Metabolic hormones and ethnic aspects in obesity

Hoekx, C.A.

CHAPTER 2

CIRCULATING FGF21 IS LOWER IN SOUTH ASIANS COMPARED TO EUROPIDS WITH TYPE 2 DIABETES MELLITUS

Carlijn A. Hoekx[1,2], Borja Martinez-Tellez[1,2,3,4,5], Maaike E. Straat[1,2], Maurice B. Bizino[1,6], Huub J. van Eyk[1,2], Hildebrandus J. Lamb[6], Johannes W.A. Smit[7], Ingrid M. Jazet[1,2], Kimberly J. Nahon[1,2], Laura G.M. Jansse[1,2], Patrick C.N. Rensen[1,2,ϕ], Mariëtte R. Boon[1,2,ϕ]

1. Division of Endocrinology, Department of Medicine, Leiden University Medical Center, Leiden, The Netherlands
2. Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Nursing Physiotherapy and Medicine, SPORT Research Group (CTS-1024), CERNEP Research Center, University of Almería, Almería, Spain
4. Biomedical Research Unit, Torrecárdenas University Hospital, 04009 Almería, Spain
5. CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Granada, Spain
6. Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands
7. Department of Medicine, Radboud University Medical Center, Nijmegen, The Netherlands

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ABSTRACT

Objective

Inflammation contributes to the development of type 2 diabetes mellitus (T2DM). While South Asians are more prone to develop T2DM than Europids, the inflammatory phenotype of the South Asian population remains relatively unknown. Therefore, we aimed to investigate potential differences in circulating levels of inflammation-related proteins in South Asians compared to Europids with T2DM.

Method

In this secondary analysis of three randomized controlled trials, relative plasma levels of 73 inflammation-related proteins were measured using the Olink Target Inflammation panel and serum FGF21 concentration using an ELISA kit in Dutch South Asians (n=47) and Dutch Europids (n=49) with T2DM.

Results

Of the 73 inflammation-related proteins, the relative plasma levels of 6 proteins were higher (SCF, CASP-8, CCL28, IFN-gamma, ST1A1, CST5; q-value<0.05), while relative levels of 6 proteins were lower (FGF21, MMP-1, IL8, CCL4, CXCL6, MCP-1; q-value<0.05) in South Asians compared to Europids. Of these, the effect size of FGF21 was the largest, particularly in females. We validated this finding by assessing FGF21 concentration in serum. FGF21 concentration was indeed lower in South Asians compared to Europids with T2DM in both males (-42.2%; P<0.05) and females (-58.5%; P<0.001).

Conclusion

Relative plasma levels of 12 inflammation-related proteins differed between South Asians and Europids with T2DM, with a significantly pronounced reduction in FGF21. In addition, serum FGF21 concentration was significantly lower in South Asian males and females compared to Europids. Whether low FGF21 is an underlying cause or consequence of T2DM in South Asians remains to be determined.

INTRODUCTION

One in ten adults is living with diabetes, and this number is expected to rise to one in nine by 2040 (1, 2). Of those affected, ninety percent have type 2 diabetes mellitus (T2DM), with ethnic groups showing different susceptibility to developing T2DM and associated diseases (3). For example, South Asians originating from the Indian subcontinent develop T2DM at a significantly younger age and a lower body mass index (BMI) than other ethnic counterparts, and given that they present nearly a quarter of the world's population, understanding the underlying mechanism is of utmost importance (4, 5). The increased risk of the development of T2DM in South Asians is thought to be partially attributable to their metabolic phenotype, characterized by more central obesity, dyslipidemia, and more insulin resistance relative to Europids (6-8). However, these factors alone do not entirely explain the increased risk (9).

In recent years, research has shown that inflammation plays a prominent role in developing T2DM and its associated complications (10, 11). Inflammation results from stress on the adipose tissue, leading to the attraction of immune cells by releasing cytokines and chemokines that promote inflammation (12-14). Interestingly, previous studies suggest the presence of a more proinflammatory phenotype in the South Asian population. Our prior research revealed higher C-reactive protein (CRP) levels already in cord blood from South Asian neonates compared to Europid neonates (15). Additionally, South Asians living with T2DM display a more activated interferon (IFN)-signaling pathway than Europids living with T2DM (16).

Considering the pivotal role of inflammation in the development of T2DM and its associated comorbidities, identifying the inflammation-related protein signature could improve our understanding of South Asians' high T2DM risk. It may also provide valuable insight into the applicability of novel treatment modalities in this population. Therefore, in the current study, we aimed to investigate potential differences in circulating inflammation-related proteins in South Asians compared to Europid individuals living with T2DM.

METHODS

Participants and study design

Participants

This study is a secondary analysis of three previously performed randomized, double-blinded, placebo-controlled clinical trials.

The first two clinical trials were designed to investigate the effect of a 26-week liraglutide treatment on glycemic endpoints and ectopic fat deposition in participants with overweight, obesity, and T2DM (17, 18). In total, 50 patients of Dutch Europid (hereinafter: 'Europid') origin (study 1) (17) and 47 of Dutch South Asian (hereinafter: 'South Asian') origin (study 2) (18) were included. South Asian ethnicity was defined as having four grandparents who originally descended from Surinam, Bangladesh, India, Nepal, Pakistan, Afghanistan, Bhutan, or Sri Lanka. Inclusion criteria were males and females aged 18-69 years, BMI ≥ 25 kg/m^2, and Hemoglobin A_{1c} (HbA_{1c}) levels of 7.0-10.0% (53-86 mmol/mol) despite the use of metformin, sulfonylurea derivatives, and insulin. The main exclusion criteria were the use of other glucose-lowering therapy and renal, hepatic, or cardiovascular disease (i.e., presence of congestive heart failure New York Heart Association (NYHA) classification III-IV, uncontrolled hypertension (systolic blood pressure > 180 mmHg and/or diastolic blood pressure > 110 mmHg) or an acute coronary or cerebrovascular accident within 30 days prior to study inclusion); gastric bypass surgery; chronic pancreatitis or previous acute pancreatitis; pregnancy or lactation and MRI contra-indications. Both trials were performed between 2013 and 2018.

The third clinical trial was a randomized, double-blinded, placebo-controlled cross-over study designed to assess the effect of cold exposure and mirabegron on plasma lipids, energy expenditure, and brown adipose tissue fat fraction in 10 healthy lean South Asian males versus 10 age—and BMI-matched Europid males. Inclusion criteria were age 18-30 and healthy BMI between 18-25 kg/m^2. The study was performed between June 2017 and June 2018.

Study approval

All three trials were conducted according to the principles of the revised Declaration of Helsinki (19). Before inclusion, written informed consent was obtained from all participants. The local ethics committee approved all trials, which were conducted at Leiden University Medical Center and registered at clinicaltrial.gov (NCT01761318, NCT02660047, and NCT03012113).

Study designs

The designs of all trials have been extensively described elsewhere (17, 18, 20).

At baseline, all participants from the three studies arrived at the outpatient clinic after at least a 6-hour fast for those with T2DM and a 10-hour overnight fast for those without T2DM. Initial assessment in all trials included body composition and bioelectrical impedance analysis (BIA; Bodystat 1500, Bodystat Ltd., Douglas, UK), followed by the collection of venous blood samples. In the trials with patients with T2DM, after the initial blood sample collection, individuals underwent an MRI and proton magnetic resonance spectroscopy (^{1}H-MRS) to measure subcutaneous and visceral adipose tissue and hepatic triglyceride content (HTGC).

Blood collection

Following the collection of venous blood samples, serum (BD Vacutainer® SST II Advanced tubes) and plasma (BD Vacutainer® EDTA tubes) were obtained by centrifugation in all studies and stored at -80°C until further analysis.

To measure relative levels of circulating inflammation-related proteins in patients with T2DM, the commercially available protein biomarker panel "Target 96 Inflammation" from Olink proteomics (Olink Bioscience, Uppsala, Sweden) was used. Olink Proteomics performed quality control; samples were excluded when their incubation and detection control deviated by more than ± 0.3 Normalized Protein Expression (NPX) from the plate median (21). This resulted in the exclusion of 1 sample. 73 of the 96 (i.e., 76%) proteins were detected in at least 75% of the plasma samples included in the analysis.

Plasma levels of total cholesterol, high-density lipoprotein-cholesterol (HDL-C), triglycerides, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured on a Modular P800 analyzer (Roche Diagnostic, Mannheim, Germany) for the patients with T2DM. Low-density lipoprotein-cholesterol (LDL-C) was calculated according to the Friedewald formula (22). HbA_{1c} was initially measured with boronate-affinity high-performance liquid chromatography (Primus Ultra; Siemens Healthcare Diagnostics, Breda, the Netherlands) due to logistical reasons and later with ion-exchange high-performance liquid chromatography (Tosoh G8, Sysmex Nederland B.V., Etten-Leur, the Netherlands). To ensure accurate and consistent results, HbA_{1c} levels obtained from the boronate affinity method were corrected based on the correlation coefficient obtained from validation samples measured on both analyzers. Plasma CRP concentrations were measured on a Roche Modular analyzer (Roche Diagnostics). Serum fibroblast growth factor 21 (FGF21) concentrations in samples from all three

trials were measured using the human FGF21 Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA).

MRI

The patients with T2DM underwent an MRI in the supine position at baseline, using a 3.0 Tesla MRI scanner (Ingenia, Philips Healthcare, Best, The Netherlands) to assess visceral, abdominal adipose tissue volumes and HTGC, as extensively described previously (18).

Statistical analysis

Data are expressed as mean ± standard deviation. The normality of data was confirmed using the Shapiro-Wilk test, visual histograms, and Q-Q plots. Baseline characteristics for the patients with T2DM were compared between ethnicities and sexes using a Chi-square test for binary values (i.e., use of diabetic medication) and an independent t-test for normally distributed data. Not normally distributed data were log10 transformed (i.e., subcutaneous adipose tissue, visceral adipose tissue, visceral/subcutaneous adipose tissue ratio, total cholesterol, triglycerides, and CRP). Non-parametric tests were performed on data not normally distributed after log 10 transformation (i.e., age, diabetes duration, body fat percentage, HTGC, HbA_{1c}, LDL-C, AST, ALT, and metformin dose).

Olink data were analyzed using the Mann-Whitney U test to determine the difference in relative plasma levels of inflammation-related proteins between ethnicities. All proteomic analyses were corrected for multiple testing using Benjamin-Hochberg's false discovery rate (FDR). FDR corrected p-value (i.e., q-value) was set at < 0.05. Volcano plots were constructed by calculating the fold change (FC) between South Asians and Europids on a log2 scale. From here on, all data was split for both sexes.

To study the difference between serum FGF21 concentrations between ethnicities, an independent t-test was performed with log10 transformed data. To determine whether the observed differences in FGF21 levels between ethnic groups were attributable to baseline phenotypical characteristics of the two cohorts, we conducted sensitivity analyses (**Supplemental Table 2**). In these analyses, we examined FGF21 level differences between ethnicities while adjusting separately for age, BMI, waist circumference, VAT, HTGC, TC, LDL-C, AST, ALT, metformin dose, and T2DM duration. The adjustments did not alter the results, indicating that the observed differences in FGF21 levels were not explained by these variables. Furthermore, nonparametric Spearman-rank correlations (rho) were applied to examine the association between relative plasma FGF21 levels from the Olink database and relative plasma IFN-gamma

levels, HTCG, and serum triglycerides and between serum FGF21 concentrations with serum CRP, HTGC, and serum triglycerides, as the data were not normally distributed.

The statistical analysis of the baseline characteristics of the cohort without T2DM has been extensively described elsewhere (23). To compare serum FGF21 concentrations between ethnicities, an independent t-test was performed with log10 transformed data of baseline values to attain a normal distribution. All statistical analyses of the Olink database were performed using RStudio (version 4.3.2, 2023), and other statistical analyses were performed using Statistical Package for the Social Sciences 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 for the analysis of the difference in relative plasma levels of inflammation-related proteins between ethnicities was set at $q < 0.05$ and for all other analyses at $P < 0.05$.

RESULTS

Baseline characteristics

At baseline, significant differences were seen in the characteristics between the South Asians and Europids with T2DM, as described previously (**Supplemental Table 1**) (17, 18). In short, South Asians exhibited a lower age ($P = 0.012$), weight ($P < 0.001$), length ($P < 0.001$), BMI ($P = 0.002$), waist circumference ($P < 0.001$), waist-to-hip ratio ($P < 0.001$), visceral adipose tissue volume ($P = 0.006$), HTGC ($P < 0.001$), total cholesterol ($P = 0.003$), LDL-C ($P = 0.003$), AST ($P < 0.001$) and dose of metformin ($P = 0.037$) compared to Europids. On the other hand, South Asians had a longer duration of diabetes ($P < 0.001$) and higher levels of ALT ($P < 0.001$) than their Europid counterparts. When splitting the groups per sex, the differences in body composition and clinical parameters between South Asians and Europids persisted (**Supplemental Table 1**).

Relative plasma levels of inflammation-related proteins differ between South Asians versus Europids with T2DM.

Relative plasma levels of six inflammation-related proteins were higher in South Asians compared to Europids with T2DM (i.e., stem cell factor (SCF), caspase-8 (CASP-8), C-C motif chemokine ligand 28 (CCL28), interferon-gamma (IFN-gamma), sulfotransferase 1A1 (ST1A1), cystatin D (CST5); fold change > 0.27, $q < 0.05$; **Fig. 1A**). Also, relative plasma levels of six inflammation-related proteins were lower in South Asians compared to Europids (i.e., FGF21, human fibroblast collagenase (MMP-1), interferon-8 (IL-8), C-C motif chemokine ligand 4 (CCL4), C-X-C motif chemokine ligand 6 (CXCL6), monocyte chemoattractant protein-1 (MCP-1); fold change < -0.24, $q < 0.05$; **Fig. 1A**). When splitting the data per sex, the effects appeared to be specific for females, since no significant

differences in relative plasma levels were found in South Asian versus Europid males (**Fig. 1B)**. In contrast, in South Asian versus Europid females, relative plasma levels of two inflammation-related proteins were higher (i.e., ST1A1, IFN-gamma; fold change > 0.74, q < 0.04) while relative plasma levels of six proteins were lower (i.e., FGF21, MCP-1, MMP-1, IL-8, vascular endothelial growth factor A (VEGFA), CXCL6; fold change < -0.28, q < 0.04; **Fig. 1C**).

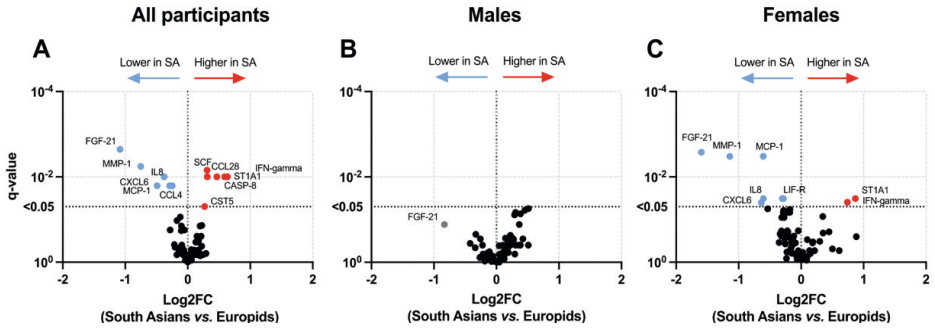

Figure 1. Comparison of plasma levels of inflammation-related proteins in South Asian compared to Europid males and females with type 2 diabetes mellitus.
Volcano plot showing the comparison of relative plasma levels of 73 inflammation-related proteins in all South Asians (SA) (n=47) compared to all Europids (EU) (n=48) with type 2 diabetes mellitus **(A)**. Additionally, comparisons are shown for South Asian (n=19) versus Europid (n=27) males with type 2 diabetes mellitus **(B)** and for South Asian (n=28) versus Europid (n=20) females with type 2 diabetes mellitus **(C)**. The x-axes show the log2 fold change (log2FC) between South Asians and Europids; the y-axes show the q-value on a log scale. P-values were obtained from a Wilcox-U test and then corrected using Benjamin-Hochberg's false discovery rate to yield q-values. Red circles represent significantly higher relative protein levels in South Asians, and blue circles represent significantly lower relative protein levels in South Asians, compared to Europids (q < 0.05). The value of one Europid male was excluded due to failure of the Quality Control of Olink, one Europid male was excluded as insufficient plasma was available to perform protein analysis, and data of 23 proteins were omitted from the 96-inflammation panel because their levels were below the detection limit in more than 75% of the samples.

Circulating FGF21 concentrations are significantly lower in South Asians versus Europids with T2DM.

Of note, of the 73 inflammation-related proteins measured, the relative plasma level of FGF21 differed most between South Asians and Europids with T2DM. The relative plasma level of FGF21 was lower in South Asians compared to Europids with T2DM, both in males and females combined (fold change -1.08; q = 0.002, **Fig 1A**) and in females (fold change -1.60; q = 0.003; **Fig 1C, Supplementary Fig. 1B**). No significant difference in relative plasma level of FGF21 was observed between South Asian males compared to Europid males with T2DM (fold change -0.82; q = 0.120; **Fig 1B, Supplementary Fig. 1A**).

To validate this finding, we next quantified FGF21 concentrations in the serum of the same cohort of South Asians and Europids with T2DM by ELISA. A strong correlation was found between relative plasma levels of FGF21 and serum FGF21 concentration (rho > 0.860; P < 0.001) (data not shown). Similarly to the relative plasma levels, serum FGF21 concentrations were lower in South Asians compared to Europids with T2DM (215 ± 136 pg/ml *vs.* 420 ± 337 pg/ml; -48.8%; P < 0.001; **Fig. 2A**). In addition, we now also observed lower serum FGF21 concentrations in South Asian compared to Europid males (182 ± 100 pg/mL *vs.* 315 ± 249 pg/mL; -42.2%; P = 0.020; **Fig. 2B**) and in females (238 ± 154 pg/mL *vs.* 574 ± 393 pg/mL; -58.5%; P < 0.001; **Fig. 2C**). Due to significant differences in baseline characteristics, we repeated the analysis with adjustments for potential confounders. However, this did not affect the results (**Supplemental Table 2**).

When measuring FGF21 concentrations in the serum of males without T2DM, serum FGF21 levels were generally lower compared to the males with T2DM, and without differences between South Asians and Europids (83 ± 58 pg/mL *vs.* 96 ± 76 pg/mL; -13.1%; P = 0.675; **Supplementary Fig. 2**).

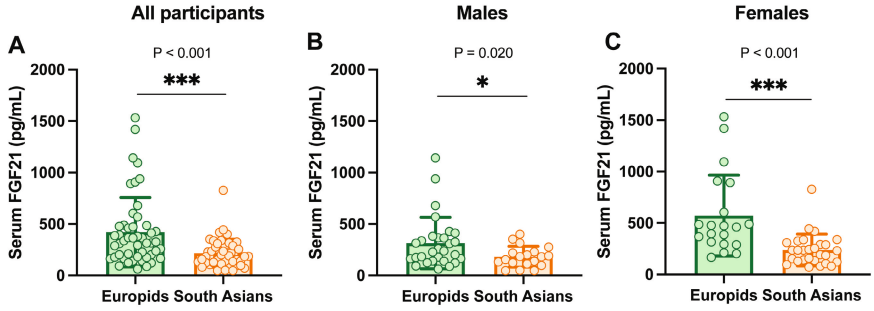

Figure 2. Comparison of serum fibroblast growth factor 21 concentration in South Asian and Europid males and females with type 2 diabetes mellitus.
Box plots showing serum concentration of fibroblast growth factor 21 (FGF21) in all South Asians (n=47, orange circles) compared to all Europids (n=49, green circles) with type 2 diabetes mellitus **(A).** In addition, FGF21 concentrations are shown for South Asian (n=19, orange circles) compared to Europid (n=29, green circles) males **(B)** and South Asian (n=28, orange circles) compared to Europid (n=20, green circles) females **(C)**. Circles represent individual values, boxes represent means, and deviations represent standard deviations.

Circulating FGF21 does not correlate with inflammation markers in both sexes.

Since we previously showed that South Asians have a more activated IFN-signaling pathway compared to Europids with T2DM (16) and FGF21 is known for its anti-inflammatory properties in the context of T2DM (24), we next assessed whether relative FGF21 levels and circulating FGF21 concentration were related to inflammation markers. First, we performed correlations with IFN-gamma levels. However, we did not find a significant correlation between relative plasma levels of FGF21 and IFN-gamma in South Asian males (rho = 0.088, P = 0.721) and Europid males (rho = -0.318, P = 0.106) (**Supplementary Fig. 3A**), or in South Asian females (rho = -0.171, P = 0.385) and Europid females (rho = 0.268, P = 0.254) (**Supplementary Fig. 3C**). In addition, we did not find a significant correlation between serum FGF21 concentrations and plasma CRP levels in South Asian males (rho = 0.125, P = 0.632) and Europid males (rho = 0.141, P = 0.466) (**Supplementary Fig. 3A**) or in South Asian females (rho = 0.034, P = 0.866) and Europid females (rho = -0.056, P = 0.819) (**Supplementary Fig. 3B**).

FGF21 relative levels and concentration positively correlate with serum triglycerides and hepatic triglyceride content in South Asians with T2DM

FGF21 is mainly synthesized by the liver and is known to play a role in lipid metabolism. (25) Therefore, we next assessed whether relative plasma FGF21 levels and serum FGF21 concentrations were related to HTGC and serum triglyceride levels. We found that relative plasma FGF21 levels tended to be positively associated with HTGC in South Asian males (rho = 0.486, P = 0.035; **Supplementary Fig. 4A**) and were significantly positively related to HTGC in South Asian females (rho = 0.460, P = 0.014; **Supplementary Fig. 4C**). However, relative plasma FGF21 levels did not correlate with HTCG in both Europid males (rho = 0.155, P = 0.458; **Supplementary Fig. 4A**) and females (rho = 0.368, P = 0.110; **Supplementary Fig 4B**). Of note, relative plasma FGF21 levels were positively correlated with serum triglycerides in South Asian males (rho = 0.639, P = 0.003; **Supplementary Fig. 4B**) and females (rho = 0.528, P = 0.004; **Supplementary Fig. 4D**). Relative plasma FGF21 levels did tend to positively relate to serum triglycerides in Europid males (rho = 0.358, P = 0.067, **Supplementary Fig. 4B**). However, no such correlation was found in Europid females (rho = 0.224, P = 0.342, **Supplementary Fig. 4D**). Comparable results were found for serum FGF21 concentrations with both serum triglycerides and HTCG levels (**Supplementary Fig. 5**).

DISCUSSION

In this study, we compared the relative plasma levels of 73 inflammation-related proteins between South Asians and Europids with T2DM. Relative plasma levels of six inflammation-related proteins were higher, and relative plasma levels of six proteins were lower in South Asians compared to Europids with T2DM. FGF21 was the most distinctive of all inflammation-related proteins measured and was lower in South Asians compared to Europid females with T2DM. We could validate this finding by measuring circulating serum FGF21 concentrations and observed lower concentrations in both male and female South Asians compared to Europids with T2DM. However, serum FGF21 concentrations did not differ in healthy South Asian versus Europid males, suggesting that the difference in FGF21 levels between ethnicities develops later in life. Furthermore, we found a tendency towards a positive correlation between relative plasma FGF21 levels, serum FGF21 concentration, and both hepatic triglycerides and circulating triglycerides in especially South Asians with T2DM.

Among the six inflammation-related proteins with higher relative levels in South Asians (i.e., SCF, CASP-8, CCL28, IFN-gamma, ST1A1, and CST5), all proteins are described in pro-inflammatory pathways (26-31). Additionally, our findings align with our previous research showing higher mRNA levels of B-cell markers and interferon signaling genes, indicating a more activated IFN signaling pathway at the gene level in the blood of South Asians compared to Europids with T2DM (16). In this study, we replicate these findings at the protein level, showing a higher relative protein level of IFN-gamma and ST1A1, a central IFN-gamma intracellular mediator, in South Asians compared to Europids. Given the potential role of IFN-gamma in inducing insulin resistance in metabolic tissues, these findings may at least in part underly the increased insulin resistance among South Asians compared to Europids (30).

Significant differences in relative levels of inflammation-related proteins between South Asians and Europids were only found in females. However, serum FGF21 concentrations were significantly different in both males and females. Given that we assessed 73 inflammatory-related proteins simultaneously, we adjusted our statical analysis to account for multiple comparisons by controlling for the false discovery rate. Consequently, it is possible that, if we had measured solely FGF21 protein in plasma, we might also have a significant difference in FGF21 among males. Furthermore, the substantial difference in relative protein levels between South Asians and Europids in females only also suggests a potential influence of sex hormones on inflammation-related protein levels. The role of sex hormones on inflammation is a known factor (32, 33). Females exhibit higher inflammatory markers (C-reactive protein, tumor necrosis

factor-alpha, and interleukin 6) compared to males during their reproductive years and variations in inflammatory markers throughout the menstrual cycle (34, 35). Furthermore, pro-inflammatory markers are typically increased in postmenopausal females (36). In addition, sex hormones contribute to differences in body composition, particularly in fat distribution between males and females (37). Unfortunately, we have not measured sex hormones in this population nor have information on the menstrual cycles, use of hormonal anticonception, or menopausal status in this population. However, in this study, both South Asian and Europid females with T2DM had significantly higher body fat percentages compared to the males. Higher fat percentage is positively associated with more inflammatory markers (38), which could contribute to the significant differences in relative protein levels between females but not males of both ethnicities.

Serum FGF21 concentrations were lower both in male and female South Asians compared to Europids with T2DM. FGF21 is an essential mediator of lipid metabolism by regulating lipolysis in white adipose tissue and increasing substrate utilization by increasing fatty acid oxidation in the liver (39, 40). It is mainly released from the liver and adipose tissue upon different metabolic stressors on the body, such as fasting, cold exposure, and overfeeding (41). In addition, FGF21 has anti-inflammatory properties (42). People with obesity have a higher concentration of FGF21 than healthy people (43), likely in response to the increased metabolic stress on the body. Despite this, in Europids, 48 weeks of treatment with the FGF21 analog Pegbelfermin improved signs of metabolic dysfunction-associated steatohepatitis (MASH) (44). The increased FGF21 concentration in people living with obesity could indicate a compensatory mechanism for the possible reduction of FGF21 sensitivity in obesity. In our study, serum FGF21 concentration was significantly lower in South Asians compared to Europids with T2DM. This could indicate that South Asians may not adequately compensate for this reduced sensitivity by increasing the FGF21 levels, potentially contributing to their increased risk of developing obesity-associated complications, including T2DM. Furthermore, exogenous FGF21 treatment could hold promise as a potential preventative and therapeutical option for obesity-associated complications in South Asians. Alternatively, since South Asians are known to exhibit higher inflammation compared to Europids, lower FGF21 concentration observed in South Asians could be a compensatory response to the increased inflammation in this population (16, 45). However, we did not find a significant negative correlation between the circulating FGF21 concentrations and pro-inflammatory markers CRP and IFN-gamma. Given the complex mechanisms regulating FGF21, this does not rule out the possibility of a compensatory mechanism to increased inflammation. We did not observe significant differences in serum FGF21 levels between young South Asians and Europids without T2DM, suggesting that the

differences observed in individuals with T2DM may contribute to the development of metabolic diseases. However, potential (epi-)genetic factors influencing the South Asian phenotype later in life cannot be ruled out. Therefore, further research is necessary to identify the potential underlying factors. Measuring circulating FGF21 levels in larger groups of lean individuals without metabolic disease or in individuals with obesity and pre-diabetes could provide more insight into its potential relationship with the development of T2DM.

South Asians are known to develop T2DM at a lower BMI and younger age compared to Europids. This pattern is consistent with our study population as BMI and waist circumference were lower in the South Asians compared to Europids, while their diabetes duration was more prolonged. A recent study analyzed a panel of inflammation-related proteins among people living with obesity, with and without metabolic syndrome. They found a significant upregulation of FGF21 among those with both obesity and metabolic syndrome compared to people living with obesity without metabolic syndrome (46). Given that South Asians in our study had lower total cholesterol and hepatic triglyceride content than Europids, we cannot exclude that they were less metabolically compromised than Europids, potentially explaining their lower FGF21 concentration.

On the other hand, despite the lower HTGC in South Asians, the hepatic stress marker ALT was significantly elevated compared to Europids. This suggests that despite the lower HTGC, South Asians may still have elevated (metabolic) stress on the liver. Additionally, South Asians had a significantly longer duration of T2DM and consequently (beneficial) treatment, which might have impacted HTGC. Therefore, while the lower HTGC observed in the South Asian population could explain the lower FGF21 concentration compared to Europids, elevated liver stress indicated by higher ALT levels could suggest that other factors may contribute to the lower FGF21 concentration observed.

One of the strengths of our study is the extensive analysis of inflammation-related proteins in a large sample of males and females of two ethnicities, allowing us to identify differences between ethnicities objectively. In addition, our population's almost equal distribution of sexes will enable us to explore potential sex differences. However, our study is not without limitations. Due to differences in the metabolic phenotype in South Asians compared to Europids, matching both ethnic groups remains difficult. In addition, we have only blood samples to provide information about inflammation. Ideally, we would have measured inflammation proteins in metabolically active tissues, like the liver and white adipose tissue. This would allow us to localize the assessment of the sources of inflammation.

In conclusion, we showed that of the 73 measurable inflammation-related proteins, the relative plasma levels of 12 proteins were significantly different in South Asians compared to Europids with T2DM, with FGF21 being the most prominent concerning effect size. This observation was further supported by the finding of lower serum FGF21 concentration in South Asians compared to Europids with T2DM. The difference in FGF21 between these ethnicities warrants further tissue-specific studies, given its potential as a metabolic target.

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Supplemental Table 1. Baseline characteristics

	Europids		South Asians		Europids	South Asians
	Males (n=29)	Females (n=20)	Males (n=19)	Females (n=28)	Combined (n=49)	Combined (n=47)
Demographics						
Age, years	60.8 ± 6.3	57.7 ± 6.5	56.0 ± 9.9	54.1 ± 10.4	59.6 ± 6.5	54.9 ± 10.1$^{\#}$
Diabetes duration, years	12.6 ± 7.6	8.9 ± 4.5	18.3 ± 11.1	17.6 ± 9.3**	11.1 ± 6.7	17.9 ± 10.0$^{\#\#\#}$
Clinical parameters						
Body weight, kg	97.1 ± 13.1	95.2 ± 14.2	85.4 ± 11.1**	75.9 ± 10.8***	96.3 ± 13.4	79.7 ± 11.8$^{\#\#\#}$
Body length, cm	178.7 ± 5.2	165.6 ± 7.1	172.9 ± 7.0**	159.0 ± 4.1***	173.3 ± 8.8	164.6 ± 8.7$^{\#\#\#}$
BMI, kg/m^2	30.3 ± 3.0	34.6 ± 3.6	28.6 ± 3.9	30.0 ± 4.0***	32.1 ± 3.9	29.5 ± 4.0$^{\#\#\#}$
Waist circumference, cm	108.8 ± 8.3	111.9 ± 10.3	102.5 ± 7.9*	100.0 ± 10.4***	110.1 ± 9.2	101.0 ± 9.5$^{\#\#\#}$
Hip circumference, cm	104.0 ± 6.1	112.0 ± 6.8	100.8 ± 7.0	106.4 ± 8.1*	107.2 ± 7.5	104.1 ± 8.1
Waist-to-hip ratio	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1**	1.0 ± 0.1	1.0 ± 0.1$^{\#\#\#}$
Body fat percentage, %	29.3 ± 3.7	46.4 ± 5.0	27.7 ± 4.2	43.1 ± 5.4**	36.4 ± 9.5	37.1 ± 9.1
Subcutaneous adipose tissue, cm^2	276 ± 91	442 ± 97	283 ± 107	347 ± 126**	344 ±124	321 ± 121
Visceral adipose tissue, cm^2	211 ± 64	197 ± 89	169 ± 49	165 ± 61	206 ± 75	166 ± 56$^{\#\#}$
Visceral/subcutaneous adipose tissue ratio	0.8 ± 0.3	0.5 ± 0.2	0.7 ± 0.3	0.5 ± 0.3	0.7 ± 0.3	0.6 ± 0.3
Hepatic Triglyceride Content, %	14.6 ± 8.7	23.2 ± 10.2	8.7 ± 6.4*	10.1 ± 10.9***	18.3 ± 10.2	9.5 ± 9.3$^{\#\#\#}$
HbA_{1c}, mmol/mol	67.4 ± 10.5	63.1 ± 10.8	68.7 ± 11.9	67.2 ± 11.0	65.6 ± 10.7	67.8 ± 11.3
Total cholesterol, mmol/L	4.6 ± 1.0	5.1 ± 1.0	4.0 ± 1.0*	4.4 ± 0.9**	4.8 ± 1.0	4.2 ± 0.9$^{\#\#}$
HDL-C, mmol/L	1.2 ± 0.3	1.3 ± 0.3	1.1 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.3
LDL-C, mmol/L	1.2 ± 0.3	2.8 ± 0.9	1.9 ± 0.8*	2.3 ± 0.8*	2.6 ± 0.9	2.1 ± 0.8$^{\#\#}$
Triglyceride, mmol/L	2.1 ± 1.5	2.2 ± 0.8	2.0 ± 2.0	1.7 ± 1.0*	2.1 ± 1.3	1.8 ± 1.4
AST, IU/L	28.7 ± 8.6	40.1 ± 22.7	26.7 ± 12.6	20.4 ± 5.9***	33.4 ± 16.7	22.9 ± 9.6$^{\#\#\#}$
ALT, IU/L	13.3 ± 5.5	14.6 ± 6.3	29.4 ± 21.3***	20.9 ± 9.1**	13.9 ± 5.8	24.3 ± 15.6$^{\#\#\#}$
CRP, mg/L	2.3 ± 1.8	3.1 ± 2.1	2.2 ± 2.0	4.9 ± 4.6	2.6 ± 1.9	3.8 ± 4.0

Supplemental Table 1. Baseline characteristics (continued)

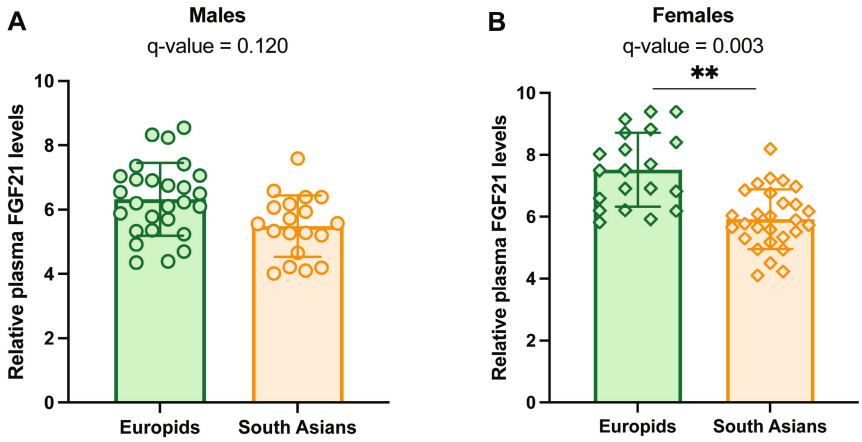
	Europids		South Asians		Europids	South Asians
	Males (n=29)	**Females (n=20)**	**Males (n=19)**	**Females (n=28)**	**Combined (n=49)**	**Combined (n=47)**
Diabetes medication						
Metformin use, n, %	*n*=29, 100%	*n*=20, 100%	*n*=18, 95%	*n*=27, 96%	*n*=49, 100%	*n*=45, 96%
Metformin, mg/day	2012 ± 627	2038 ± 609	1808 ± 694	1693 ± 622	2022 ± 613	1739 ± 646$^{\#}$
Sulfonylurea, n, %	*n*=6, 21%	*n*=8, 40%	*n*=2, 11%	*n*=6, 21%	*n*=14, 29%	*n*=8, 17%
Insulin use, n, %	*n*=19, 66%	*n*=13, 65%	*n*=14, 74%	*n*=22, 79%	*n*=32, 65%	*n*=36, 77%

Data adapted from the original data of South Asian (1) and Europid (2) individuals with type 2 diabetes mellitus. Data are presented as mean ± standard deviation. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; HbA_{1c}, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Asterisk signs (*) indicate significant differences between ethnicities within a specific sex group *P < 0.05, **P < 0.01, ***P < 0.001. Hash sign ($^{\#}$) indicates significant differences between ethnicities when both sex groups are combined $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001.

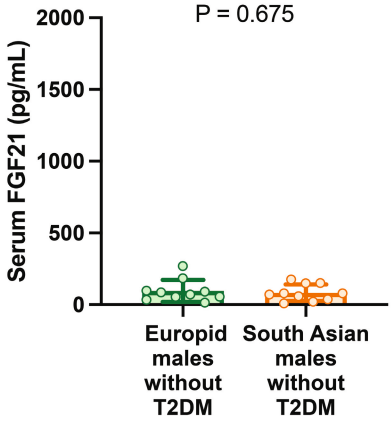
Supplemental Table 2. Differences in serum FGF21 concentrations between South Asians and Europids corrected for baseline values

	Males	Females	Combined
Adjusting for:			
Age	P = 0.075	P < 0.001	P < 0.001
BMI	P = 0.020	P < 0.001	P < 0.001
Waist circumference	P = 0.098	P = 0.002	P = 0.004
Visceral adipose tissue	P = 0.131	P < 0.001	P = 0.002
Hepatic Triglyceride Content	P = 0.072	P = 0.015	P = 0.013
Total cholesterol	P = 0.041	P < 0.001	P = 0.001
LDL-C	P = 0.006	P < 0.001	P < 0.001
AST	P = 0.027	P = 0.049	P = 0.009
Dose of metformin	P = 0.052	P < 0.001	P < 0.001
Duration T2DM	P = 0.013	P = 0.002	P < 0.001
ALT	P = 0.084	P < 0.001	P < 0.001

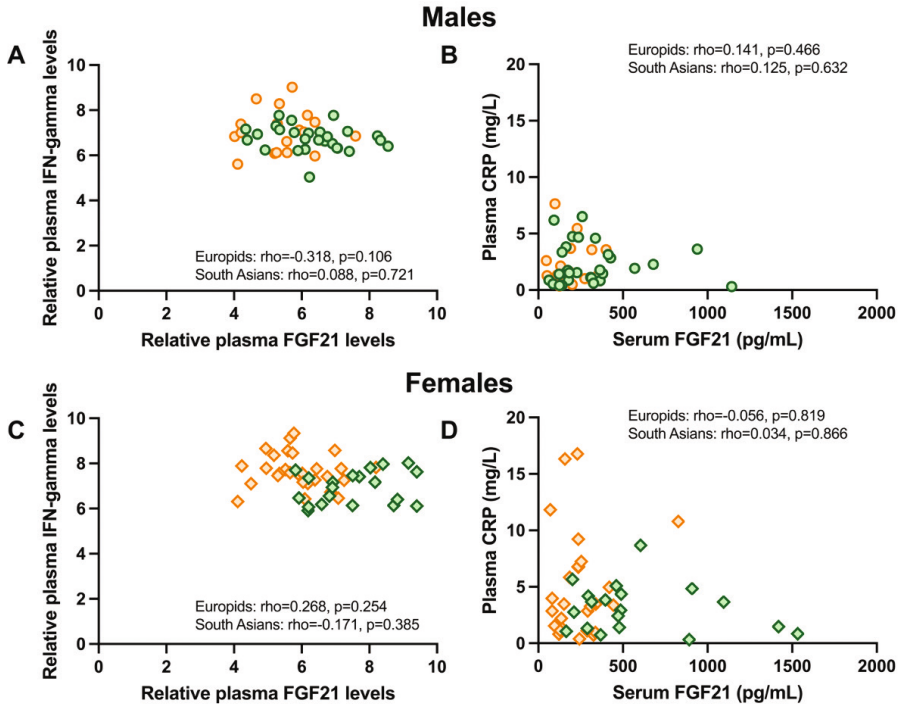
P values of the differences in serum FGF21 concentrations between South Asian males, females and combined compared to Europids corrected for the baseline characteristics that were significantly different between the two ethnicities. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; LDL-C, low-density lipoprotein-cholesterol; T2DM, type 2 diabetes mellitus.



Supplementary Figure 1. Comparison of relative plasma levels of fibroblast growth factor-21 in South Asian and Europid males and females with type 2 diabetes mellitus.
Box plots showing relative plasma levels of fibroblast growth factor 21 (FGF21) in South Asian (n=19; orange circles) versus Europid (n=27; green circles) males with type 2 diabetes mellitus (**A**) and in South Asian (n=28; orange diamonds) versus Europid (n=20; green diamonds) females with type 2 diabetes mellitus (**B**). The value of one Europid male was excluded due to failure of the Quality Control of Olink; one Europid male was excluded as insufficient plasma was available to perform the protein analysis. Circles and diamonds represent individual values; boxes represent means, and deviations represent standard deviations.

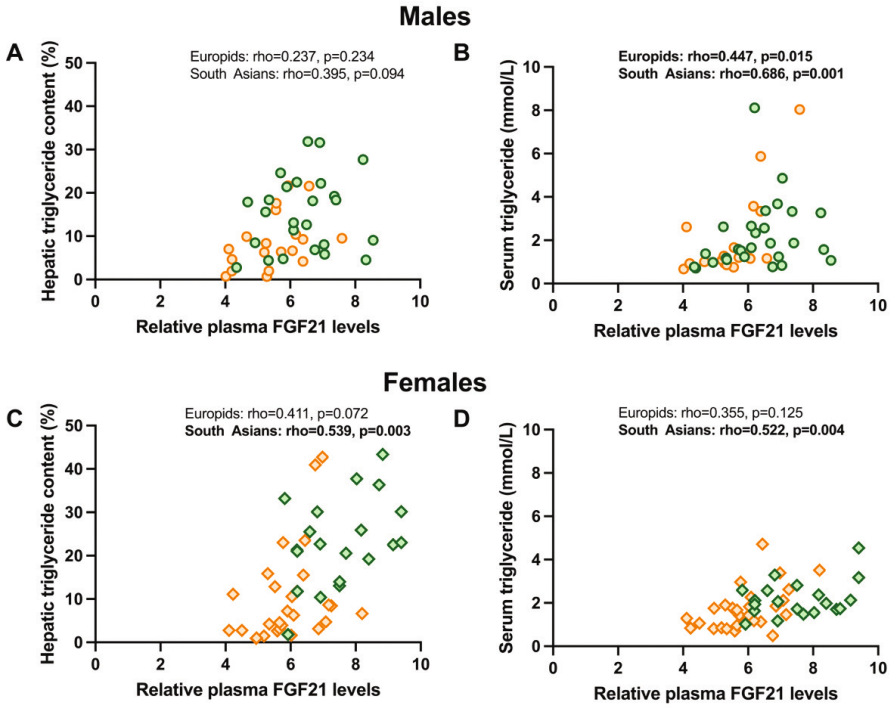


Supplementary Figure 2. Comparison of fibroblast growth factor-21 serum concentration in South Asians and Europid males without type 2 diabetes mellitus.
Box plots showing serum concentration of fibroblast growth factor 21 (FGF21) in South Asian males (n=10, orange circles) compared to Europid males (n=10, green circles) without T2DM. Circles represent individual values, boxes represent means, and deviations represent standard deviations.



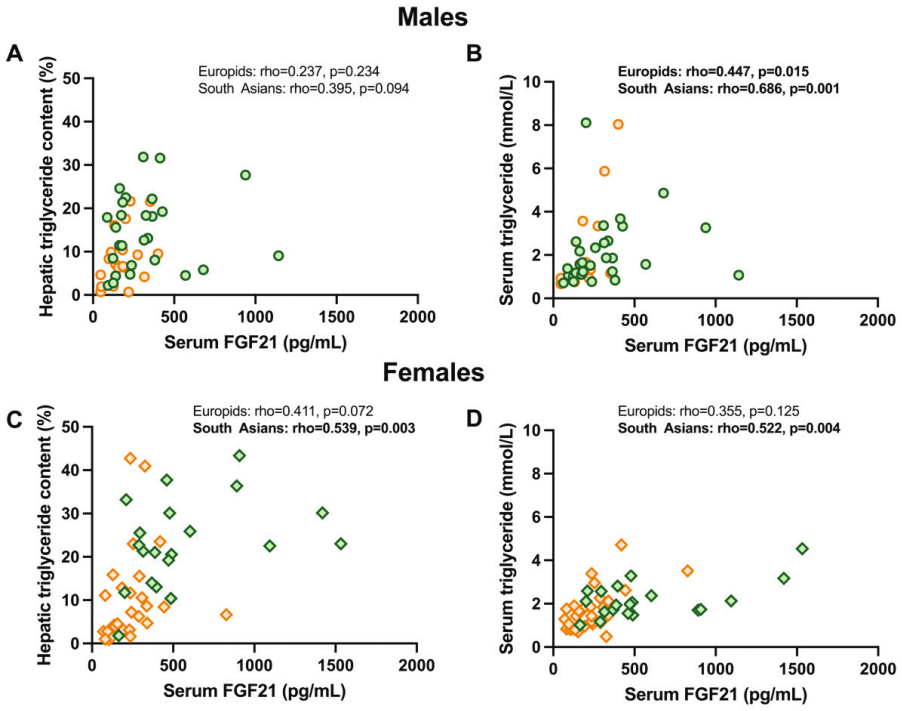
Supplementary Figure 3. Correlations between relative plasma levels of fibroblast growth factor 21 and interferon-gamma and serum fibroblast growth factor 21 concentrations and plasma C-reactive protein concentrations in South Asian compared to Europid males and females with type 2 diabetes mellitus.

Spearman correlations, in both South Asians and Europids, between relative plasma levels of fibroblast growth factor 21 (FGF21) and interferon-gamma (IFN-gamma) in South Asian (n=19, orange circles) and Europid (n=27, green circles) males with type 2 diabetes mellitus **(A)** and in South Asian (n=28, orange diamonds) and Europid (n=20, green diamonds) females with type 2 diabetes mellitus **(C)**. Spearman correlation of serum FGF21 concentration and plasma concentration of C-reactive protein (CRP) in South Asian (n=17, orange circles) and Europid (n=29, green circles) males with type 2 diabetes mellitus **(B)** and in South Asian (n=27, orange diamonds) and Europid (n=19, green diamonds) females with type 2 diabetes mellitus **(D)**. The value of one Europid male was excluded due to failure of the Quality Control of Olink; one Europid male was excluded as insufficient plasma was available to perform the protein analysis. Two South Asian males had plasma CRP levels below the detection limit, one South Asian female had insufficient plasma material for CRP analysis, and one Europid female had serum CRP levels out of range and therefore excluded.



Supplementary Figure 4. Correlations between relative plasma fibroblast growth factor 21 levels, hepatic triglyceride content, and serum triglyceride concentration in South Asian compared to Europid males and females with T2DM.

Spearman correlations, in both South Asians and Europids, between relative plasma Fibroblast Growth Factor 21 (FGF21) levels and hepatic triglyceride content (HTGC) in South Asian (n=19, orange circles) and Europid (n=25, green circles) males with type 2 diabetes mellitus **(A)** and in South Asian (n=28, orange diamonds) and Europid (n=20, green diamonds) females with type 2 diabetes mellitus **(C)**. Spearman correlation of relative plasma FGF21 levels and serum triglyceride concentration in South Asian (n=19, orange circles) and Europid (n=27, green circles) males with type 2 diabetes mellitus **(B)** and in South Asian (n=28, orange diamonds) and Europid (n=20, green diamonds) females with type 2 diabetes mellitus **(D)**. The hepatic triglyceride content was missing for two Europid males due to technically unsuccessful ^{1}H-MRS of the liver. The value of one Europid male was excluded due to failure of the Quality Control of Olink; one Europid male was excluded as insufficient plasma was available to perform protein analysis.



Supplementary Figure 5. Correlations between serum fibroblast growth factor 21 concentration, hepatic triglyceride content, and serum triglyceride concentration in South Asian compared to Europid males and females with T2DM.

Spearman correlations, in both South Asians and Europids, between serum Fibroblast Growth Factor 21 (FGF21) concentration and hepatic triglyceride content (HTGC) in South Asian (n=19, orange circles) and Europid (n=27, green circles) males with type 2 diabetes mellitus **(A)** and in South Asian (n=28, orange diamonds) and Europid (n=20, green diamonds) females with type 2 diabetes mellitus **(C)**. Spearman correlation of serum FGF21 concentration and serum triglyceride concentration in South Asian (n=19, orange circles) and Europid (n=29, green circles) males with type 2 diabetes mellitus **(B)** and in South Asian (n=28, orange diamonds) and Europid (n=20, green diamonds) females with type 2 diabetes mellitus **(D)**. The hepatic triglyceride content was missing for two Europid males due to technically unsuccessful ^{1}H-MRS of the liver.

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