig. 2B**). In Europid males, we did find a positive correlation between plasma leptin/ghrelin ratio and BMI (rho = 0.676, P = 0.011; **Suppl. Fig. 2A**) but not with HOMA-IR (rho = 0.214, P = 0.482; **Suppl. Fig. 2B**). In South Asian females, no correlations were found between leptin/ghrelin ratio and BMI (rho = 0.252, P = 0.430; **Suppl. Fig. 2C**) and HOMA-IR (rho = 0.210, P = 0.513; **Suppl. Fig. 2D**). Similarly, in Europid females no correlations were found between leptin/ghrelin ratio and BMI (rho = 0.210, P = 0.513; **Suppl. Fig. 2C**) and HOMA-IR (rho = 0.252, P = 0.430; **Suppl. Fig. 2D**).

Baseline plasma leptin levels positively correlate with fat mass and fat percentage in South Asian males and females

Given that fat percentage is higher in South Asians, which is not reflected by a higher BMI at least for females, we reasoned that plasma leptin levels may be a better marker for adiposity. Therefore, we next assessed whether baseline plasma leptin levels were related to fat mass and fat percentage in both South Asian and Europid males and females.

Baseline plasma leptin levels strongly positively correlated with fat mass in South Asian males (rho = 0.825, P < 0.001; **Fig. 5A**), and South Asian females (rho = 0.587, P = 0.045; **Fig. 5C**). A similar correlation was observed in Europid males (rho = 0.587, P = 0.035; **Fig. 5A**) but not Europid females (rho = 0.420, P = 0.175; **Fig. 5C**). Likewise, baseline plasma leptin levels were strongly positively correlated with fat percentage in South Asian males (rho = 0.909, P < 0.001; **Fig. 5B**) and South Asian females (rho = 0.608, P = 0.036; **Fig. 5D**). No such correlation was observed in Europid males (rho = 0.489, P = 0.090; **Fig. 5B**) and Europid females (rho = 0.266, P = 0.404; **Fig. 5D**).

In contrast, baseline plasma ghrelin and plasma PYY levels did not correlate with fat mass or fat percentage in South Asian or Europid males and females (data not shown).

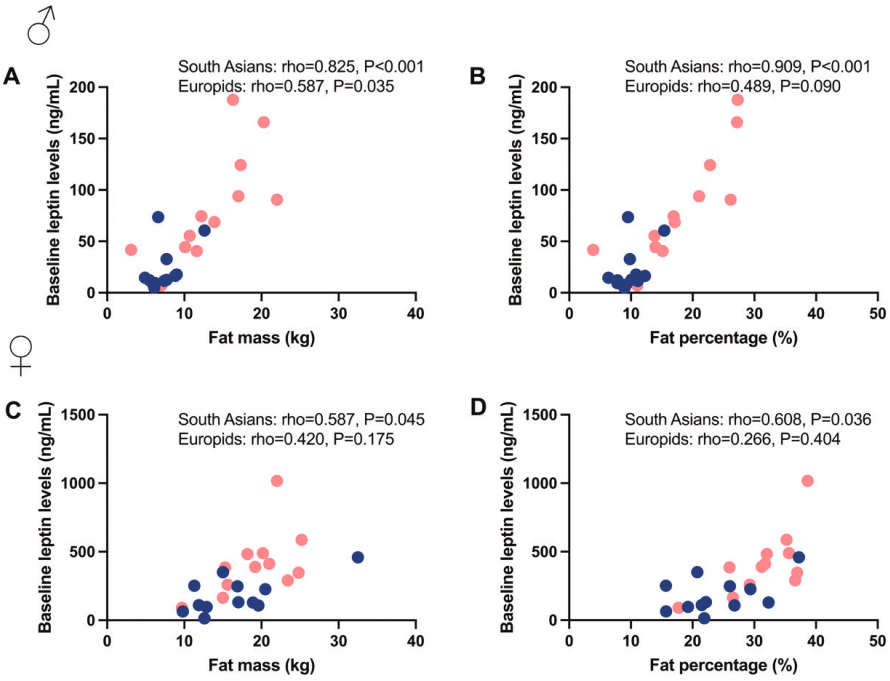

Figure 5. Correlations between baseline circulating plasma leptin levels and fat mass and fat percentage in South Asian compared to Europid males and females

Spearman correlation plots between baseline plasma leptin levels and fat mass in South Asian males (n=12, pink circles) and Europid males (n=13, blue circles) **(A)** and in South Asian females (n=12, pink circles) and Europid females (n=12, blue circles) **(C)**. Spearman collections of baseline plasma leptin levels and fat percentage in South Asian males (n=12, pink circles) and Europid males (n=13, blue circles) **(B)** and South Asian females (n=12, pink circles) and Europid females (n=12, blue circles) **(D)**.

DISCUSSION

In this study, we aimed to investigate the baseline levels of the hunger and satiety hormones ghrelin, leptin, and PYY, as well as their response to an extended MMTT, in young and lean South Asians compared to Europids. We observed several differences: Discussion

In this study, we aimed to investigate the baseline levels of the hunger and satiety hormones ghrelin, leptin, and PYY, as well as their response to an extended MMTT, in young and lean South Asians compared to Europids. We observed several differences: lower plasma ghrelin levels at baseline and throughout the MMTT in South Asian males, and higher plasma leptin levels at baseline and throughout the MMTT in both South Asian males and females. This resulted in a significantly higher leptin/ghrelin ratio especially in South Asian compared to Europid males. Furthermore, baseline plasma leptin levels were strongly positively correlated with both fat mass and fat percentage in South Asian males and females. The potential contribution of high leptin levels to the disadvantageous metabolic phenotype in South Asians remains to be determined.

With respect to the satiety hormone PYY, we did not observe differences between the two ethnicities at baseline and during the MMTT. While PYY has been generally described to peak 1-2 hours after a meal in healthy individuals (19), we did not observe a peak of PYY in our participants. However, this is in line with a previous study where PYY was measured in healthy individuals in an extended MMTT with multiple meals during different time points. This study showed no major change in circulating PYY levels after the first meal up to 180 minutes, after which another meal was provided (31). Theoretically, we may have missed a potential change of plasma PYY after the meal, as our study stopped after 240 minutes. A more prolonged study using various meals could give more information on PYY excursion in South Asians compared to Europids.

In contrast, plasma levels of the hunger hormone ghrelin were consistently lower in South Asian compared to Europid males at baseline and during the MMTT. Likewise, a previous study showed a lower fasting acylated ghrelin concentration in healthy South Asian *vs.* Europid men (32). Notably, they measured only the acylated ghrelin levels, while our study assessed total ghrelin, which includes both acylated and des-acyl ghrelin. Des-acyl ghrelin has both independent and opposing functions to acylated ghrelin (33, 34). Based on the disadvantageous metabolic phenotype of South Asians, lower plasma ghrelin levels would seem counterintuitive. However, both acylated and des-acyl ghrelin excursions have been found to be decreased in people at risk for T2DM after an oral glucose tolerance test (35). The underlying mechanisms behind the

observed lower ghrelin levels in this population, as well as the consequences, remain unclear and require further research.

The largest difference observed between South Asians and Europids throughout the MMTT in the current study was in circulating leptin levels. At baseline and throughout the MMTT circulating leptin levels were higher in both South Asian males and females compared to Europids, resulting in higher $tAUC_{0\text{-}240}$ in South Asians. Higher leptin levels in females compared to males within the same ethnicity and in South Asians compared with other ethnicities, including Europids, are in line with previous studies (32, 36, 37). Since leptin is secreted by adipocytes, adipocyte size and mass influence leptin secretion. Indeed, South Asians are known to have larger adipocytes compared to Europids (38). Since adipocyte size has been shown to be positively associated with leptin secretion (36), this could at least in part explain the higher circulating leptin levels observed in South Asians. Unfortunately, we did not measure adipocyte size in our cohort to study this directly. However, we did show that fat mass and fat percentage strongly positively correlated with leptin levels in both South Asian males and females and, to a lesser extent, in Europid males, suggesting that this may largely explain the higher leptin levels. In fact, the correlation between plasma leptin levels and fat mass and fat mass percentage was particularly strong in South Asian men, with a rho of 0.91 for fat mass percentage. Since BMI does not accurately reflect body composition in South Asians, these data suggest that leptin may form a more reliable measure for body fat in South Asians, especially in men. Interestingly, leptin levels were particularly high in South Asian females. Although this could at least in part be explained by their higher fat mass, potentially also a more leptin resistant state could be present in females, further contributing to higher leptin levels.

As ghrelin and leptin have opposing effects with respect to hunger and satiety, we reasoned the leptin/ghrelin ratio would provide insight into the balance between hunger and satiety signals. We observed a higher plasma leptin/ghrelin ratio throughout the MMTT in both South Asian compared to Europid males and females. This elevated ratio in males was mainly driven by a higher baseline ratio in South Asians. For females, the differences in plasma leptin/ghrelin ratio seemed more pronounced in the postprandial state, with higher ratios at 30, 180, and 240 min during the MMTT. Higher leptin/ghrelin ratios, whether in fasting conditions or postprandially, have been observed in people living with obesity (28). Interestingly, a higher leptin/ghrelin ratio is also associated with lower resting metabolic rate and insulin resistance (28, 29, 39), characteristics also known in the South Asian population (11, 40). This suggests that a higher leptin/ghrelin ratio in South Asians could potentially reflect a more compromised metabolic status, particularly in males. However, we did not find a significant correlation between the

baseline leptin/ghrelin ratio and BMI or HOMA-IR. The elevated plasma leptin/ghrelin ratio in South Asians could potentially influence the outcome of some intervention strategies. Previous research showed that individuals living with obesity with a higher baseline leptin/ghrelin ratio lose less body weight and fat mass after 12 weeks on a low-calorie diet (39). Whether the observed difference in the leptin/ghrelin ratio exacerbates as South Asians become more metabolically compromised, and how it affects the efficacy of caloric restriction in this population, or upon anti-obesity medication, remains to be seen.

A strength of this study is our ability to measure various hormones during an extended MMTT, lasting up to 240 minutes postprandially. The inclusion of a young and lean cohort of South Asian males and females allows us to gain insight into this population before significant metabolic derangements occur, as well as to observe differences between the sexes. However, this study does have its limitations. We used a 200 mL liquid meal, and while it offers a more comprehensive nutrient composition than an OGTT, the relatively small volume may have resulted in some hormonal differences being overlooked. Furthermore, while we extended the MMTT duration to 240 minutes, there remains the possibility that we missed observing changes in excursions of hormones that peak later than 240 minutes.

In conclusion, in South Asians *vs*. Europids, plasma ghrelin levels are lower and leptin levels are higher at baseline and during an MMTT, resulting in a higher leptin/ghrelin ratio particularly in South Asian men. Although the exact impact of these differentially regulated satiety hormones on the adverse metabolic profile of South Asians warrants further investigation.

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SUPPLEMENTAL DATA

Supplemental Table 1. Overview of the total and incremental areas under the curve in South Asian compared to Europid males

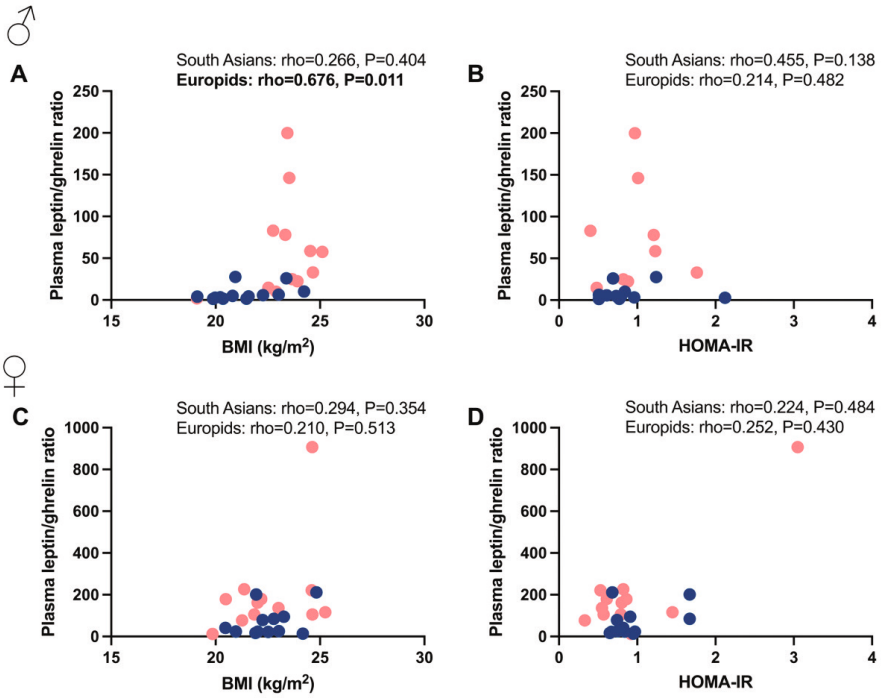
	Europid males	South Asian males	P values	
	$tAUC_{0-240}$	$tAUC_{0-240}$	$tAUC_{0-240}$	$P_{Interaction}$
Hunger & satiety hormones				
Plasma PYY (ng/mL * min)	323±99[a]	269±108[b]	0.168[c]	0.708[c]
Plasma Ghrelin (ng/mL *min)	958±532[a]	556±261[b]	0.060[c]	0.484[c]
Plasma Leptin (ng/mL * min)	3168±2173[a]	14404±9543[b]	<0.001[c]	0.015[c]

Means and standard deviations of the total area under the curve (tAUC) of the PYY, ghrelin, and leptin excursions for both South Asian and Europid males. P-values for the comparison between the two ethnicities were obtained via the non-parametric Man-Whitney U test and the p-value of the interaction was analyzed via repeated measurement ANOVA. PYY, peptide YY. Letters indicate n values of each ethnicity, *a*n=13; *b*n=12; and *c*n=25.

Supplemental Table 2. Overview of the total and incremental areas under the curve in South Asian compared to Europid females

	Europid females	South Asian females	P values	
	$tAUC_{0-240}$	$tAUC_{0-240}$	$tAUC_{0-240}$	$P_{Interaction}$
Hunger & satiety hormones				
Plasma PYY (ng/mL * min)	266±69[a]	348±171[b]	0.310[c]	0.490[c]
Plasma Ghrelin (ng/mL * min)	851±29[a]	701±288[b]	0.247[c]	0.295[c]
Plasma Leptin (ng/mL * min)	34046±23463[a]	55491±22860[b]	0.041[c]	0.047[c]

Means and standard deviations of the total area under the curve (tAUC) for both South Asian and Europid females with the p-value of the comparison between the two ethnicities via the non-parametric Man-Whitney U test and the p-value of the interaction analyzed via a repeated measurement ANOVA. PYY, peptide YY. Letters indicate n values of each ethnicity, *a*n=12; *b*n=9; and *c*n=21.



Supplemental Figure 1. Correlations between baseline circulating plasma leptin/ghrelin ratio and body mass index and HOMA-IR in South Asian compared to Europid males and females
Spearman correlation plots between baseline plasma leptin/ghrelin ratio and body mass index (BMI) in South Asian males (n=12, pink circles) and Europid males (n=13, blue circles) **(A)** and in South Asian females (n=12, pink circles) and Europid females (n=12, blue circles) **(C)**. Spearman collections of baseline plasma leptin/ghrelin ratio and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) in South Asian males (n=12, pink circles) and Europid males (n=13, blue circles) **(B)** and South Asian females (n=12, pink circles) and Europid females (n=12, blue circles) **(D)**.