South Asian men have lower expression of IFN signalling genes in white adipose tissue and skeletal muscle compared with white men

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RESEARCH LETTER

South Asian men have lower expression of IFN signalling genes in white adipose tissue and skeletal muscle compared with white men

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Abbreviations

CXCL10	Chemokine (C-X-C) motif ligand 10
IPA	Ingenuity pathway analysis
WAT	White adipose tissue

To the Editor: South Asians, who originate from the Indian subcontinent, make up 20% of the global population and have a higher risk of developing type 2 diabetes compared with white Europids [1]. Albeit central obesity and insulin resistance are more prevalent in the South Asian population [1], these classic predisposing risk factors cannot fully explain the excess risk of developing type 2 diabetes. Inflammation is thought to play a role in obesity-associated metabolic disorders but our understanding of the mechanisms involved is still limited [2]. Despite the high risk of type 2 diabetes in South Asian people and increasing evidence on the links between inflammation and the development of type 2 diabetes, comprehensive data on the inflammatory state in the South Asian population are lacking. Therefore, the aim of the current study was to compare transcriptomic levels of a large panel of inflammatory, immune regulating and immune cell subset markers in the blood, skeletal muscle and white adipose tissue (WAT) of overweight South Asian men at risk of diabetes and BMI- and age-matched white men.

Ten overweight, middle-aged South Asian men from the Netherlands who were at risk of diabetes and ten BMI- and age-matched white men were included in this study, which was originally designed to assess the effect of L-arginine vs placebo on energy expenditure and mitochondrial function. A 4 h fasted blood sample was drawn and biopsies from skeletal muscle and subcutaneous WAT were taken after an overnight fast. Subsequently, RNA was isolated from blood, WAT and skeletal muscle samples and a dual-colour reverse transcriptase multiplex ligation-dependent probe amplification (dcRT-MLPA) assay [3] on 144 genes was performed on these samples. Further, plasma levels of the cytokines IFNα2a, the chemokine (C-X-C) motif ligand 10 (CXCL10) and IL-6 were quantified by ELISA (Meso Scale Discovery, Rockville, Maryland, USA). See the electronic supplementary material (ESM) for further details on the methods used in this study. The study (ClinicalTrial.gov registration no. NCT02291458) was approved by the Ethics Committee of Maastricht University Medical Center and the Leiden University Medical Center (the Netherlands). Procedures were conducted

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according to the principles of the Declaration of Helsinki and all participants provided written informed consent.

The two groups had comparable age, BMI and fasting glucose (ESM Table 1). Transcriptomic profiles of immune markers in the blood are listed in ESM Table 2. Several markers were expressed at higher levels in South Asian compared with white men, including *GNLY* (+ 79%), *NOD2* (+ 40%), *IL2RA* (+ 33%), *CCL5* (+ 32%), *NLRP3* (+ 27%) and *PRF1* (+ 23%). In contrast, the expression of *CCL19* (− 47%), *IL6* (− 30%), *FPR1* (− 29%), *DSE* (− 24%), *CXCL10* (− 22%) and *TGFBR2* (− 12%) was lower in South Asian men.

In muscle, the expression of many immune markers was below the detection limit (ESM Table 2). Nevertheless, expression levels of individual genes including *LAG3* (+ 79%), *TNIP1* (+ 49%), *IL23A* (+ 39%) and *NLRC4* (+ 32%) were higher in the South Asian group. The two genes that were expressed most significantly lower in South Asian men relative to white men were the IFN signalling genes *IFI44* (− 51%, $p < 0.01$) and *IFIT3* (− 45%, $p < 0.001$) (Fig. 1a). In addition, *CTLA4*

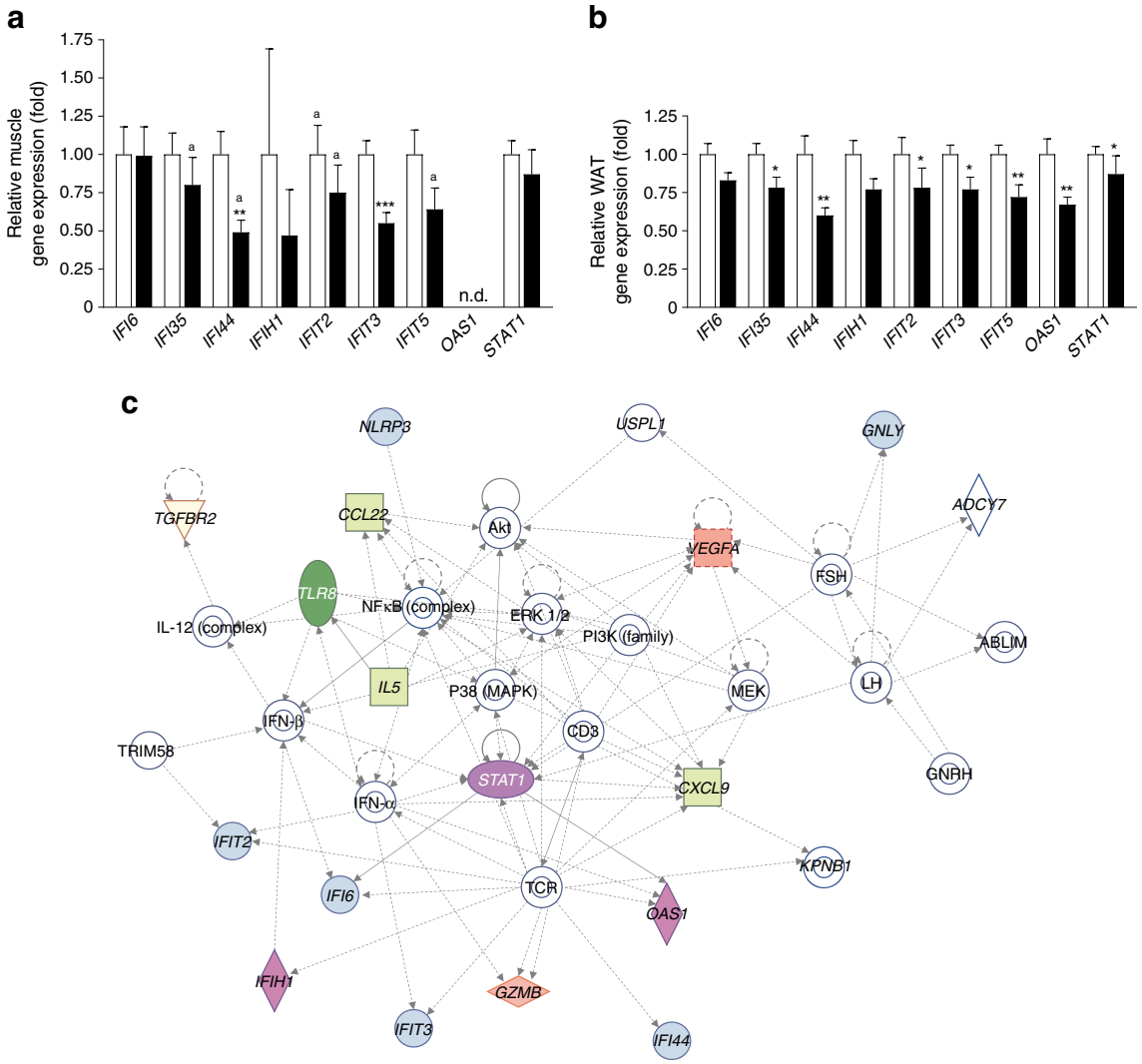

Fig. 1 Expression of IFN signalling genes is reduced in muscle and WAT of South Asian men compared with white men. Relative expression of IFN signalling genes in (**a**) muscle and (**b**) WAT of white and South Asian men. White bars, white men; black bars, South Asian men; n.d., not detectable. Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs white men. [a]Gene expression of four or more individuals was not detectable. (**c**) IPA was performed on the list of genes with a (tendency for [$p < 0.1$]) differential expression between South Asian and white men, which are marked with coloured symbols in the network. Genes in white symbols were not analysed or identified as differentially expressed in our experiment and were integrated into the computationally generated network based on the evidence stored in the IPA knowledge memory indicating relevance to this network. The top canonical pathway was IFN signalling. Gene and gene relationship symbols: square, cytokine; dashed square, growth factor; vertical diamond, enzyme; horizontal diamond, peptidase; triangle, kinase; horizontal oval, transcription factor; vertical oval, transmembrane receptor; double circle, group or complex; single circle, other. Arrow, acts on; continuous line, direct interaction; dashed line, indirect interaction. ABLIM, actin binding LIM protein 1; ERK, extracellular signal-regulated kinase; FSH, follicle-stimulating hormone; GNRH, gonadotropin-releasing hormone; LH, luteinising hormone; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PI3K, phosphoinositide 3-kinase; TCR, T cell receptor; TRIM58, tripartite motif-containing protein 58

(− 44%), *CXCL10* (− 39%), *NCAM1* (− 38%), *TNFRSF1A* (− 38%), *IL13* (− 36%), *SEC14L1* (− 33%) and *TGFB1* (− 14%) were expressed at lower levels in South Asian men.

In WAT, several immune markers having a diverse range of immunological functions were differentially expressed between ethnicities, including *GNLY* (+ 58%), *BPI* (− 46%), *CCL22* (− 34%), *CXCL9* (− 28%) and *TGFBR2* (− 17%) (ESM Table 2). Interestingly, in line with skeletal muscle, of the 12 genes with significantly lower expression in South Asian men, seven were IFN signalling genes, including *IFI35* (− 22%, $p < 0.05$), *IFI44* (− 40%, $p < 0.01$), *IFIT2* (− 22%, $p < 0.05$), *IFIT3* (− 23%, $p < 0.05$), *IFIT5* (− 28%, $p < 0.01$), *OAS1* (− 33%, $p < 0.01$) and *STAT1* (− 13%, $p < 0.05$). The expression of *IFI6* (− 17%) and *IFIH1* (− 23%) was also non-significantly reduced in this group (Fig. 1b).

To identify the signalling pathways that are differentially regulated in WAT, genes with a tendency for differential expression between the South Asian and white men ($p < 0.1$) were fed into the ingenuity pathway analysis (IPA). The top canonical pathway enriched with differentially expressed genes between the two ethnicities was IFN signalling (Fig. 1c), confirming our results of differentially expressed individual genes in WAT between these ethnic groups.

Subsequently, the IFNα2a protein level was measured in plasma by ELISA (Meso Scale Discovery, Rockville, Maryland, USA); however, this was found to be below the detection range of the assay (0.6 pg/ml) in most participants. Therefore, protein levels of the more stable downstream marker of IFN signalling CXCL10 and the general marker of inflammation IL-6 were determined in plasma. Plasma CXCL10 levels did not differ between groups (ESM Fig. 1a), while plasma IL-6 was non-significantly higher in South Asian men (+ 42%, $p = 0.09$; ESM Fig. 1b). Interestingly, plasma IL-6 levels increased in both white and South Asian men during an OGTT and plasma IL-6 levels were higher in South Asian men compared with white men 120 min after a glucose bolus (ESM Fig. 2a). Plasma IL-6 levels correlated positively with BMI ($R^2 = 0.21$, $p < 0.05$), fat mass ($R^2 = 0.28$, $p < 0.05$) and visceral adipose tissue volume ($R^2 = 0.33$, $p < 0.05$; ESM Fig. 2b–d). We also assessed the protein levels of the IFN signalling factors IFI44 and OAS1 in WAT by Western Blot (antibodies used at 1:1000; purchased from Abcam, Cambridge, UK). No differences were observed possibly as a result of considerable inter-individual variation (not shown).

Of note, the most differentially expressed gene in WAT was *IL5* (− 66%), mainly because of a lack of *IL5* expression in most of the South Asian participants (ESM Fig. 3a). Remarkably, *IL5* expression in WAT of white participants (as expression was undetectable in South Asian participants) was negatively correlated with BMI ($R^2 = 0.43$, $p < 0.05$) and fat mass ($R^2 = 0.69$, $p < 0.01$; ESM Fig. 3b,c).

Taken together, many of the investigated panel of immune markers from blood, muscle and WAT that are involved in a range of immunological functions, are differentially expressed in South Asian compared with white men. The major finding of our study was that, in both WAT and muscle, IFN signalling genes were expressed at consistently lower levels in South Asian participants. IPA corroborated the finding that the anti-inflammatory type 1 IFN signalling pathways, i.e. IFN-α and -β, were downregulated in South Asian men. Interestingly, adipose tissue-specific knockout of *Ifna1* or *Ifnb1* in mice reduces high-fat-diet-induced weight gain, insulin resistance and glucose intolerance [4]. IFNβ1 induces cellular glucose uptake via the phosphoinositide 3-kinase (PI3K)/Akt pathway [5] and overexpression of *Ifnb1* suppresses adipose tissue inflammation and protects against diet-induced obesity and glucose intolerance [6]. Together, this suggests that impaired IFN signalling in South Asian people may, at least partly, contribute to their predisposition for development of obesity as well as type 2 diabetes.

The main limitation of our study is the small sample size. Future research into the immune profile of South Asian people would benefit from performing analysis of protein levels and flow cytometry in a larger population. The strengths of our study include the extent of the panel of immune markers measured, not only in blood but also in two important major metabolic tissues within the same individuals. Furthermore, the individuals of different ethnicities were well-matched for metabolic variables that could interfere with inflammation, such as age and BMI.

In conclusion, the immune profile in blood, muscle and WAT is differentially primed in South Asian men compared with white men as evident by a consistently lower expression of IFN signalling genes in the metabolic tissues of South Asian men as the most prominent feature. The pathophysiological significance of these findings in the development of obesity-associated metabolic disorders remains to be determined.

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Data availability The data generated or analysed during the current study that are not included in this published article (and its supplementary information files) are available from the corresponding author on reasonable request. Raw data from the dual-colour reverse transcriptase multiplex ligation-dependent probe amplification (dcRT-MLPA) assay are provided in ESM Table 3.

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Duality of interest The authors declare no potential conflicts of interest relevant to this article.

Contribution statement ADvD helped to conceptualise the project, perform the experiments and analyse the data, contributed to the discussion, wrote and critically reviewed the manuscript. MJWH helped to perform the experiments, analyse the data and critically reviewed the manuscript. RvE, EQ, HCS, CJMH, THMO and MCH helped to perform the experiments, analyse the data and critically review the manuscript. IMJ and WDvML helped to conceptualise the project and to critically review the manuscript. PCNR and MRB helped to conceptualise the project and perform the experiments, supervised the project, contributed to the discussion and edited the manuscript. All authors gave approval for the final version to be published. ADvD, MJWH, PCNR and MRB are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Raji A, Seely EW, Arky RA, Simonson DC (2001) Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. J Clin Endocrinol Metab 86:5366–5371
2. Lumeng CN, Saltiel AR (2011) Inflammatory links between obesity and metabolic disease. J Clin Invest 121:2111–2117
3. Haks MC, Goeman JJ, Magis-Escurra C, Ottenhoff TH (2015) Focused human gene expression profiling using dual-color reverse transcriptase multiplex ligation-dependent probe amplification. Vaccine 33:5282–5288
4. Wieser V, Adolph TE, Grander C et al (2016) Adipose type I interferon signalling protects against metabolic dysfunction. Gut https://doi.org/10.1136/gutjnl-2016-313155
5. Burke JD, Platanias LC, Fish EN (2014) Beta interferon regulation of glucose metabolism is PI3K/Akt dependent and important for antiviral activity against coxsackievirus B3. J Virol 88:3485–3495
6. Alsaggar M, Mills M, Liu D (2016) Interferon beta overexpression attenuates adipose tissue inflammation and high-fat diet-induced obesity and maintains glucose homeostasis. Gene Ther 24:60–66