Metabolic hormones and ethnic aspects in obesity

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CHAPTER 3

GLP-1, GIP, AND GLUCAGON EXCURSIONS DURING A MIXED MEAL TOLERANCE TEST IN YOUNG AND LEAN SOUTH ASIANS VERSUS EUROPIDS

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Submitted

ABSTRACT

Objectives

South Asians exhibit an unfavorable metabolic phenotype characterized by visceral obesity, insulin resistance and dyslipidemia. Since various hormones play a critical role in regulating energy metabolism, we aimed to study the meal-induced excursion of incretin hormones and glucagon in South Asians and Europids.

Method

49 young, lean South Asian (n=24), and Europid (n=25) males and females underwent an extended (up to 240 min) mixed meal tolerance test (MMTT). At seven time points circulating incretins (active and total GLP-1 and GIP), glucagon, and parameters related to glucose and lipid metabolism were measured.

Results

While a single peak (t=30 min) in circulating glucose levels was observed in Europids, a biphasic peak (t=30 and t=90 min) was found in South Asian males and females. In addition, South Asian males exhibited an increased insulin response, with elevated levels at the corresponding glucose peaks. On the other hand, South Asian females demonstrated a drop in circulating glucagon at t=90 min, and double peaks of total and active GLP-1 and GIP (t=30 and t=120 min). Postprandial lipid excursions did not differ between ethnicities.

Conclusion

South Asians respond to an MMTT with a biphasic peak in glucose levels, without differences in postprandial lipid excursions. Potentially as a consequence, female South Asians demonstrated a second peak of active GLP-1 and GIP and a drop in glucagon. Interestingly, an increased insulin response was only observed in South Asian males. We speculate that these effects result from biphasic gastric emptying in South Asians.

INTRODUCTION

Obesity is defined by the World Health Organization as abnormal or excessive fat accumulation that presents a health risk (1). Currently, 16 percent of adults are living with obesity worldwide (1). This number has more than doubled since 1980 and is expected to continue to rise (1, 2). Obesity not only impacts health directly but also increases the risk of various obesity-related diseases, such as type 2 diabetes mellitus (T2DM), cardiovascular diseases, and various forms of cancer (3, 4). Therefore, understanding the underlying mechanisms of obesity development is important to effectively prevent and treat obesity.

Certain ethnic populations, such as the South Asian population, are more prone to develop obesity and obesity-related diseases. For instance, South Asians have a fourfold increased risk of developing T2DM compared to other ethnicities (i.e., Whites, Chinese Americans, African Americans, and Hispanics) (5). In addition, they develop obesity-related diseases at a younger age and a lower BMI than Europids (6, 7). The underlying mechanisms for their increased risk to develop T2DM are only partly known. South Asians exhibit an unfavorable metabolic phenotype characterized by higher fat mass, especially in visceral and ectopic areas, lower muscle mass, and dyslipidemia (8). They typically also show insulin resistance, which is already observable at birth (9-12). Furthermore, they have lower resting energy expenditure, and brown adipose tissue volume, which may further contribute to increased fat storage (13).

While various metabolic hormones released by the gastrointestinal tract play a role in regulating energy balance (e.g., satiety and energy expenditure) and insulin sensitivity, their potentially different regulation in South Asians versus Europids has not been studied in detail. Incretin hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), secreted by the intestinal L-cells and K-cells, respectively, are important mediators in lowering postprandial glucose levels. These hormones bind to their respective receptors (GLP-1R and GIPR) on the beta cells in the pancreas (14-16), and promote the release of insulin in a glucose-dependent manner (17). Both GLP-1 and GIP also induce satiety in the hypothalamus and hindbrain (15, 17, 18). Glucagon is released from the alpha cells of the pancreas in response to a drop in circulating glucose levels. It regulates glucose homeostasis by stimulating glycogenolysis and gluconeogenesis, with the aim to increase plasma glucose levels. Furthermore, it has been shown to contribute to energy balance by increasing energy expenditure (19). A novel therapeutical intervention for the treatment of obesity and T2DM is based on the potential of GLP-1, GIP, and glucagon to concomitantly induce satiety and increase energy expenditure (20). Understanding the release of these

hormones in response to a meal in South Asians versus Europids could provide valuable insights into underlying mechanisms that contribute to the higher cardiometabolic disease risk in South Asians. Moreover, it could offer insight in the potential benefit of interventions based on these hormones in South Asians. The mixed meal tolerance test (MMTT) is a low-invasive and well-established method, used to assess pancreatic beta cell function and the release of incretin and glucagon hormones, with the variety of nutrients in the used liquid meal providing a more accurate reflection of postprandial metabolic processes compared to the common used oral glucose tolerance test (21-23).

Therefore, in the current study, we aimed to investigate the effect of an extended MMTT (up to 240 min) on the excursion of GLP-1, GIP, and glucagon and parameters related to glucose and lipid metabolism (i.e., glucose, insulin, FFA, triglycerides, and total cholesterol) in young and lean South Asians and Europids.

METHODS

Study Design

This study uses samples and data obtained from the CAMI project (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 (24). 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 18-25 kg/m^2 and age of 18-30 years. We included an additional Europid male (resulting in 13 instead of 12 Europid males) 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 4 grandparents originally descent 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.

Procedure

Participants were asked to withhold from vigorous exercise 48 hours preceding the study days and not to drink alcohol or caffeinated drinks 24 hours preceding the study days. In addition, they were instructed to eat a standardized meal (prepared supermarket meal including pasta or noodles, ranging from 450–600 kcal, or similar meal prepared by themselves) in the evening before the experiment and not to eat or drink anything other than water until the completion of the study day.

Questionnaires and anthropometric measurements

After an overnight fast, participants arrived at the LUMC at 08:00 am, where they underwent questionnaires about their medical history and current health. Thereafter, body weight and body composition were assessed using bioelectrical impedance analysis (BIA) (InBody720, InBody CO., Ltd., CA, USA). In addition, height, waist, and hip circumference were obtained using a measuring lint. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Mixed meal tolerance test

A catheter was inserted in the antecubital vein for venous blood sampling, whereafter a screening sample was obtained using Vacutainer SST II Advance Gel and EDTA tubes, to determine inclusion into the study. Measurements included full blood count, glucose, insulin, kidney function, liver function, and lipid metabolism. If the participants were eligible for inclusion they proceeded with the study. Next, a baseline sample prior to ingestion of the mixed meal test was obtained using Vacutainer SST II Advance Gel tubes, a BD™ P800 collection tube, and an EDTA tube. At approximately 9.00 am, within 5 minutes, the participants ingested 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). Blood samples were collected at 7 time points in total (-10 or baseline, 30, 60, 90, 120, 180, and 240 minutes). The BD™ P800 collection tubes and EDTA tubes were directly stored on ice after obtaining blood, and blood obtained using the Vacutainer SST II

Advance Gel tubes was clotted for at least 30 minutes at room temperature. Thereafter, the samples were centrifuged to obtain plasma or serum, respectively, and stored at -80°C until batch-wise analyses. Plasma levels of total and active GLP-1, total and active GIP, and glucagon were measured from blood collected in the BD™ P800 collection tubes using a U-Plex Assay Platform (Meso-Scale Diagnostics, Gaithersburg, MD, USA). Commercially available kits were used for the measurements of serum free fatty acids (FFA) (Wako chemicals, Neuss, Germany), serum triglycerides and serum total cholesterol (Roche Diagnostics, Woerden, the Netherlands), plasma glucose (Instruchemie, Delfzijl, the Netherlands), and serum insulin (Chrystal Chem, Elk Grove Village, IL, USA).

Statistical analysis

Data are expressed as mean ± standard deviation. The normality of data was assessed using the Shapiro-Wilk test, 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, obtained by BIA, from the total body weight. 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 this by 22.5.

Baseline characteristics of the participants 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). Not normally distributed data were log10 transformed to yield normal distribution (i.e., total cholesterol) and analyzed using an independent t-test. Non-parametric tests were performed on data that were not normally distributed even after log 10 transformation (i.e., waist circumference, waist-hip ratio, body fat percentage, fasting insulin, HOMA-IR, and triglycerides).

For the comparison of the excursion of GLP-1, GIP, and parameters for glucose and lipid metabolism in response to a mixed meal test, we calculated the total area under the curve ($tAUC_{0\text{-}240}$, $tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$) with the trapezoid rule (25). To determine the incremental AUC ($iAUC_{0\text{-}240}$, $iAUC_{0\text{-}60,}$ and $iAUC_{60\text{-}240}$), defined as the AUC corrected for baseline, we subtracted the area below the baseline value from the $tAUC_{0\text{-}240}$. To compare the tAUC and iAUC between the two ethnicities, the non-parametric Mann-Whitney U test was used, as not all the data was normally distributed. In addition, we used a two-way repeated measures ANOVA with the within-subject factor 'time' and the between-subject factor 'ethnicity' for the comparison of the response of various hormones throughout a mixed meal test between ethnicities. We compared the means of each time point

between ethnicities of the general linear model using the estimated marginal means comparison corrected with the Bonferroni methods to correct for multiple tests.

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). Significance was set at $P < 0.05$.

RESULTS

Baseline characteristics

All groups were comparable with respect to age. Both South Asian males and females were significantly shorter than their Europid counterparts (males: P = 0.015; females P = 0.003; **Table 1**). In males, there was no difference in body weight between ethnicities, which resulted in a higher BMI in South Asians (P = 0.004). On the other hand, South Asian females had a lower body weight compared to Europid females (P = 0.020), which combined with the shorter stature resulted in a similar BMI for the two ethnicities. The South Asian females had a lower lean mass compared to the Europid females ($P < 0.001$). Furthermore, the South Asian males had a higher fat mass (P = 0.002), and both South Asian males and females had a higher body fat percentage compared to the Europids (males: $P < 0.001$; females: P = 0.020). Fasting glucose, insulin and, as a consequence, HOMA-IR did not differ between ethnicities in both males and females. Lastly, the South Asian males had a higher serum total cholesterol compared to Europid males (P = 0.027).

South Asians exhibit biphasic glucose excursions during an MMTT

Following the mixed meal, South Asian and Europid males and females showed a single peak in circulating plasma glucose levels after 30 minutes (**Fig. 1**). Interestingly, South Asian males and females exhibited an additional glucose peak at 90 minutes, with glucose levels being significantly higher compared to both Europid males and females (P = 0.026 and P = 0.044, respectively) which pursued until 120 min in South Asian females (P = 0.028). Although these changes did not result in significant differences over time between the ethnicities (males: $P_{Interaction}$ = 0.368; females: $P_{Interaction}$ = 0.156; **Fig. 1**, **Suppl. Tables 1 and 2**), this biphasic response led to a significantly elevated $tAUC_{0\text{-}240}$ of the glucose excursion in South Asian compared to Europid females (P = 0.043, **Suppl. Table 2 and Fig. 1**). After evaluating both peaks individually and calculating $tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$ of the glucose response, we observed no difference in $tAUC_{0\text{-}60}$, while $tAUC_{60\text{-}240}$ was significantly higher in South Asian compared to Europid females (P = 0.020; **Suppl. Fig. 1**. Furthermore, the $iAUC_{0\text{-}240}$ for the glucose excursion did not differ between ethnicities in either sex (P = 0.949 and P = 0.114, **Suppl. Tables 1 and 2** and **Fig. 1**).

Table 1. Baseline characteristics

	Males		Females	
	Europids (*n*=13)	**South Asians (*n*=12)**	**Europids (*n*=12)**	**South Asians (*n*=12)**
Age, years	21.7 ± 2.9	23.3 ± 3.2	23.1 ± 2.1	23.3 ± 3.3
Body length, m	1.86 ± 0.07	1.79 ± 0.06*	1.74 ± 0.08	1.63 ± 0.06**
Body weight, kg	73.6 ± 6.0	74.9 ± 7.2	68.1 ± 9.1	60.3 ± 5.7*
BMI, kg/m^2	21.3 ± 1.5	23.3 ± 1.5**	22.5 ± 1.2	22.6 ± 1.8
Waist circumference, cm	69.9 ± 12.9	75.7 ± 5.9	69.8 ± 4.6	68.9 ± 3.8
Hip circumference, cm	93.3 ± 3.1	94.1 ± 5.0	89.8 ± 6.4	87.9 ± 5.9
Waist to hip ratio	0.7 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0
Lean mass, kg	66.4 ± 5.3	61.4 ± 8.2	51.5 ± 6.6	41.2 ± 3.6***
Fat mass, kg	7.3 ± 2.0	13.5 ± 5.5**	16.6 ± 6.1	19.1 ± 4.6
Fat percentage, %	9.8 ± 2.3	18.0 ± 7.2***	24.1 ± 6.5	31.5 ± 5.9*
Fasting glucose, mmol/L	4.9 ± 0.5	4.9 ± 0.3	4.8 ± 0.3	4.8 ± 0.3
Fasting insulin, mU/L	4.0 ± 2.0	4.4 ± 1.8	4.4 ± 1.6	4.4 ± 3.3
HOMA-IR	0.8 ± 0.5	0.8 ± 0.5	0.9 ± 0.4	0.9 ± 0.7
Serum Total Cholesterol, mmol/L	3.1 ± 0.4	3.6 ± 0.5*	3.4 ± 0.5	3.7 ± 0.9
Serum Triglycerides, mmol/L	0.6 ± 0.2	0.8 ± 0.7	0.5 ± 0.2	0.6 ± 0.3

Asterisk signs (*) indicate significant differences between ethnicities within a specific sex. *P < 0.05, **P < 0.01, ***P < 0.001. BMI, Body Mass Index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

In South Asian males, serum insulin levels were higher compared to Europid males at 30 and 90 minutes (**Fig. 2**). This resulted in significantly different levels of circulating serum insulin over time (P = 0.046), a higher $tAUC_{0\text{-}240}$ (P = 0.044), and a higher $iAUC_{0\text{-}240}$ (P = 0.016) in South Asian compared to Europid males (**Suppl. Table 1** and **Fig. 2**) but not females (**Suppl. Table 2** and **Fig. 2**). For males and females, both the $tAUC_{0\text{-}60}$ and the $tAUC_{60\text{-}240}$ for the insulin excursion was not significantly different between South Asians and Europids (**Suppl. Fig. 2**).

South Asian females have lower circulating plasma glucagon levels at 90 min after MMTT compared to Europid females

In males, we did not find any difference in excursion of circulating plasma glucagon levels between South Asians and Europids over time ($P_{Interaction}$ = 0.409). Correspondingly, $tAUC_{0\text{-}240}$ and $iAUC_{0\text{-}240}$ did not differ between ethnicities (P = 0.242 and P = 0.671, respectively; **Suppl. Table 1** and **Fig. 3**). In females, however, South Asians exhibited lower circulating plasma glucagon levels at 30 and 90 minutes (P = 0.040 and P =

0.013, respectively; **Fig. 3**). This resulted into significant differences in circulating plasma glucagon levels over time during the MMTT between South Asian and Europid females ($P_{Interaction}$ = 0.045, **Suppl. Table 2** and **Fig. 3**). The $tAUC_{0-240}$ tended to be lower (P = 0.095) in South Asian compared to Europid females (**Suppl. Table 2** and **Fig. 3**). We did not find a significant different $tAUC_{0-60}$ or $tAUC_{60-240}$ for the glucagon excursion; **Suppl. Fig. 3**). Furthermore, $iAUC_{0-240}$ did not differ between ethnicities in females.

South Asian males exhibit lower circulating plasma total GLP-1 levels while females exhibit higher active GLP-1 during an MMTT compared to Europids

Next, we assessed the excursions of plasma incretins during the MMTT. In males, circulating plasma levels of both total GLP-1 and active GLP-1 were not significantly different at different time points and over time between ethnicities (total GLP-1: $P_{Interaction}$ = 0.774, active GLP-1: $P_{Interaction}$= 0.655; **Fig. 4 and 5**). However, in South Asian males, the $tAUC_{0-240}$ of circulating total GLP-1, but not active GLP-1, was significantly lower compared to Europids (total GLP-1: P = 0.030; active GLP-1: P = 0.487; **Fig 4 and 5** and **Suppl. Table 1**). For excursions of total GLP-1, $tAUC_{0-60}$ tended to be lower and $tAUC_{60-240}$ was significantly lower in South Asian males compared to Europids (P = 0.098 and P = 0.026, respectively, **Suppl. Fig. 4**). The $iAUC_{0-240}$ for circulating total and active GLP-1 excursions were not significantly different in males in either ethnicity (total GLP-1: P = 0.936; active GLP-1: P = 0.487; **Fig 4 and 5** and **Suppl. Table 1**).

In females, South Asians had higher levels of circulating plasma total and active GLP-1 at time point 120 minutes during the MMTT (P = 0.016 and P = 0.002, respectively; **Fig. 4 and 5**). Circulating plasma active GLP-1 was also higher at 240 minutes in South Asian females compared to Europid females (P = 0.003; **Fig 5**). However, this did not result in a different excursion of circulating plasma total and active GLP-1 over time between ethnicities (total GLP-1: $P_{Interaction}$ = 0.394, active GLP-1: $P_{Interaction}$= 0.387; **Fig. 4 and 5** and **Suppl. Table 2**) or $tAUC_{0-240}$ (total GLP-1: P = 0.148; active GLP-1: P = 0.058) and $iAUC_{0-240}$ (total GLP-1: P = 0.219; active GLP-1: P = 0.129) (**Suppl. Table 2**, **Fig. 4 and 5**). Interestingly, the $tAUC_{60-240}$ for active GLP-1 was significantly higher in South Asian compared to Europid females (P = 0.015; **Suppl. Fig. 4**).

South Asian females exhibit higher circulating plasma total and active GIP levels during an MMTT compared to Europid females

In males, circulating plasma total and active GIP levels did not differ over time between both ethnicities (total GIP: $P_{Interaction}$ = 0.715, active GIP: $P_{Interaction}$= 0.729; **Suppl. Table 1** and **Fig. 6**). As a result, $tAUC_{0-240}$ did not differ between ethnicities (P = 0.538 and P = 0.319, respectively), also not for the $tAUC_{0-60}$ and the $tAUC_{60-240}$ (**Suppl. Fig 5**). However,

the $iAUC_{0\text{-}240}$ was significantly higher in South Asian compared to Europid males (total GIP: P = 0.040; active GIP: P = 0.033).

In females, circulating plasma total and active GIP levels were higher in South Asians at 120, 180, and 240 minutes during the MMTT (**Suppl. Table 2** and **Fig. 6 and 7**). This resulted in a tendency towards higher circulating plasma total GIP and active GIP levels over time ($P_{Interaction}$ = 0.069 and $P_{Interaction}$ = 0.052, respectively; **Suppl. Table 2**, **Fig. 6 and 7**). The $tAUC_{0\text{-}240}$ and $iAUC_{0\text{-}240}$ of both circulating plasma total and active GIP were not significantly different in females between South Asians and Europids. However, similarly to active GLP-1 in South Asian females, plasma active GIP increased during the second part of the MMTT with a significantly higher $tAUC_{60\text{-}240}$ of plasma active GIP (P = 0.041; **Suppl. Fig. 5**).

Postprandial lipid excursions do not differ between South Asian and Europid males and females

Finally, we assessed lipid levels (serum FFA, triglycerides, and total cholesterol) during the MMTT. In males, circulating serum FFA levels did not differ between time points or over time between South Asians and Europids (**Suppl. Table 1** and **Suppl. Fig. 6**). In females, circulating serum FFA levels remained suppressed longer over time in South Asians compared to Europids, which resulted in lower circulating serum FFA levels at 180 minutes (P = 0.038) and over time ($P_{Interaction}$ = 0.022; **Suppl. Table 2** and **Suppl. Fig. 6**). While the $tAUC_{0\text{-}240}$ of circulating serum FFA levels did not differ (P = 0.557; **Suppl. Tables 1 and 2**), the iAUC was lower in South Asians compared to Europids (P = 0.029). The responses of circulating serum triglyceride levels were similar in both South Asian males and females compared to their Europid counterparts (**Suppl. Tables 1 and 2** and **Suppl. Fig. 6**).

In males, circulating serum total cholesterol levels were higher in South Asians compared to Europids during all time points (all P < 0.05). Since no difference in excursion in total cholesterol was observed over time in both ethnicities, this resulted in no significant difference over time ($P_{Interaction}$ = 0.306), but in a significantly higher $tAUC_{0\text{-}240}$ (P = 0.007) in South Asian males compared to Europid males, with no significant different $iAUC_{0\text{-}240}$ (P = 0.059) (**Suppl. Table 1** and **Suppl. Fig. 6**). In females, circulating serum cholesterol levels remained similar between South Asians and Europids at the various time points and over time (**Suppl. Table 2** and **Suppl. Fig. 6**).

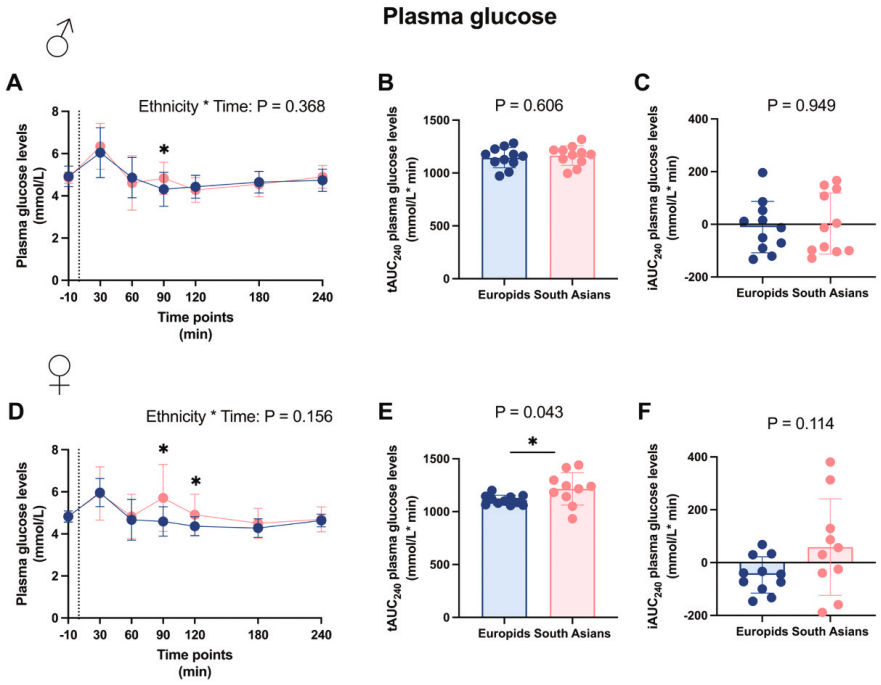

Figure 1. Plasma glucose levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graphs showing plasma glucose levels before and during a mixed meal tolerance test (MMTT) in South Asian (n=11) compared to Europid (n=11) males **(A)**. Box plots showing total area under the curve ($tAUC_{0\text{-}240}$) **(B)** and incremental area under the curve ($iAUC_{0\text{-}240}$) **(C)** in South Asian and Europid males. Similarly, line graphs showing plasma glucose levels during an MMTT in South Asian females (n=10) compared to Europid females (n=11) **(D)** and box plots showing $tAUC_{0\text{-}240}$ **now** and $iAUC_{0\text{-}240}$ **(F)** for South Asian and Europid females **(F)**. Circles represent means in **A** and **D** and individuals' values in **B, C, E,** and **F**, and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. Dotted lines represent the time of the ingestion of the liquid meal (t=0). We were unable to retrieve a blood sample of one South Asian male at two time points, and from one Europid male, two South Asian females, and one Europid female at one time point.

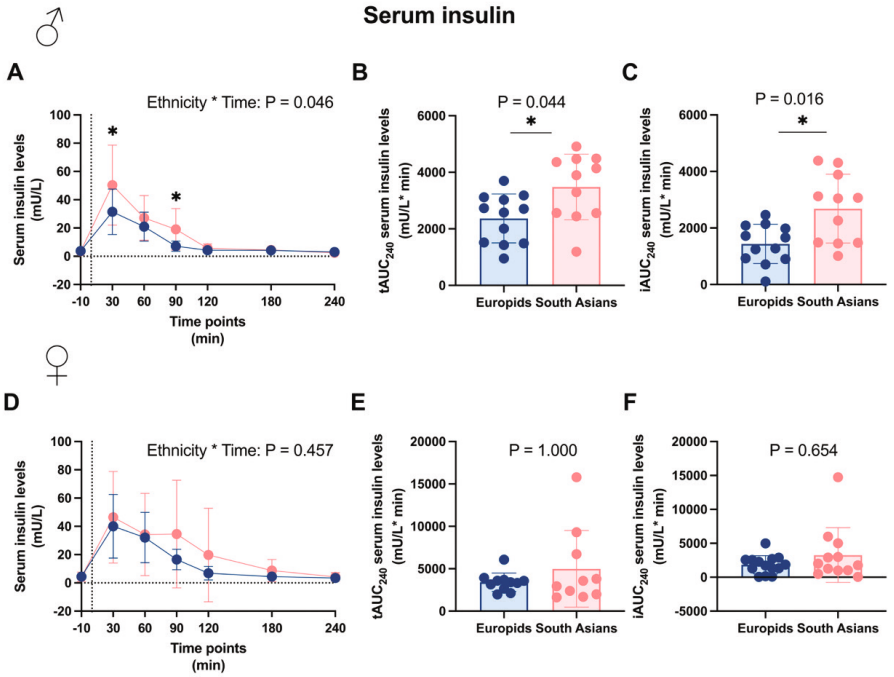

Figure 2. Serum insulin levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graphs showing serum insulin levels before and during a mixed meal tolerance test (MMTT) in South Asian (n=11) compared to Europid (n=12) males **(A)**. Box plots showing the total area under the curve ($tAUC_{0\text{-}240}$) **(B)** and incremental area under the curve ($iAUC_{0\text{-}240}$) **(C)** in South Asian and Europid males. Similarly, line graphs showing serum insulin levels during an MMTT in South Asian females (n=10) compared to Europid females (n=11) **(D)** and box plots showing the $tAUC_{0\text{-}240}$ **(E)** and $iAUC_{0\text{-}240}$ **(F)** for South Asian and Europid females **(F)**. Circles represent means in **A** and **D**, and individuals' values in **B, C, E,** and **F**, and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. Dotted lines represent the time of the ingestion of the liquid meal (t=0). We were unable to retrieve a blood sample of one South Asian female at two time points, and from one South Asian male, Europid male, one South Asian female, and one Europid female at one time point.

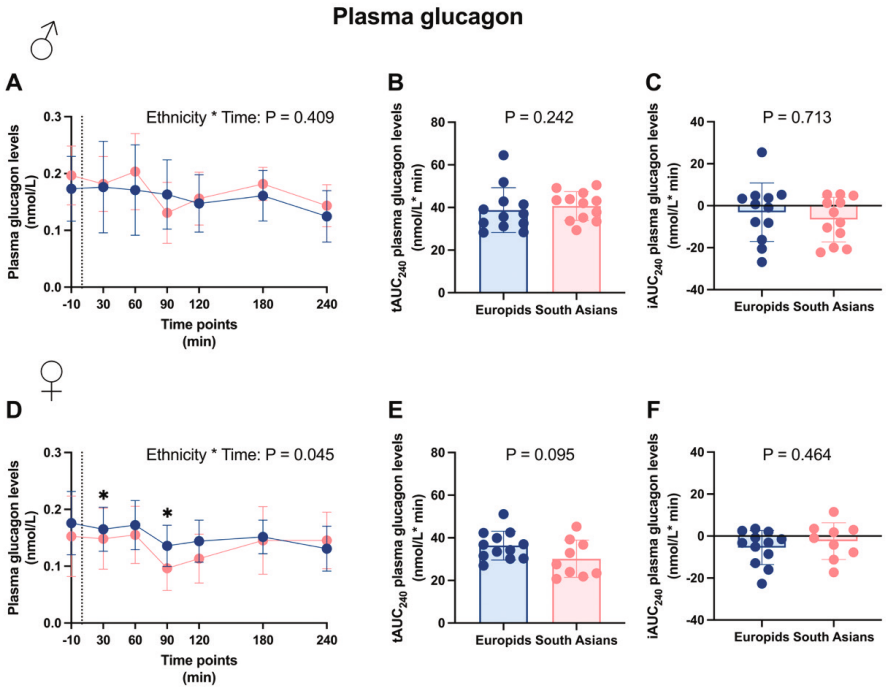

Figure 3. Plasma glucagon levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graphs showing plasma glucagon levels before and during a mixed meal tolerance test (MMTT) in South Asian (n=12) compared to Europid (n=12) males **(A)**. Box plots showing the total area under the curve ($tAUC_{0\text{-}240}$) **(B)** and incremental area under the curve ($iAUC_{0\text{-}240}$) **(C)** in South Asian and Europid males. Similarly, line graphs showing plasma glucagon levels during an MMTT in South Asian females (n=9) compared to Europid females (n=12) **(D)** and box plots showing the $tAUC_{0\text{-}240}$ **(E)** and $iAUC_{0\text{-}240}$ **(F)** for South Asian and Europid females **(F)**. Circles represent means in **A** and **D**, and individuals' values in **B, C, E,** and **F**, and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. Dotted lines represent the time of the ingestion of the liquid meal (t=0). Due to a technical error, one sample of one Europid male is missing and we were unable to retrieve a blood sample and of three South Asian females at one time point.

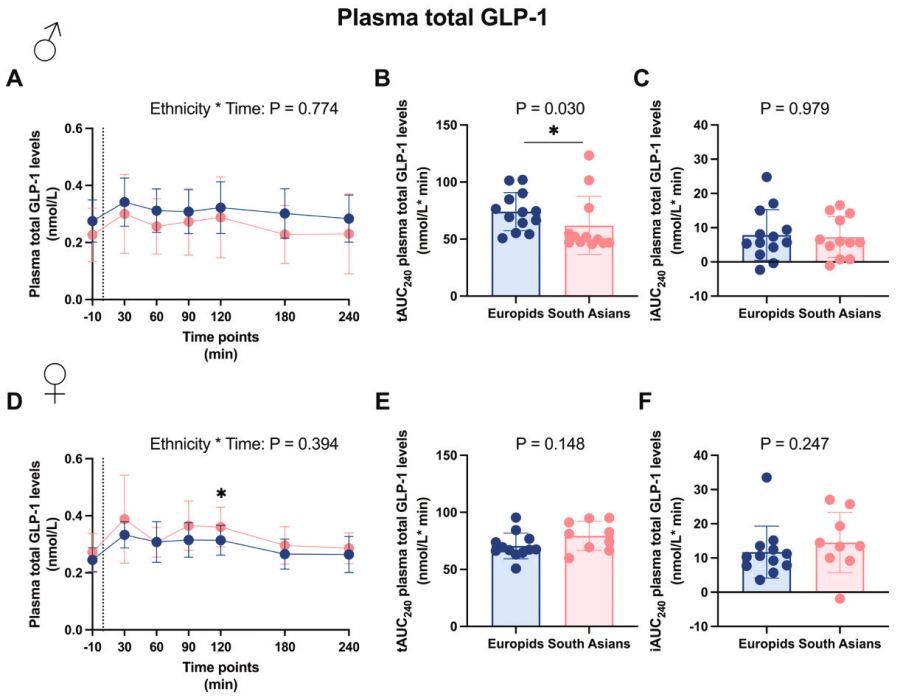

Figure 4. Plasma total glucagon-like peptide-1 levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graphs showing plasma total glucagon-like peptide-1 (GLP-1) levels before and during a mixed meal tolerance test (MMTT) in South Asian (n=12) compared to Europid (n=13) males **(A)**. Box plots showing the total area under the curve ($tAUC_{0\text{-}240}$) **(B)** and incremental area under the curve ($iAUC_{0\text{-}240}$) **(C)** in South Asian and Europid males. Similarly, line graphs showing the plasma GLP-1 levels during an MMTT in South Asian females (n=9) compared to Europid females (n=12) **(D)** and box plots showing the $tAUC_{0\text{-}240}$ **(E)** and $iAUC_{0\text{-}240}$ **(F)** for South Asian and Europid females **(F)**. Circles represent means in **A** and **D**, and individuals' values in **B, C, E,** and **F**, and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. Dotted lines represent the time of the ingestion of the liquid meal (t=0). We were unable to retrieve a blood sample of three South Asian females at one time point.

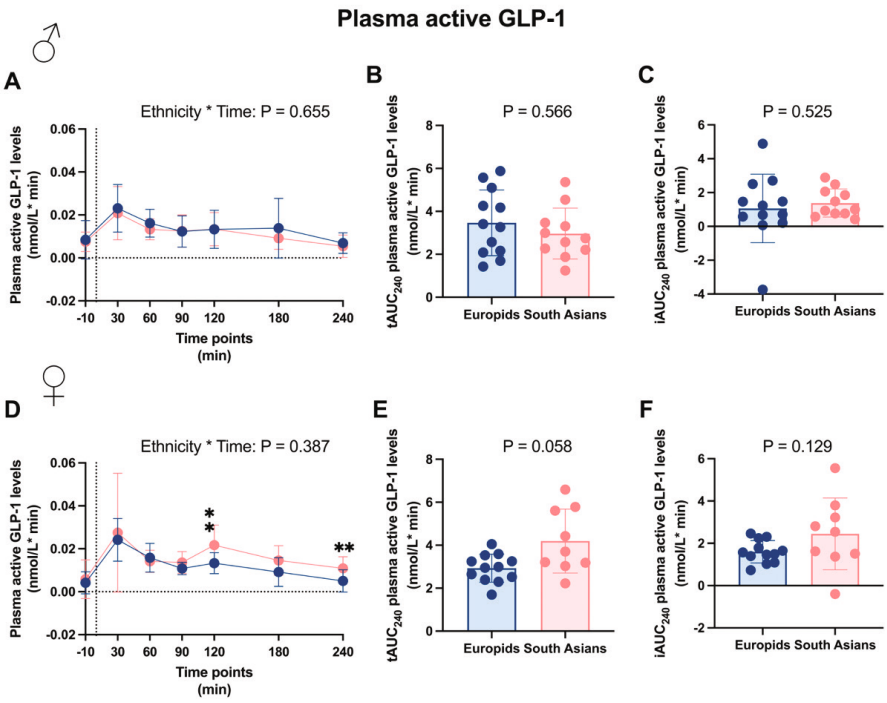

Figure 5. Plasma active glucagon-like peptide-1 levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graphs showing plasma active glucagon-like peptide-1 (GLP-1) levels before and during a mixed meal tolerance test (MMTT) in South Asian (n=12) compared to Europid (n=13) males **(A)**. Box plots showing the total area under the curve ($tAUC_{0\text{-}240}$) **(B)** and incremental area under the curve ($iAUC_{0\text{-}240}$) **(C)** in South Asian and Europid males. Similarly, line graphs showing the plasma active GLP-1 levels during an MMTT in South Asian females (n=9) compared to Europid females (n=12) **(D)** and box plots showing the $tAUC_{0\text{-}240}$ **(E)** and $iAUC_{0\text{-}240}$ **(F)** for South Asian and Europid females **(F)**. Circles represent means in **A** and **D**, and individuals' values in **B, C, E,** and **F**, and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. Dotted lines represent the time of the ingestion of the liquid meal (t=0). We were unable to retrieve a blood sample of three South Asian females at one time point.

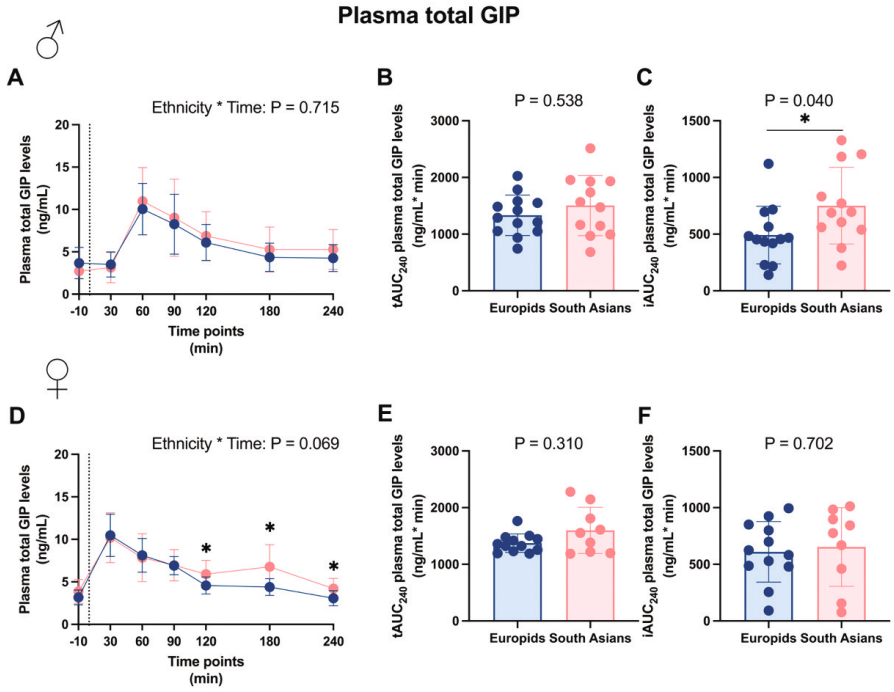

Figure 6. Plasma total glucose-dependent insulinotropic polypeptide levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graphs showing plasma total glucose-dependent insulinotropic polypeptide (GIP) levels before and during a mixed meal tolerance test (MMTT) in South Asian (n=12) compared to Europid (n=13) males **(A)**. Box plots showing the total area under the curve ($tAUC_{0-240}$) **(B)** and incremental area under the curve ($iAUC_{0-240}$) **(C)** in South Asian and Europid males. Similarly, line graphs showing the plasma GIP levels during an MMTT in South Asian females (n=9) compared to Europid females (n=12) **(D)** and box plots showing the $tAUC_{0-240}$ **(E)** and $iAUC_{0-240}$ **(F)** for South Asian and Europid females **(F)**. Circles represent means in **A** and **D**, and individuals' values in **B, C, E,** and **F**, and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. Dotted lines represent the time of the ingestion of the liquid meal (t=0). We were unable to retrieve a blood sample of three South Asian females at one time point.

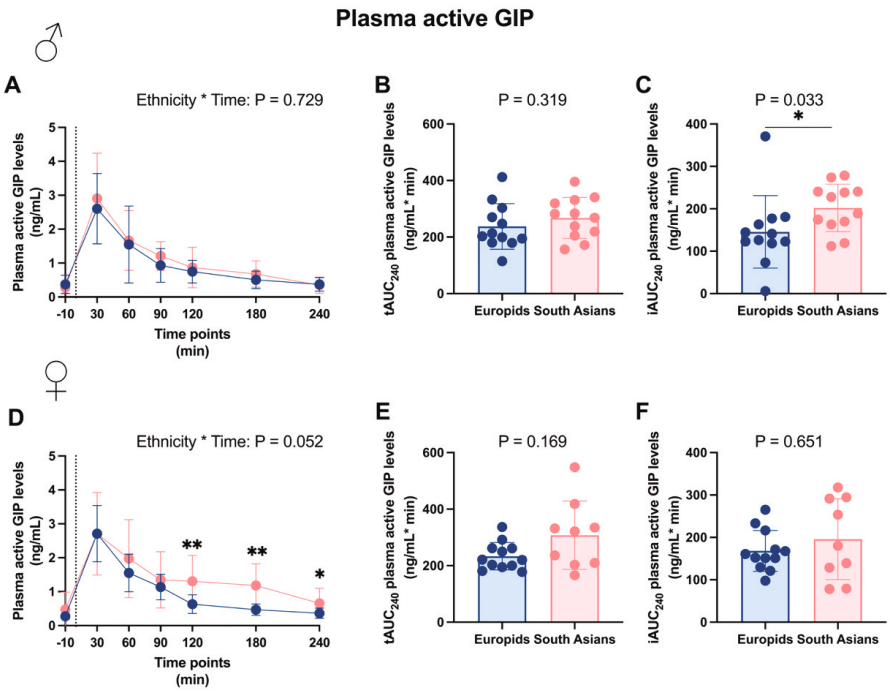

Figure 7. Plasma active glucose-dependent insulinotropic polypeptide levels before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graphs showing plasma active glucose-dependent insulinotropic polypeptide (GIP) levels before and during a mixed meal tolerance test (MMTT) in South Asian (n=12) compared to Europid (n=12) males **(A)**. Box plots showing the total area under the curve ($tAUC_{0\text{-}240}$) **(B)** and incremental area under the curve ($iAUC_{0\text{-}240}$) **(C)** in South Asian and Europid males. Similarly, line graphs showing the plasma active GIP levels during an MMTT in South Asian females (n=9) compared to Europid females (n=12) **(D)** and box plots showing the $tAUC_{0\text{-}240}$ **(E)** and $iAUC_{0\text{-}240}$ **(F)** for South Asian and Europid females **(F)**. Circles represent means in **A** and **D**, and individuals' values in **B, C, E,** and **F**, and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. Dotted lines represent the time of the ingestion of the liquid meal (t=0). We were unable to retrieve a blood sample of three South Asian females at one time point and of one Europid male one time point is missing due to a technical failure.

DISCUSSION

In this study, we aimed to compare the excursions of GLP-1, GIP, and glucagon as well as the response of markers related to glucose and lipid metabolism between young and lean South Asians and Europids in response to an extended MMTT. We observed several differences between the two ethnicities: South Asian males and females both exhibited a biphasic rather than monophasic glucose excursion. South Asian males exhibited an increased insulin response, with elevated levels at the corresponding glucose peaks. South Asian females demonstrated higher active GLP-1 and active GIP excursion of in the second phase of the MMTT, and a tendency towards lower $tAUC_{0\text{-}60}$ of circulating glucagon compared to Europid females.

Firstly, we found that both South Asian males and females exhibited a biphasic glucose response following the MMTT, which was not observed in Europids. In healthy individuals, the circulating glucose levels in response to a meal or an oral glucose tolerance test often follow either a single peak or a biphasic curve, both of which are part of a normal physiological response with the biphasic curve often resulting in a lower $tAUC_{0\text{-}240}$ than with a monophasic curve (26, 27). Of note, a more pronounced biphasic glucose curve also seemed to be found in healthy South Asians males following an MMTT in a recent study in healthy lean South Asian and Europid males (28), supporting an ethnic variation in glucose excursion following an MMTT. We speculate that delayed gastric emptying rates in South Asians compared to Europids at least in part contributed to the biphasic glucose peak. Interestingly, variations of gastric emptying rates between other ethnicities (i.e., Mexican Americans and American Indians compared to Caucasians) have been described in (29). The consequences of the observed biphasic glucose curve for healthy South Asians and the possible development of T2DM later in life remain unknown and warrant further research. However, considering the well-established link between elevated glucose levels and cardiometabolic diseases, the enhanced glucose $tAUC_{0\text{-}240}$ that accompanied the biphasic glucose excursion in South Asians might already contribute to an enhanced T2DM risk.

Postprandial increases in glucose levels are an important stimulus for the release of the incretin hormones GLP-1 and GIP by intestinal L-cells and K-cells, respectively. Indeed, this is consistent with the biphasic peaks in total and active GLP-1 and GIP we found in South Asian females. More specifically, for GLP-1, the increase in both total and active GLP-1 levels in South Asian females from 90 minutes onwards coincided with the increased $tAUC_{60\text{-}240}$ of plasma glucose observed in South Asian females compared to Europids. This pattern was also found for active GIP, having higher $tAUC_{60\text{-}240}$ in South Asians compared to Europid females. The excursions of postprandial incretin hormones

have only scarcely been studied in healthy lean South Asians compared to Europids. Our finding of higher postprandial GLP-1 levels in South Asian females is in line with a previous study executed in lean Dutch South Asians compared to Dutch Europid males, in which a higher GLP-1 peak was found in the South Asian men following an oral glucose tolerance test (30). However, in that study, only a single increased peak of GLP-1 was found in South Asians, which may have been due to the fact that an oral glucose load rather than a mixed meal was used. Remarkably, in our study, plasma total GLP-1 excursion was lower in the South Asian compared to Europid males. This could have been explained by the fact that baseline levels of total GLP-1 levels seemed to be lower, which persisted throughout the MMTT. An important question is how this sex difference in GLP-1 excursions can be explained between ethnicities. Potentially, South Asian males are more sensitive to the effects of GLP-1 compared to females and therefore require lower postprandial GLP-1 levels, which warrants further investigation. Regarding the mechanism, the lower total GLP-1 excursion throughout the MMTT in South Asian males could indicate a reduced release of GLP-1 by the enterocytes in response to the mixed meal. To the best of our knowledge, DPP4 activity has not been investigated in South Asians yet. Importantly, GLP-1 promotes the glucose-stimulated release of insulin by pancreatic beta cells, thereby lowering circulating glucose levels postprandially (31). Even though we observed a lower $tAUC_{0\text{-}240}$ of plasma total GLP-1 levels in South Asian males compared to Europids, their insulin response was higher. This could indicate that the diminished exposure to postprandial circulating plasma GLP-1 levels at least did not affect serum insulin excursion. However, GIP also plays an important role in the release of insulin postprandially. Of note, although in males, total GIP levels were equal between South Asians and Europids, the $iAUC_{240}$ was higher for both total and active GIP, supporting a steeper increase in GIP release during the MMTT. Indeed, for total GIP, this was especially evident between 30 and 60 minutes during the MMTT.

The biphasic peak in circulating glucose levels accompanied by the differences in excursions of GLP-1 (South Asian females) and GIP (South Asian males) could have contributed to the elevated insulin response in South Asians compared to Europids. The higher $tAUC_{0\text{-}240}$ of plasma glucose, along with the higher peak of serum insulin, which was especially evident in South Asian males, could indicate that, even in our young and lean cohort, South Asian males are already exhibiting decreased insulin sensitivity. However, this was not reflected in a difference in HOMA-IR index. Our data are in line with a recent study in which a mixed meal tolerance test was performed in young and lean South Asian and Europid males both at baseline and after 5-7% weight gain following 4-6 weeks of overfeeding. Although in that study the HOMA-IR index and fasting and postprandial glucose levels did not differ between South Asian and

Europid males, postprandial insulin levels were 62% higher in South Asians at baseline compared to their Europid counterparts (28).

Insulin decreases glucagon levels to help maintain glucose homeostasis. However, despite the higher serum insulin excursion in South Asian males, we did not observe a significant difference in plasma glucagon levels compared to Europid males. In females, however, plasma glucagon excursion was significantly different during the MMTT in South Asians compared to Europids, with a trend toward a lower total $AUC_{0\text{-}240}$, primarily driven by lower plasma glucagon levels at 30 and 90 minutes. The reduced plasma glucagon levels in South Asian females do not appear to be driven by higher insulin levels, as we did not observe significantly higher serum insulin levels in this group. However, the lower glucagon levels at 30 and 90 minutes coincided with the biphasic glucose curve observed in our cohort. The increased glucose levels in South Asian females could be contributing to lower glucagon levels by acting directly on the pancreatic alpha cells (32) or through other hormones, such as GLP-1 (33), which was significantly higher after 120 minutes in South Asian females compared to Europid females. Given that glucagon increases energy expenditure (34), the lower plasma glucagon levels in South Asian females could contribute to the lower energy expenditure known in the South Asian population (13). However, to our knowledge, differences in (postprandial) glucagon levels between South Asians and Europids and their relationship to variations in resting energy expenditure have not been previously reported.

Various incretin-based pharmacological interventions are emerging for the treatment of obesity and T2DM, which improve insulin sensitivity and reduce nutrient intake by inducing satiety (35). If the lower $tAUC_{0\text{-}240}$ of total GLP-1 levels, driven by lower baseline levels, observed in South Asian males in our study indeed impairs satiety, then these interventions could be especially beneficial for the South Asian population, especially the males. These treatments might be initiated earlier, given the already existing differences observed in young and lean South Asians compared to Europids in the current study. A previous study from our group showed similar beneficial effects in South Asians and Europids for improving the glycemic index and improving body composition in the treatment of T2DM with GLP-1 receptor agonist liraglutide (36, 37). Interestingly, a recent meta-analysis showed that GLP-1 receptor agonists have more benefit in cardiovascular outcomes in Asians compared to whites (38). Although the applicability for South Asian males remains to be determined, the fact whether South Asians benefit more from and potentially earlier treatment with incretin-based pharmacological intervention is an interesting topic for future studies.

A strength of this study is that we were able to measure a wide variety of hunger and satiety hormones during the MMTT up to 240 minutes after food ingestion in both males and females. However, this study is not without its limitations. Despite having a young and lean population with a healthy BMI, we already noticed metabolic differences. Matching South Asians and Europids remains a challenge due to differences in fat mass, fat percentage, and possibly insulin sensitivity already observed. Therefore, finding additional markers to match these ethnicities, could overcome these discrepancies. Furthermore, similar to the standard MMTT procedure, all participants ingested the same amount of liquid meal. However, due to variations in body composition between sexes and ethnicities, the caloric content of the liquid meal may represent a different proportion of energy intake relative to their basal metabolic rate.

In conclusion, South Asians respond differently to an MMTT compared to Europids, with a noticeable biphasic peak in glucose levels, and, potentially as a consequence, higher active GLP-1 and active GIP levels towards the end of the MMTT in South Asian compared to Europid females. Our findings suggest that various metabolic hormones are differentially regulated in South Asians compared to Europids, although the precise contribution to their disadvantageous metabolic phenotype remains to be determined.

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SUPPLEMENTAL DATA

Supplemental Table 1. Overview of the area under the curve of the excursions of glucose, insulin, hormones, and lipids during a mixed meal tolerance test in South Asian compared to Europid males

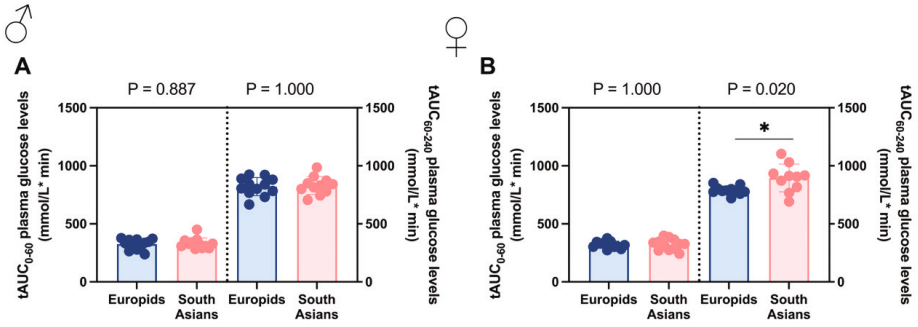
	Europids		South Asians				
	Males		Males		P values		
	$tAUC_{0\text{-}240}$	$iAUC_{0\text{-}240}$	$tAUC_{0\text{-}240}$	$iAUC_{0\text{-}240}$	$tAUC_{0\text{-}240}$	$iAUC_{0\text{-}240}$	$P_{Interaction}$
Glycemic parameters							
Plasma Glucose (mmol/L * min)	1145±99^{c}	1107±94^{c}	1165±93^{c}	1126±94^{c}	0.606^{w}	0.949^{w}	0.368^{w}
Serum Insulin (mU/L * min)	2369±865^{b}	2338±855^{b}	3483±1161^{c}	3457±115^{c}	0.044^{x}	0.016^{x}	0.046^{x}
Plasma Glucagon (nmol/L * min)	38.8±10.5^{b}	37.4±10.4^{b}	40.7±6.7^{b}	39.1±6.5^{b}	0.242^{y}	0.713^{y}	0.409^{y}
Incretin hormones							
Plasma Total GLP-1 (nmol/L * min)	74.1±16.7^{a}	71.9±16.2^{a}	62.0±25.5^{b}	60.2±24.7^{b}	0.030^{z}	0.979^{z}	0.774^{z}
Plasma Active GLP-1 (nmol/L * min)	3.5±1.5^{b}	3.4±1.5^{b}	3.0±1.2^{c}	2.9±1.2^{c}	0.566^{x}	0.525^{x}	0.655^{x}
Plasma Total GIP (ng/mL * min)	1333±357^{a}	1308±349^{a}	1505±532^{b}	1483±522^{b}	0.538^{z}	0.040^{z}	0.715^{z}
Plasma Active GIP (ng/mL * min)	238±81^{c}	234±80^{c}	268±72^{c}	266±71^{c}	0.319^{w}	0.033^{w}	0.660^{w}
Lipids							
Serum FFA (mmol/L * min)	63.2±19.0^{b}	59.3±17.6^{b}	73.7±25.5^{c}	69.7±24.0^{c}	0.316^{x}	0.651^{x}	0.921^{x}
Serum TG (mmol/L * min)	132.3±53.4^{b}	128.3±52.1^{b}	114.8±29.4^{c}	110.9±28.6^{c}	0.413^{x}	0.169^{x}	0.416^{x}
Serum TC (mmol/L * min)	708±104^{b}	683±101^{b}	847±114^{c}	819±110^{c}	0.007^{x}	0.059^{x}	0.306^{x}

Table showing the mean and standard deviation of the total area under the curve ($tAUC_{0\text{-}240}$) and incremental area under the curve ($iAUC_{0\text{-}240}$) of the excursions of glucose, insulin, hormones, and lipids during a mixed meal tolerance test for both South Asian and Europid males. P-values of the comparisons between the two ethnicities were obtained via the non-parametric Man-Whitney U test and the p-values of the interactions were analyzed via a repeated measurement ANOVA. FFA, free fatty acids; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; TC, total cholesterol; TG, triglycerides. Letters indicate n values of each ethnicity, *a*n=13; *b*n=12; *c*n=11; *d*n=10, and *w*n=22; *x*n=23; *y*n=24, *z*n=25.

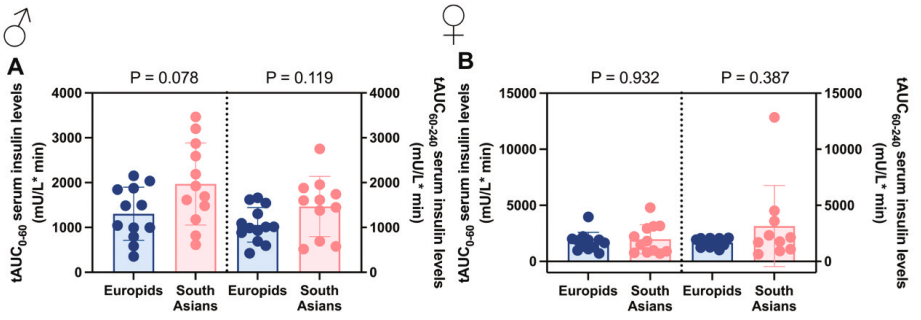
Supplemental Table 2. Overview of the area under the curve of the excursion of glucose, insulin, hormones, and lipids during a mixed meal tolerance test in South Asian compared to Europid females

	Europids		South Asians				
	Females		Females		P values		
	$tAUC_{0-240}$	$iAUC_{0-240}$	$tAUC_{0-240}$	$iAUC_{0-240}$	$tAUC_{0-240}$	$iAUC_{0-240}$	$P_{Interaction}$
Glycemic parameters							
Plasma Glucose (mmol/L * min)	1110±46[c]	1071±45[c]	1216±154[d]	1178±154[d]	0.043[v]	0.114[v]	0.156[v]
Serum Insulin (mU/L * min)	3409±1089[c]	3373±1086[c]	4988±4519[d]	4951±4506[d]	1.000[v]	0.654[v]	0.457[v]
Plasma Glucagon (nmol/L * min)	36.3±6.8[b]	35.0±6.4[b]	30.2±8.7[e]	29.1±8.3[e]	0.095[v]	0.464[v]	0.045[v]
Incretin hormones and glucagon							
Plasma Total GLP-1 (nmol/L * min)	70.5±11.3[b]	68.6±11.0[b]	79.5±12.8[e]	77.3±12.4[e]	0.148[v]	0.247[v]	0.394[v]
Plasma Active GLP-1 (nmol/L * min)	2.9±0.7[b]	2.9±0.6[b]	4.2±1.5[e]	4.1±1.5[e]	0.058[v]	0.129[v]	0.387[v]
Plasma Total GIP (ng/mL * min)	1374±164[b]	1351±164[b]	1598±408[e]	1571±402[e]	0.310[v]	0.702[v]	0.069[v]
Plasma Active GIP (ng/mL * min)	233±48[b]	231±48[b]	308±121[e]	304±118[e]	0.169[v]	0.651[v]	0.052[v]
Lipids							
Serum FFA (mmol/L * min)	94.6±25.1[c]	90.5±24.5[c]	84.2±30.0[d]	78.6±28.6[d]	0.349[v]	0.029[v]	0.022[v]
Serum TG (mmol/L * min)	137±45[c]	133±44[c]	170±72[d]	165±70[d]	0.314[v]	0.132[v]	0.538[v]
Serum TC (mmol/L * min)	805±129[c]	778±125[c]	850±195[d]	821±188[d]	0.654[v]	0.197[v]	0.454[v]

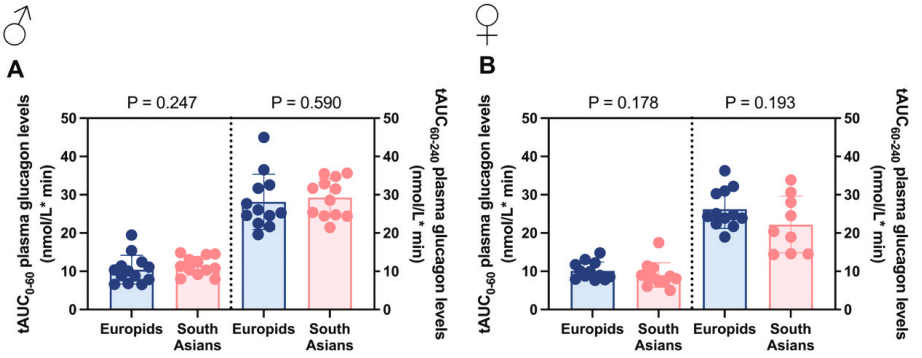
Table showing the mean and standard deviation of the total area under the curve ($tAUC_{0-240}$) and incremental area under the curve ($iAUC_{0-240}$) of the excursions of glucose, insulin, hormones, and lipid during a mixed meal tolerance test for both South Asian and Europid females. P-values of the comparisons between the two ethnicities were obtained via the non-parametric Man-Whitney U test and the p-values of the interaction were analyzed via a repeated measurement ANOVA. FFA, free fatty acids; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; TC, total cholesterol; TG, triglycerides. Letters indicate n values of each ethnicity, *b*n=12; *c*n=11; *d*n=10, *e*n=9, and *v*=21



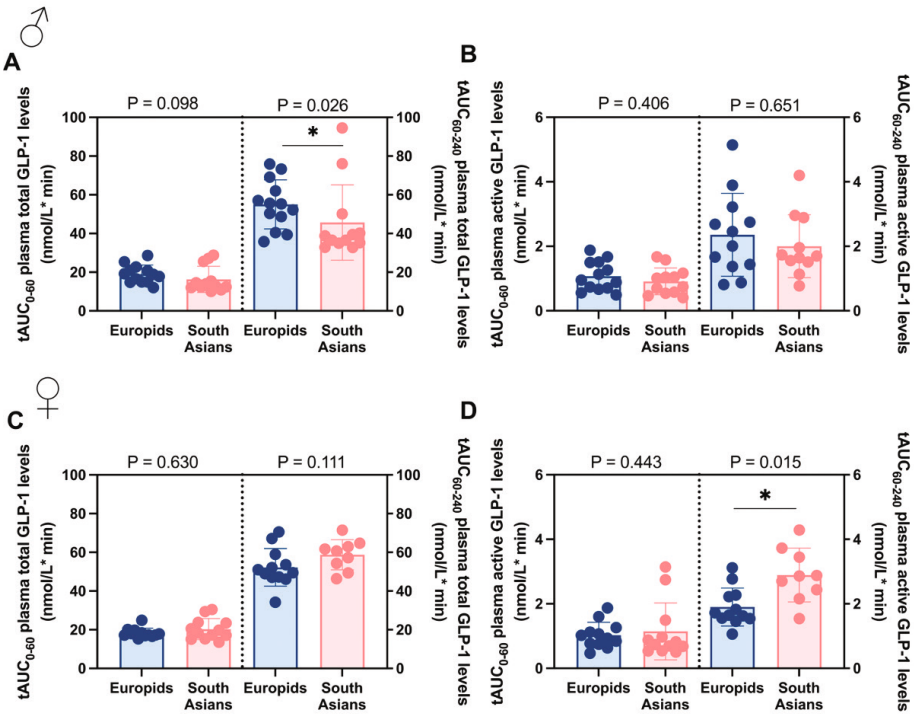
Supplemental Figure 1. Total areas under the curve of the glucose excursion within two periods during the mixed meal tolerance test in South Asian and Europid males and females
Box plots showing the total areas under the curve within two periods ($tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$) of the glucose excursion during the mixed meal tolerance test in South Asian (n=11) compared to Europid (n=12) males (**A)** and in South Asian (n=10) compared to Europid (n=11) females (**B**). Circles represent individuals' values and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. We were unable to retrieve a blood sample of one South Asian male at two time points, and of one Europid male, two South Asian females, and one Europid female at one time point.



Supplemental Figure 2. Total areas under the curve of the insulin excursion within two periods during the mixed meal tolerance test in South Asian and Europid males and females
Box plots showing the total areas under the curve within two periods ($tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$) of the insulin excursion in South Asian (n=11) compared to Europid (n=12) males (**A)** and in South Asian (n=10) compared to Europid (n=11) females (**B**). Circles represent individuals' values and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. We were unable to retrieve a blood sample of one South Asian female at two time points, and from one South Asian male, Europid male, one South Asian female, and one Europid female at one time point.

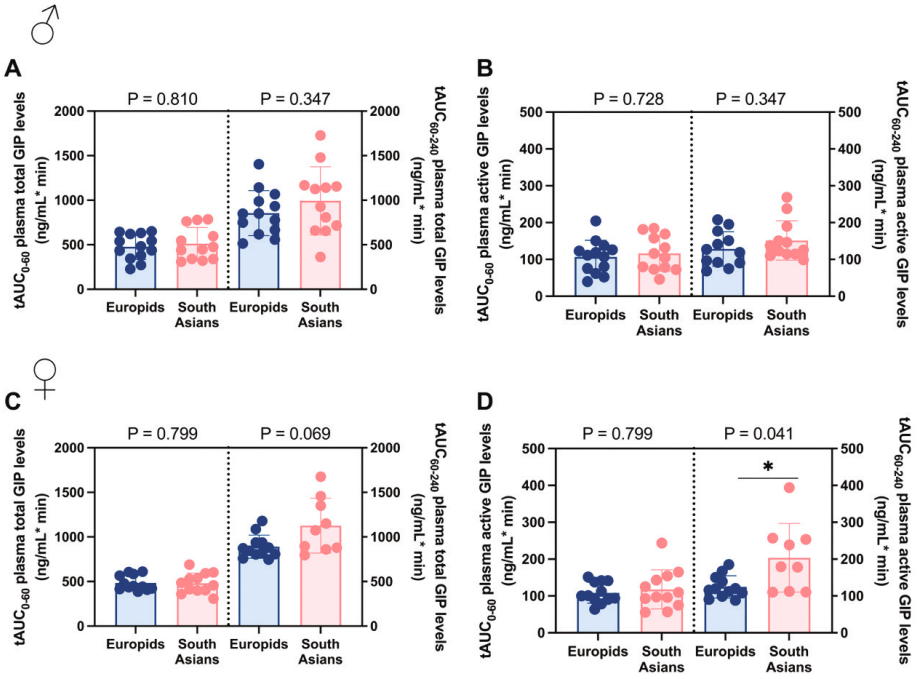


Supplemental Figure 3. Total areas under the curve of the glucagon excursion within two periods during the mixed meal tolerance test in South Asian and Europid males and females
Box plots showing the total areas under the curve within two periods ($tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$) of the glucagon excursion during the mixed meal tolerance test in South Asian (n=12) compared to Europid (n=12) males (**A)** and in South Asian (n=9) compared to Europid (n=12) females (**B**). Circles represent individuals' values and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. Due to a technical error, one sample of one Europid male is missing and we were unable to retrieve a blood sample of three South Asian females at one time point.



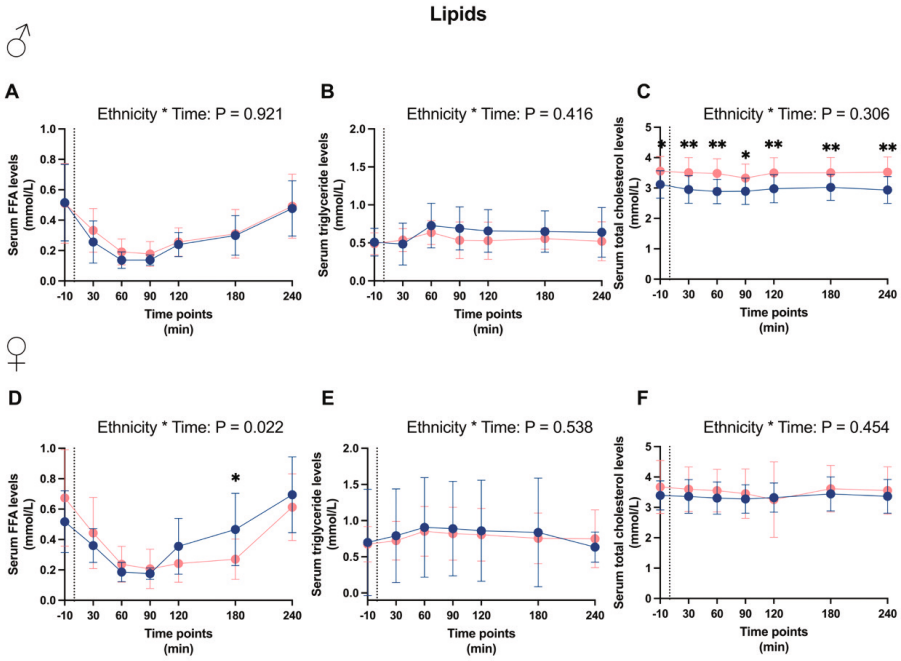
Supplemental Figure 4. Total areas under the curve of the total and active glucagon-like peptide-1 excursions within two periods during the mixed meal tolerance test in South Asian and Europid males and females

Box plots showing the total areas under the curve within two periods ($tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$) of the total glucagon-like peptide-1 (GLP-1) excursion in South Asian (n=12) compared to Europid (n=13) males (**A**) and box plots showing the $tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$ of active GLP-1 excursion in South Asian (n=11) and Europid (n=12) males (**B**). Box plots showing the $tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$ of the GLP-1 excursions in South Asian (n=9) compared to Europid (n=12) females (**C**) and box plots showing the $tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$ of active GLP-1 excursion in South Asian (n=9) and Europid (n=12) females (**D**). Circles represent individuals' values and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. We were unable to retrieve a blood sample of one South Asian female at two time points, and from one South Asian male, Europid male, one South Asian female, and one Europid female at one time point.



Supplemental Figure 5. Total areas under the curve of total and active glucose-dependent insulinotropic polypeptide excursions within two periods during the mixed meal tolerance test in South Asian and Europid males and females

Box plots showing the total areas under the curve within two periods ($tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$) of the total glucose-dependent insulinotropic polypeptide (GIP) excursions during the mixed meal tolerance test (MMTT) in South Asian (n=12) compared to Europid (n=13) males (**A**) and box plots showing the $tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$ of active GIP excursions in South Asian (n=12) and Europid males (n=12) (**B**). Box plots showing $tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$ of total GIP excursion during the MMTT in South Asian (n=9) compared to Europid (n=12) females (**C**) and box plots showing $tAUC_{0\text{-}60}$ and $tAUC_{60\text{-}240}$ of active GIP excursions in South Asian (n=9) and Europid (n=12) females (**D**). Circles represent individuals' values and deviations are the standard deviations. Blue circles, lines, and boxes represent Europids, and pink circles, lines, and boxes represent South Asians. We were unable to retrieve a blood sample of three South Asian females at one time point and of one Europid male one time point missing due to a technical failure.



Supplemental Figure 6. Free fatty acids, triglycerides, and total cholesterol excursions before and during a mixed meal tolerance test in South Asian and Europid males and females

Line graphs showing in South Asian (n=12) compared to Europid (n=13) males the free fatty acid (FFA) (**A)**, triglyceride **(B)**, and total cholesterol **(C)** excursions before and during a mixed meal tolerance test (MMTT). Similarly, showing in South Asian (n=12) compared to Europid (n=12) females line graphs showing the FFA, triglyceride, and total cholesterol excursions during an MMTT. 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 line is the time of the ingestion of the liquid meal. We were unable to retrieve a blood sample of one South Asian female at two time points, and from one South Asian male, Europid male, one South Asian female, and one Europid female at one time point.