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ORIGINAL ARTICLE

# The tumour necrosis factor (*TNF*)-α –308GA promoter polymorphism is related to prenatal growth and postnatal insulin resistance

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## Summary

**Objective** Variation in the tumour necrosis factor gene, (*TNF*) has been associated with insulin resistance traits. We questioned whether the *TNF* –308G/A polymorphism is associated with birthweight and insulin resistance in children born small for gestational age (SGA), a patient population known to be at risk for insulin resistance.

**Design** A cross-sectional, hospital-based study assessing insulin sensitivity in SGA children.

**Patients** One hundred and ninety-eight school-age children born either SGA ($n = 90$, age $7{\cdot}4 \pm 4{\cdot}5$ years) or appropriate for gestational age (AGA, $n = 108$, age $8{\cdot}7 \pm 4{\cdot}0$ years).

**Measurements** All children were genotyped for the *TNF* –308G/A polymorphism; a biochemical profile was also performed in prepubertal SGA ($n = 58$) and AGA ($n = 57$) subjects.

**Results** Genotype frequencies for the *TNF* –308G/A single nucleotide polymorphisms (SNPs) (GG and GA/AA) differed between SGA and AGA children (86% *vs.* 72% and 14% *vs.* 28%, respectively; $P = 0{\cdot}025$). The GG genotype was associated with lower birthweight and birth length ($2747{\cdot}0 \pm 23{\cdot}3$ g *vs.* $2851{\cdot}0 \pm 45{\cdot}7$ g, $P = 0{\cdot}045$, and $47{\cdot}0 \pm 0{\cdot}2$ cm *vs.* $48{\cdot}2 \pm 0{\cdot}4$ cm, $P = 0{\cdot}011$, respectively) and, in AGA but not in SGA children, with higher systolic blood pressure [103·3 (95% confidence interval (CI) 96·4–110·2) mmHg *vs.* 92·8 (84·9–100·7) mmHg; $P = 0{\cdot}028$], higher blood glucose [4·8 (4·7–5·0) mmol/l *vs.* 4·5 (4·3–4·8) mmol/l; $P = 0{\cdot}042$] and higher homeostasis model assessment for insulin resistance (HOMA-IR) index [1·4 (1·1–1·7) *vs.* 0·9 (0·4–1·3); $P = 0{\cdot}005$]. In multivariate analysis, the *TNF* –308GG genotype was an independent predictor of HOMA-IR during childhood, explaining 8% of its variance.

**Conclusion** SGA children show increased frequency of the *TNF* –308G allele, an allele that is associated with prenatal growth and with postnatal insulin resistance. The *TNF* –308G/A polymorphism may have implications in the growth and metabolic abnormalities that characterise SGA children.

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## Introduction

Low birthweight has been related to increased risk for developing the metabolic syndrome in adulthood. Hyperinsulinaemia is usually present before metabolic abnormalities (hypertension, dyslipidaemia, obesity) become detectable and has been proposed as the common trigger of the constellation.[1,2] The mechanisms underlying the relationship between weight at birth and insulin sensitivity in adulthood are still unclear. Hales and Barker[2] have postulated that the foetal adaptation to an adverse intrauterine environment involves altered programming of endocrine pathways, leading to permanent metabolic changes, including reduced insulin sensitivity. Others have proposed that this association is genetically mediated.[3] The links between low birthweight and insulin resistance may also explain the pathogenesis of reduced prenatal growth in small for gestational age (SGA) individuals.[4]

Tumour necrosis factor-α (TNF-α) is a strong determinant of insulin sensitivity.[5–7] The induction of insulin resistance by this cytokine is mediated through its ability to induce serine phosphorylation of insulin receptor substrate 1.[8]

TNF-α signals through two known cell-surface receptors (TNFR1 and 2).[9] Although specific signalling pathways and cellular responses have been documented for the different TNF-α receptors, both molecules are ubiquitously expressed, both are critically involved in the establishment of host defence, and both have been related to the insulin resistance phenotype.[6,10–12]

The soluble fractions of these receptors, sTNFR1 and sTNFR2, result from proteolytic cleavage of the cell-surface forms upon TNF-α binding; they are thought to both inhibit and stabilize the bioactivity of TNF-α, depending on the experimental conditions.[13] Serum levels of sTNFR are considered to be good surrogates of TNF-α

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because they reflect local tissue action of this cytokine[14] and because they are fairly stable over time.[15]

Recent genetic studies have shown that the *TNF* −308G/A single nucleotide polymorphism (SNP) is associated with insulin resistance traits in humans. The base change (G to A) in the *TNF* gene has been associated with increased transcriptional activity,[16] with insulin resistance-associated hypertension,[17] increased fat mass and leptin levels and decreased insulin sensitivity in healthy subjects,[18] and with obesity in population-based studies.[19]

We hypothesized that children born SGA manifest a proinflammatory genotype (i.e. increased frequency of the A allele in the *TNF* gene) that can modify the risk for insulin resistance conferred by a low weight at birth. To test this we genotyped children born SGA or appropriate for gestational age (AGA) for the above-mentioned SNP and studied its associations with both birthweight and insulin resistance in these children. As a secondary aim, we also studied the plasma concentrations of sTNFR1 and sTNFR2 in prepubertal children and studied their associations with birthweight and insulin resistance in these subjects.

## Subjects and methods

### Subjects

One hundred and ninety school-age children born either SGA ($n = 90$; 34 boys and 56 girls; age 7·4 ± 4·5 years) or AGA ($n = 108$; 59 boys and 49 girls; age 8·7 ± 4·0 years) were genotyped for the *TNF* −308G/A SNP. Among these, 58 SGA and 57 AGA were prepubertal at the time of the study, as based on the standards by Marshall and Tanner,[20,21] and comprised the group for biochemical analyses.

For a given value of $P = 0{\cdot}05$, the study had an 80% power to detect a significant difference in GG genotype frequencies between SGA and AGA children (difference of at least 20%) as well as significant correlations between endocrine–metabolic parameters (Pearson coefficient of at least 0·3).

SGA and AGA were defined as gestational age- and sex-adjusted birthweight below the 10th centile and above the 25th centile, respectively. SGA children were recruited among patients seen at the outpatient endocrinology clinic in the Barcelona hospital. AGA children were selected from healthy children seen at the outpatient paediatric clinic in the same hospital. Five SGA and four AGA children were born preterm at between 32 and 36 weeks of gestation.

The exclusion criteria were: evidence for a syndromic, chromosomal or infectious aetiology of low birthweight; gestational diabetes and/or hypertension; evidence of acute or chronic illness or medication use within the previous month of inclusion; GH deficiency (defined as an abnormal GH response to both clonidine and L-dopa tests in the context of suggestive auxological data); abnormal thyroid function; current or previous treatment with recombinant human GH; and first-degree relatives with type 2 diabetes mellitus. Girls with short stature (either SGA or AGA) underwent a karyotype analysis to rule out Turner's syndrome. Informed written consent was obtained from the parents after the purpose, nature and potential risks of the study were explained to the parents and subjects. The experimental protocol was approved by the Institutional Review Board of Sant Joan de Déu Hospital, Barcelona.

### Measurements

Anthropometric measurements were performed by a single observer (P.C.-S.). Weight was measured to the nearest 0·5 kg using a hospital balance beam scale. Height was measured to the nearest 0·5 cm using a wall-mounted stadiometer. Age- and sex-adjusted standard deviation scores (SDS) for current weight, height and body mass index (BMI) were calculated using regional normative data.[22]

Waist circumference was measured at the minimal circumference between the umbilicus and xiphoid process with the child standing; the hips were measured at maximum extension of the buttocks.

Blood pressure was measured in the sitting position on the right arm after a 10-min rest; a standard sphygmomanometer of appropriate cuff size was used and the first and fifth phases were recorded. Values used in the analysis are the average of two readings taken at 5-min intervals.

Birthweight and birth length were obtained from standard medical records. Gestational age- and sex-adjusted SDS for birthweight and height were calculated using regional normative data.[23]

### Analytical methods

Serum glucose, total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol and triglycerides were measured using standard enzymatic methods. Fasting serum insulin was assessed using a solid-phase, enzyme-labelled, chemiluminescent sequential immunometric assay (Immulite 2000, DPC DIPESA S.A., Madrid, Spain). The lower limit of detection was 14·4 pmol/l. The intra- and interassay coefficients of variation (CVs) were less than 8%.

Insulin resistance and insulin secretion were calculated using the homeostasis model assessment for insulin resistance [HOMA-IR = (fasting insulin in mU/l) × (fasting glucose in mM)/22·5] and β-cell function [HOMA-β-cell = (fasting insulin in mU/l × 20)/(fasting glucose in mM − 3·5)].[24]

sTNFR1 and sTNFR2 levels were analysed using commercially available solid-phase enzyme-amplified sensitivity immunoassays (EASIA™, Biosource Technologies, Inc. Europe S.A., Fleunes, Belgium). The intra- and interassay CVs were < 7% and < 9%, respectively. sTNFR1 EASIA has no cross-reactivity with sTNFR2, and TNF-α does not interfere with the assay.

### Genetic analysis

Genomic DNA was purified from peripheral blood leucocytes using QIAGEN QIAmpBlood kits. The amount of DNA used for PCR amplification was approximately 100 ng.

A 'G' to 'A' transition polymorphism in the promoter (position −308) of the *TNF* gene was studied by allelic discrimination analysis using an ABI Prism 7000 sequence detector and TaqMan technology. Sequences for minor groove binder (MGB) probes were 5′-CCGTCCCCATGCC-3′ and 5′-CCGTCCTCATGCC-3′ for GG and AA alleles, respectively, and sequences for primers were 5′-GAAATGGAGGCAATAGGTTTTGAG-3′ and 5′-GTAGGACCCTGGAGGCTGAAC-3′. The reaction was performed in a final volume of 25 μl. DNA was amplified after 50 cycles with an initial denaturation of 10 min at 95 °C. The cycle programme consisted of 15 s denaturation

at 92 °C and 1 min annealing and extension at 60 °C. Positive controls that had been characterized by restriction fragment length polymorphism analysis, as described previously,[18] were included in all reactions. The frequencies of the alleles in the control population were 86% and 14% for G and A alleles, respectively. The populations of children born AGA and those born SGA were in Hardy–Weinberg equilibrium ($\chi^2 = 0{\cdot}0$, $P = 1{\cdot}0$ and $\chi^2 = 0{\cdot}05$, $P = 0{\cdot}83$, respectively).

### *Statistical methods*

Statistical analyses were performed using SPSS version 10·0. Parameters that did not show a normal distribution were log transformed for subsequent analyses. The relationships between variables were analysed by simple correlation (Pearson's test) and multiple linear regression analyses. The $\chi^2$-test and logistic regression analyses were used to study differences in genotype frequencies between groups. Student's *t*-test and general lineal models were used to study differences in continuous variables among genotype groups and between SGA and AGA children. Levels of statistical significance were set at $P < 0{\cdot}05$.

## Results

The relationship between *TNF* genotypes and birth anthropometry was studied in 90 SGA (age 7·4 ± 4·5 years) and 108 AGA (age 8·7 ± 4·0 years) children, whose clinical characteristics at birth are shown in Table 1. To avoid the influence of puberty on insulin sensitivity,

**Table 1.** Clinical and laboratory variables in the study subjects

| | SGA | AGA | *P* |
|---|---|---|---|
| At birth | | | |
| *N* | 90 | 108 | |
| Sex (male/female) | 34/56 | 59/49 | 0·022 |
| Gestational age (weeks) | 39·2 ± 1·9 | 39·3 ± 1·4 | ns |
| Birthweight (g) | 2309·9 ± 380·5 | 3216·2 ± 399·0 | < 0·0001 |
| Birthweight SDS | −2·1 ± 0·5 | −0·2 ± 0·7 | < 0·0001 |
| Birth length (cm) | 45·3 ± 3·4 | 48·8 ± 2·3 | < 0·0001 |
| Birth length SDS | −2·1 ± 0·9 | −0·6 ± 1·1 | < 0·0001 |
| At time of study | | | |
| *N* | 58 | 57 | |
| Sex (male/female) | 25/33 | 34/23 | ns |
| Age (years) | 6·0 ± 2·7 | 6·9 ± 2·8 | ns |
| Weight SDS | −1·0 ± 1·4 | 0·1 ± 1·3 | < 0·0001 |
| Height SDS | −1·5 ± 1·5 | −0·2 ± 1·3 | < 0·0001 |
| BMI SDS | −0·3 ± 1·4 | 0·1 ± 1·2 | 0·054 |
| Waist to hip ratio | 0·93 ± 0·1 | 0·95 ± 0·1 | ns |
| SBP (mmHg) | 98·4 ± 11·2 | 103·4 ± 15·7 | ns |
| DBP (mmHg) | 50·6 ± 9·3 | 52·1 ± 10·7 | ns |
| Glucose (mmol/l) | 4·8 ± 0·4 | 4·8 ± 0·5 | ns |
| Insulin (pmol/l) | 28·7 (19·4–41·6) | 29·4 (14·4–54·5) | ns |
| HOMA-IR | 1·0 (0·7–1·3) | 0·89 (0·4–1·6) | ns |
| HOMA-β-cell | 73·9 (53·0–108·7) | 70·7 (51·7–125·2) | ns |
| Cholesterol (mmol/l) | 4·5 ± 0·8 | 4·5 ± 0·7 | ns |
| LDL-cholesterol (mmol/l) | 2·6 ± 0·8 | 2·5 ± 0·6 | ns |
| HDL-cholesterol (mmol/l) | 1·6 ± 0·4 | 1·7 ± 0·4 | ns |
| Triglycerides (mmol/l) | 0·63 (0·48–0·77) | 0·55 (0·42–0·66) | 0·031 |
| sTNFR1 (μg/l) | 1·56 (1·36–1·80) | 1·47 (1·27–1·68) | ns |
| sTNFR2 (μg/l) | 4·00 (3·41–4·99) | 4·73 (4·03–5·78) | 0·056 |
| After adjustment for sex, age and current weight or height | | | |
| Insulin (pmol/l) | 39·5 (34·4–44·7) | 33·0 (28·3–37·8) | 0·010 |
| HOMA-IR | 1·2 (1·0–1·4) | 1·0 (0·8–1·2) | 0·008 |
| Triglycerides (mmol/l) | 0·67 (0·58–0·77) | 0·58 (0·49–0·67) | 0·065 |
| sTNFR1 (μg/l) | 1·6 (1·5–1·7) | 1·5 (1·4–1·6) | ns |
| sTNFR2 (μg/l) | 4·3 (3·7–4·8) | 5·0 (4·5–5·6) | 0·020 |

AGA, appropriate for gestational age; SGA, small for gestational age; SDS, age- and sex-adjusted standard deviation score; BMI, body mass index; SBP/DBP, systolic and diastolic blood pressure; HOMA, homeostasis model assessment of insulin resistance (IR) and beta cell function (β-cell); sTNFR1 and 2, soluble TNF-α receptors 1 and 2. For nonadjusted means, data are expressed as mean ± SD for Gaussian variables and as median and interquartile range for non-Gaussian variables. Otherwise data are mean and 95% CI. *P*-values are from Student's *t*-test (nonadjusted means) and from general lineal models (adjusted means). Data for adjusted means of sTNFR2 are for lean prepubertal children.

**Table 2.** Genotype frequencies of *TNF* −308G/A SNP

| | SGA ($n = 90$) | AGA ($n = 108$) | $P$ | OR (95% CI) |
|---|---|---|---|---|
| *TNF* −308G/A | | | | |
| GG | 77 (85·6%) | 78 (72·2%) | | |
| GA | 13 (14·4%) | 29 (26·9%) | | |
| AA | 0 (0%) | 1 (0·9%) | 0·025* | 2·3 (1·1–4·7)* |

*For GG homozygous subjects compared to GA/AA genotypes.

the relationships among *TNF* genotypes, current anthropometry and endocrine–metabolic parameters were restricted to 58 prepubertal SGA and 57 prepubertal AGA children whose clinical data at birth did not differ from those of the group as a whole, except for a nonstatistically significant difference in gender (Table 1).

### *Current anthropometry and endocrine–metabolic parameters in prepubertal SGA and AGA children*

Prepubertal SGA children were lighter and shorter (both $P < 0·0001$) than prepubertal AGA children, had higher serum triglycerides ($P = 0·031$) and tended to have lower sTNFR2 ($P = 0·056$; Table 1). After adjusting for age, sex and current weight or height in general lineal models, prepubertal SGA children also showed higher serum insulin and HOMA-IR values than AGA children ($P = 0·01$ and $P = 0·008$, respectively). Further analysis revealed that the difference in serum sTNFR2 was restricted to lean subjects (arbitrarily defined as having BMI SDS values below the 50th centile, $P = 0·020$). In SGA children, being overweight caused a significant increase in serum sTNFR2 concentrations [from 4·3 (95% confidence interval (CI) (3·7–4·8) μg/l to 5·0 (4·5–5·6) μg/l, $P = 0·028$], which were very similar to those observed in AGA children [5·0 (4·6–5·4) μg/l].

### *TNF genotypes in SGA and AGA children*

Genotype frequencies for the *TNF* −308G/A SNP are shown in Table 2. The frequency of the G allele in the *TNF* gene for the AGA population was similar to that reported in healthy adults from the same geographical area.[18] Among SGA children, the frequency of GG homozygotes was unexpectedly higher than in AGA children (86% *vs.* 72%, $P = 0·025$). The risk of being SGA when having the GG genotype was 2·3 (95% CI 1·1–4·7; $P = 0·026$).

### *TNF genotypes and birth anthropometry*

In addition to the increased prevalence in SGA children, the GG genotype was weakly associated with lower birth length in the AGA group (adjusted means: 48·4 ± 0·3 cm *vs.* 49·5 ± 0·5 cm; $P = 0·065$; Table 3), and was associated with both lower birthweight and lower birth length in all subjects, pooled in a single group, in general lineal models that included SGA status as an independent variable (adjusted means: 2747·0 ± 23·3 g *vs.* 2851·0 ± 45·7 g, $P = 0·045$, and 47·0 ± 0·2 cm *vs.* 48·2 ± 0·4 cm, $P = 0·011$, respectively).

### *TNF genotypes and metabolic parameters in prepubertal SGA and AGA children*

In the AGA but not in the SGA group, the *TNF* −308GG genotype was associated with higher systolic blood pressure [adjusted means: 103·3 (95% CI 96·4–110·2) mmHg *vs.* 92·8 (84·9–100·7) mmHg; $P = 0·028$], and higher glucose [4·8 (4·7–5·0) mmol/l *vs.* 4·5 (4·3–4·8) mmol/l; $P = 0·042$], serum insulin [45·9 (38·3–53·5) pmol/l *vs.* 30·0 (17·9–42·0) pmol/l; $P = 0·012$] and HOMA-IR [1·4 (1·1–1·7) *vs.* 0·9 (0·4–1·3); $P = 0·005$; Table 3].

### *Correlation and regression analyses*

Simple correlation analysis revealed an association between sTNFR2 and age ($r = −0·36$, $P < 0·0001$). Because most metabolic parameters were also age- and sex-dependent, simple correlation analyses were calculated adjusting for age and sex. Serum concentrations of sTNFR2 showed significant associations with current anthropometry, i.e. current weight ($r = 0·20$, $P = 0·043$) and BMI ($r = 0·21$, $P = 0·037$). Further analyses showed that these associations were restricted to SGA subjects ($r = 0·37$, $P = 0·009$, for both current weight and BMI in SGA children). Circulating sTNFR2 was also associated with total cholesterol ($r = −0·22$, $P = 0·044$) and LDL-cholesterol ($r = −0·29$, $P = 0·010$) in all subjects studied as a group. sTNFR1 was similarly associated with total cholesterol ($r = −0·27$, $P = 0·008$) and had a borderline correlation with LDL-cholesterol ($r = −0·20$, $P = 0·072$). No other significant correlations were observed between endocrine–metabolic or anthropometric parameters and either sTNFR1 or sTNFR2, after adjustment for age and sex.

In multiple regression analyses of HOMA-IR as the dependent variable, and age, sex, BMI, birthweight and *TNF* −308GG genotype as independent variables, age was the main predictor of HOMA-IR, explaining 36% of its variance ($\beta = 0·05$; $P < 0·0001$). Additional predictors of HOMA-IR in AGA, but not in SGA, children were BMI ($\beta = 0·05$; $P = 0·001$; $R^2 = 15\%$), the *TNF* −308GG genotype ($\beta = 0·21$; $P = 0·01$; $R^2 = 8\%$) and female sex ($\beta = 0·20$; $P = 0·01$; $R^2 = 7\%$).

Multiple regression models were also constructed with sTNFR2 as the dependent variable, and age, sex, BMI, birthweight and HOMA-IR as independent variables. In these models, age was the main predictor or sTNFR2, explaining 11% of its variance ($\beta = −0·02$; $P < 0·0001$). After excluding age from these models, significant effects were also documented for HOMA-IR in AGA children ($\beta = −0·15$; $P = 0·043$; $R^2 = 7\%$) and for BMI in SGA children ($\beta = 0·02$; $P = 0·009$; $R^2 = 12\%$).

## Discussion

To our knowledge, only two previous studies have examined the relationship between the *TNF* −308GA SNP and birthweight and both failed to show significant associations between these traits.[25,26]

A major, although unexpected, finding of our study was the higher frequency of GG homozygosity at position −308 in the *TNF* gene in SGA children (it should be noted that the frequency of the G allele for AGA control children was in agreement with that described for healthy adults from the same geographical area).[18]

**Table 3.** Clinical and laboratory variables in the study subjects according to *TNF* −308G/A genotype

| | SGA | | | AGA | | |
|---|---|---|---|---|---|---|
| | GG | GA/AA | *P* | GG | GA/AA | *P* |
| At birth | | | | | | |
| *N* | 77 | 13 | | 78 | 30 | |
| Sex (male/female) | 30/47 | 4/9 | ns | 42/36 | 17/13 | ns |
| Gestational age (weeks) | 39·2 ± 1·9 | 39·7 ± 1·2 | ns | 39·4 ± 1·5 | 39·1 ± 1·2 | ns |
| Birthweight | 2289·5 ± 397·0 | 2429·2 ± 242·1 | ns | 3195·6 ± 396·2 | 3274·8 ± 422·2 | ns |
| Birthweight SDS | −2·1 ± 0·5 | −2·0 ± 0·4 | ns | −0·2 ± 0·6 | 0·0 ± 0·8 | ns |
| Birth length | 45·4 ± 2·8 | 46·6 ± 1·1 | ns | 48·6 ± 2·3 | 49·6 ± 2·4 | ns |
| Birth length SDS | −2·2 ± 0·9 | −1·7 ± 0·7 | ns | −0·7 ± 0·9 | − 0·1 ± 1·3 | ns |
| After adjustment for sex and gestational age | | | | | | |
| Birthweight | 2307·7 ± 29·8 | 2373·5 ± 71·1 | ns | 3174·6 ± 35·8 | 3295·8 ± 59·8 | ns |
| Birth length | 45·3 ± 0·2 | 46·5 ± 0·6 | ns | 48·4 ± 0·3 | 49·5 ± 0·5 | 0·065 |
| At time of study | | | | | | |
| *N* | 49 | 9 | | 40 | 17 | |
| Sex (male/female) | 23/26 | 2/7 | ns | 24/16 | 10/7 | ns |
| Age (years) | 5·6 ± 2·9 | 4·1 ± 1·4 | 0·020 | 7·0 ± 2·9 | 6·3 ± 3·1 | ns |
| Weight SDS | −1·1 ± 1·4 | −1·7 ± 1·0 | ns | 0·2 ± 1·4 | −0·3 ± 1·4 | ns |
| Height SDS | −1·7 ± 1·3 | −2·0 ± 1·5 | ns | −0·2 ± 1·4 | −0·8 ± 1·3 | ns |
| BMI SDS | −0·4 ± 1·5 | −0·9 ± 1·0 | ns | 0·3 ± 1·6 | 0·0 ± 1·3 | ns |
| Waist to hip ratio | 0·91 ± 0·1 | 1·01 ± 0·1 | ns | 0·95 ± 0·1 | 0·94 ± 0·1 | ns |
| SBP (mmHg) | 94·8 ± 10·7 | 100·3 ± 10·4 | ns | 106·6 ± 14·5 | 94·5 ± 11·4 | 0·014 |
| DBP (mmHg) | 48·8 ± 9·3 | 50·4 ± 9·3 | ns | 54·2 ± 10·7 | 49·9 ± 8·9 | ns |
| Glucose (mmol/l) | 4·7 ± 0·5 | 4·6 ± 0·4 | ns | 4·9 ± 0·5 | 4·5 ± 0·8 | 0·020 |
| Insulin (pmol/l) | 29·4 (19·6–41·6) | 24·4 (14·4–37·3) | ns | 36·6 (16·0–67·6) | 23·0 (14·4–38·0) | 0·034 |
| HOMA-IR | 0·9 (0·6–1·3) | 0·8 (0·4–1·2) | ns | 1·0 (0·5–2·2) | 0·6 (0·4–1·2) | 0·016 |
| HOMA-β-cell | 73·3 (50·0–106·5) | 66·7 (49·7–87·8) | ns | 79·2 (50·0–132·8) | 61·6 (35·0–130·2) | ns |
| Cholesterol (mmol/l) | 4·4 ± 1·0 | 4·5 ± 0·7 | ns | 4·4 ± 0·6 | 4·5 ± 0·7 | ns |
| LDL-cholesterol (mmol/l) | 2·5 ± 0·9 | 2·7 ± 0·6 | ns | 2·5 ± 0·6 | 2·5 ± 0·6 | ns |
| HDL-cholesterol (mmol/l) | 1·5 ± 0·5 | 1·5 ± 0·3 | ns | 1·7 ± 0·4 | 1·7 ± 0·3 | ns |
| Triglycerides (mmol/l) | 0·63 (0·48–0·75) | 0·61 (0·56–0·89) | ns | 0·54 (0·42–0·77) | 0·57 (0·46–0·67) | ns |
| STNFR1 (μg/l) | 1·6 (1·4–1·81) | 1·5 (1·2–1·7) | ns | 1·4 (1·2–1·6) | 1·6 (1·3–1·8) | ns |
| STNFR2 (μg/l) | 4·4 (3·5–5·2) | 3·9 (3·6–4·9) | ns | 4·6 (3·8–6·2) | 4·7 (4·1–5·7) | ns |
| After adjustment for sex, age, BMI and birthweight | | | | | | |
| SBP (mmHg) | 93·7 (90·0–97·5) | 103·0 (92·2–113·8) | ns | 103·3 (96·4–110·2) | 92·8 (84·9–100·7) | 0·028 |
| Glucose (mmol/l) | 4·6 (4·5–4·7) | 4·7 (4·5–5·0) | ns | 4·8 (4·7–5·0) | 4·5 (4·3–4·8) | 0·042 |
| Insulin (pmol/l) | 33·7 (28·6–38·8) | 30·1 (16·9–43·1) | ns | 45·9 (38·3–53·5) | 30·0 (17·9–42·0) | 0·012 |
| HOMA-IR | 1·0 (0·8–1·1) | 0·9 (0·5–1·3) | ns | 1·4 (1·1–1·7) | 0·9 (0·4–1·3) | 0·005 |

SDS, age- and sex-adjusted standard deviation score; BMI, body mass index; SBP/DBP, systolic and diastolic blood pressure; HOMA, homeostasis model assessment of insulin resistance (IR) and beta cell function (β-cell); sTNFR1 and 2, soluble TNF-α receptor 1 and 2. For nonadjusted means, data are expressed as mean ± SD for Gaussian variables and as median and interquartile range for non-Gaussian variables. Otherwise data are mean and 95% CI. *P*-values are from Student's *t*-test (nonadjusted means) and from general lineal models (adjusted means).

Infants and children born SGA have been reported to be more insulin resistant than children born AGA,[27] but also to be leaner and shorter,[28] to have decreased muscularity and decreased subcutaneous fat and relatively increased visceral fat.[29] As TNF-α is a mitogen for skeletal muscle,[30] and possibly also for adipocytes,[31] our data might indicate that the *TNF* −308G allele, which is known to be associated with lower expression of the *TNF*-α gene, is associated with smaller size at birth by influencing either muscle or fat development and thus may have important implications for later development of both muscle and fat mass and insulin sensitivity in adults born SGA.

In agreement with the higher GG homozygosity in SGA children, we also observed an association between the GG genotype and lower birthweight and length and, independently of size at birth, higher systolic blood pressure, serum glucose, insulin and HOMA-IR. It can be argued that the condition of lower birthweight and higher insulin resistance of SGA children may override these associations, which would explain why most of these differences were observed in AGA children. Alternatively, the statistical power in SGA children might not have been sufficient to disclose at least part of the associations observed in AGA children.

sTNFR1 and sTNFR2, which are considered proinflammatory molecules and surrogates of TNF-α activity, have been associated with hypertension, dyslipidaemia, obesity and insulin resistance.[6] Unexpectedly, we found that lean SGA children had lower concentrations of sTNFR2 than lean AGA children and the soluble receptor was directly correlated with current anthropometric measures in these children. In line with the above-mentioned findings, sTNFR2 may act as a positive regulator of body weight, specifically muscle mass, fat mass, or both. Of note, mice deficient in TNF-α receptor-associated factor 2 (TRAF2), an important signalling molecule for TNF-α receptor activation, are born with a systematically smaller muscle mass,[32] and we and others have observed significant positive correlations between circulating sTNFR2 and measures of muscle mass in humans.[5,33]

sTNFR2 was also associated with measurements of insulin resistance and serum lipids besides its associations with body size. Similarly, sTNFR1 was associated with total cholesterol. It is noteworthy that the TNF-α soluble receptors were negatively associated with insulin resistance traits. Because TNF-α is known to acutely induce glucose uptake, presumably in a noninsulin-dependent manner,[34,35] it is conceivable that this cytokine may be capable of exerting insulin-like properties in children, favouring glucose uptake and growth.[31] We speculate that ageing and obesity result in a predominance of the inhibitory actions of TNF-α on insulin-dependent glucose uptake and in resistance to the growth factor-like effects of this cytokine. Supporting our findings, Jefferies *et al.*[36] have recently reported lower circulating TNF-α in formerly SGA children and a positive correlation between the reduction in insulin sensitivity in these children and the lowering of plasma TNF-α concentrations.

Our data can be unified as follows: the *TNF* –308GG genotype, which is associated with lower transcription rates, causes a functional deficiency of the growth-promoting activities of TNF-α, resulting in lower size at birth. The allele may also increase the risk for postnatal insulin resistance, partly because of its association with low birthweight, partly by mechanisms that imply at least the insulin-like properties of TNF-α on glucose uptake. This hypothesis is supported by the fact that SGA children have increased frequencies of this allele and increased postnatal insulin resistance.

TNF-α may also have postnatal growth-promoting actions, which is supported by the fact that lean SGA children have lower circulating sTNFR2 and that both postnatal growth and insulin sensitivity are determinants of serum sTNFR2 concentrations. Alternatively, circulating sTNFR2 can be growth dependent during childhood. Because of the cross-sectional design of our study, the proposed mechanisms are only speculative and should be viewed as hypothesis generating.

In summary, SGA children show increased frequency of the *TNF* –308G allele, an allele that is associated with prenatal growth and postnatal insulin resistance. The *TNF* –308G/A polymorphism may have implications in the growth and metabolic abnormalities that characterize SGA children.

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