The 23K variant of the R23K polymorphism in the glucocorticoid receptor gene protects against postnatal growth failure and insulin resistance after preterm birth

GR variation on metabolism after prematurity

Martijn JJ Finken (1,2), Ingrid Meulenbelt (3), Friedo W Dekker (2), Marijke Frölich (4), Johannes A Romijn (5), P Eline Slagboom (3), Jan M Wit (1); on behalf of the Dutch POPS-19 Collaborative Study Group

1. Department of Paediatrics, Leiden University Medical Center, the Netherlands
2. Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands
3. Department of Molecular Epidemiology, Leiden University Medical Center, the Netherlands
4. Department of Clinical Chemistry, Leiden University Medical Center, the Netherlands
5. Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, the Netherlands

Participants in the Dutch POPS-19 Collaborative Study Group are: TNO Quality of Life, Leiden (E. T. M. Hille, C. H. de Groot, H. Kloosterboer-Boerrigter, A. L. den Ouden, A. Rijpstra, S. P. Verloove-Vanhorick, J. A. Vogelaar); Emma Children's Hospital AMC, Amsterdam (J. H. Kok, A. Ilse, M. van der Lans, W. J. C. Boelen-van der Loo, T. Lundqvist, H. S. A Heymans); University Hospital Groningen, Beatrix Children's Hospital, Groningen (E. J. Duiverman, W. B. Geven, M. L. Duiverman, L. I. Geven, E. J. L. E. Vrijlandt); University Hospital Maastricht, Maastricht (A. L. M. Mulder, A. Gerver); University Medical Center St Radboud, Nijmegen (L. A. A. Kollée, L. Reijmers, R. Sonnemans); Leiden University Medical Center, Leiden (J. M. Wit, F. W. Dekker, M. J. J. Finken); Erasmus MC—Sophia Children's Hospital, University Medical Center Rotterdam (N. Weisglas-Kuperus, M. G. Keijzer-Veen, A. J. van der Heijden, J. B. van Goudoever); V. U. University Medical Center, Amsterdam (M. M. van Weissenbruch, A. Cranendonk, H. A. Delemarre-van de Waal, L. de Groot, J. F. Samson); Wilhelmina Children's Hospital, UMC, Utrecht (L. S. de Vries, K. J. Rademaker, E. Moerman, M. Voogsgeerd); Máxima Medical Center, Veldhoven (M. J. K. de Kleine, P. Andriessen, C. C. M. Dielissen-van Helvoirt, I. Mohamed); Isala Clinics, Zwolle (H. L. M. van Straaten, W. Baerts, G. W. Veneklaas Slots-Kloosterboer, E. M. J. Tuller-Pikkemaat); Royal Effatha Guyot Group, Zoetermeer (M. H. Ens-Dokkum); Association for Parents of Premature Babies (G. J. van Steenbrugge).

Corresponding author (to whom reprint requests should be addressed):
Martijn J.J. Finken
Department of Paediatrics
Leiden University Medical Center
P. O. Box 9600
2300 RC Leiden
The Netherlands
Tel.: +31-71-526 2824
Fax: +31-71-526 6994
E-mail: m.j.j.finken@lumc.nl

Disclosure summary:
MJJF, IM, FWD, MF, JAR and PES have nothing to declare. JMW has received consulting fees and lecture fees from Ferring, Ipsen, Novo Nordisk and Ipsen.

Length of article:
Word count of text only: 2,467
Word count of abstract: 248
Number of tables: 3
Number of figures: 3

Acknowledgements:
This specific part of the POPS-19 study was supported by grants from the Netherlands Organisation for Scientific Research (NWO) and the Center of Medical System Biology (CMSB). The POPS-19 study was supported by grants from the Netherlands Organisation for Health Research and Development (ZonMw), Edgar Doncker Foundation, Foundation for Public Health Fundraising Campaigns, Phelps Foundation, Swart-van Essen Foundation, Foundation for Children's Welfare Stamps, TNO Quality of Life, Netherlands Organisation for Scientific Research (NWO), Dutch Kidney Foundation, Sophia Foundation for Medical Research, Stichting Astmabestrijding, Royal Effatha Guyot group.
We thank Dennis Kremer for genotyping.

Key terms:
Preterm birth
Growth
Metabolic syndrome
Glucocorticoid receptor
Abstract

Context:
Preterm birth is associated with postnatal growth failure, abdominal fat accumulation, insulin resistance and hypertension, resembling increased glucocorticoid bioactivity.

Objective:
We tested the effects of the R23K and N363S polymorphisms in the glucocorticoid receptor gene, associated with decreased and increased sensitivity to cortisol, respectively, on linear growth and the adult metabolic profile in a cohort (n=249) of men and women born <32 gestational weeks and followed prospectively from birth until 19 years of age.

Design and participants:
Birth cohort study that included 249 19-year-old survivors born at a gestational age <32 weeks from the Dutch Project On Preterm and Small-for-gestational-age infants (POPS) cohort.

Setting:
Multicenter-study.

Main outcome measures:
Linear growth and adult body composition, fasting cortisol, glucose, insulin and cholesterol concentrations, and blood pressure.

Results:
The 23K variant (n=24) was associated with lower fasting insulin levels (mean difference after log-transformation: -0.09 (95% CI: -0.16; -0.01) mU/l) and a lower HOMA-IR (mean difference after log-transformation: -0.09 (95% CI: -0.16; -0.01)), as well as with a taller stature departing from the age of 1 year onwards. 23K carriers showed complete catch-up growth between the ages of 3 months and 1 year and attained height was similar to the population reference mean, whereas stature in non-carriers was on average 0.5 SD below this mean. In contrast, the N363S polymorphism was not associated with any of the outcomes.

Conclusions:
Carriers of the 23K variant are, at least in part, protected against postnatal growth failure and insulin resistance after preterm birth.
Introduction

Functional changes in the gene encoding the glucocorticoid receptor (GR) play an important role in glucocorticoid bioactivity. To date, several functional polymorphisms in the GR gene (NR3C1) have been identified, including R23K (ER22/23EK) and N363S. The 23K variant has been associated with decreased sensitivity to glucocorticoids and a beneficial metabolic health (1). Elderly persons with this variant had lower levels of fasting insulin, and of total and LDL cholesterol (1). Thirty-six-year-old men with this variant had a taller stature, more lean body mass and a greater muscle strength, while their female contemporaries had a tendency towards a smaller waist circumference (2). Unfavourable effects were found with the 363S variant, which has been associated with increased sensitivity to glucocorticoids (3). Subjects carrying this variant were predisposed to obesity and coronary artery disease (3-6).

A number of recent studies have elucidated the long-term metabolic consequences of preterm birth. These consequences include an increased risk of short stature (7, 8), abdominal fat accumulation (9), insulin resistance (10-12), and hypertension (12-14). The clustering of postnatal growth failure and metabolic risk factors in individuals born preterm is indicative for, and/or resembles, effects of increased glucocorticoid bioactivity. Indeed, in a small sample of young adults, it was found that basal cortisol levels were higher after preterm birth (15). It is unknown whether common variants in the GR gene could explain variations in the endocrine-metabolic state of adults born prematurely.

Therefore, we tested the effects of the R23K and N363S polymorphisms on linear growth, body composition, insulin resistance, the serum lipid profile, and blood pressure in a cohort of 19-year-old men and women who were born very preterm (i.e., <32 gestational weeks).

Methods

Subjects

The Project On Preterm and Small-for gestational-age infants (POPS) study is a nationwide, multicenter, prospective follow-up study, comprising 94% of all liveborn very preterm (<32 weeks' gestation) and/or very-low-birth-weight (<1,500 g) infants born in the Netherlands in 1983 and has documented birth, growth and a number of other characteristics from birth onwards (16, 17). At the follow-up visits (at the age of 3 mo, 6 mo, 1 yr and 2 yrs post-term, and at the chronological age of 5 yrs), length/height was measured. Length until the age of 2 years was measured to the nearest 1 cm in supine position, fully extended with the heels in contact with a baseboard. Standing height at the age of 5 years was measured to 1-mm accuracy. At 19 years of age, all 637 alive subjects born with a gestational age <32 weeks who were free from congenital skeletal deformations, Down's syndrome, chromosomal abnormalities, multiple congenital deformations or inborn errors of metabolism, and who were not born to mothers with gestational diabetes, were approached by mail to participate in the POPS-19 study. Of these subjects, 395 consented to participate (62% response rate). Subjects with diabetes mellitus, or on thyroid hormone or systemic corticosteroids, as well as non-Caucasian subjects and pregnant women, were excluded for this specific study. The data of not-fasted subjects were not analyzed either. The approval of the medical ethical committees of all participating centers was obtained for the POPS-19 study.

Study protocol

Subjects who gave written informed consent to participate were seen after an overnight fast between 08.30 and 10.00 a.m. at one of the outpatient clinics of the ten participating centers. Assessors were blinded with respect to the perinatal characteristics of the subjects.

After 30 minutes in supine position, systolic and diastolic blood pressure was measured three times consecutively with an automatic blood pressure device (Dinamap, Critikon, Germany) at the non-dominant arm. The mean values of these measurements were used in the statistical analyses. The cuff size was adjusted to fit arm length and circumference. Venous blood
was subsequently obtained in supine position. Thereafter, anthropometry was performed, for which assessors had received extensive training prior to the study, and re-training during the entire study period at 2-months' intervals. Subjects were measured barefoot while wearing underclothing only. Weight was measured to the nearest 0.1 kg on a balance scale, and height to the nearest 0.1 cm with a fixed stadiometer. Waist and hip circumferences were measured at 0.1 cm-accuracy using standard methods (18). Four skinfold thickness measurements were taken in duplicate with a calibrated skinfold caliper on the left side of the body: at triceps, biceps, subscapular, and iliacal regions. From these measurements, fat mass was calculated using the equations of Durnin (19). A more detailed description of skinfold thickness measurements obtained in the POPS-19 study has been published elsewhere (9).

Laboratory analyses
Blood samples were stored at -80 °C, and thawed only once immediately before analysis. Glucose and total cholesterol were measured in a fully automated computerized laboratory system with a Hitachi 747 (Hitachi, Tokyo, Japan) chemistry analyzer. HDL and LDL were measured with a turbidimetric assay on a Hitachi 911. Cortisol was measured with a fluorescence polarization immunoassay on an Abbott TDx (Abbott Laboratories, Abbott Park, IL 60064, USA). The sensitivity of this assay is 20 nmol/l and the interassay CV ranges from 3.1 to 6.4% at different levels. Insulin and C-peptide were measured with highly sensitive radioimmunoassays (Linco, St. Charles, MO 63304, USA). The detection levels of these assays are 0.1 mU/l and 0.03 mmol/l, respectively, and the interassay CVs range from 4.7-12.2% and 3.2-9.3% at different levels, respectively. HOMeostatic Model Assessment for Insulin Resistance index (HOMA-IR) was calculated (20). Insulin and C-peptide levels, and HOMA-IR were used as parameters of insulin resistance. Fasting insulin levels and HOMA-IR correlate strongly with S, assessed by the frequently-sampled IVGTT in young persons (21, 22).

For both the R23K (rs6190) and N363S (rs6195) single-nucleotide polymorphisms (SNPs), polymerase chain reactions were performed using 2.5 ng of genomic DNA and standard reagents. SNPs were subsequently genotyped by mass spectrometry (homogeneous mass array system, Sequenom Inc., San Diego, CA, USA), using standard conditions. Genotypes were analyzed by using Genotyper 3.0 software (Sequenom Inc.). We identified 24 subjects (9.6%) who were heterozygous for the 23K variant (12 men and 12 women), and 15 (6.0%) who were heterozygous for the 363S variant (6 men and 9 women). None of the subjects was carrier of both variants. The corresponding allele frequencies of 4.8% and 3.0%, respectively, were reasonably well in range with the allele frequencies observed in healthy Dutch populations, ranging from 3 to 4.5% for the 23K variant, and from 3 to 5% for the 363S variant (1-3, 23, 24). For both SNPs, the genotype distribution was in agreement with the distribution predicted by the Hardy-Weinberg equilibrium (p=0.42 for the R23K polymorphism, and p=0.62 for the N363S polymorphism).

Statistical analysis
Auxological data at birth and on subsequent occasions were converted to standard deviation scores (SDSs), to correct for (gestational) age and sex, using Swedish references for preterm infants (25) and recently collected Dutch references (18, 26, 27), respectively. Comparisons were made between minor allele carriers and non-carriers, using the independent samples t test. Outcomes with skewed distributions (cortisol, insulin, and HOMA-IR) were 10log-transformed prior to statistical comparison. Analyses were repeated with adjustment for perinatal factors (obstetric characteristics, gestational age, and postnatal clinical course) using linear regression analysis. Modification by gender of the effect of genotype on outcomes was tested by first including the variables genotype (in which minor allele carrier=1 and non-carrier=0) and gender (in which male=1 and female=0) in a linear regression analysis followed by the inclusion of their product.

Results
Table 1 lists the perinatal characteristics of the 249 participants, showing that, apart from an unequal distribution in the numbers who had been treated with glucocorticoids as neonates, there were no statistically significant differences between the GR genotypes.

Table 2 summarizes the growth patterns of the groups up to adult height. 23K carriers and non-carriers showed a similar degree of catch-down growth between birth and the age of 3 months. Between the ages of 3 months and 1 year, 23K carriers showed more rapid catch-up growth than non-carriers. Stature at 1 year and beyond was greater than or similar to the population reference mean in carriers of the 23K variant, whereas in non-carriers it was on average 0.5 SD below this mean. Correction for perinatal factors only slightly reduced the strength of these associations (data not shown). Figures 1A-C show that the difference in linear growth between 23K carriers and non-carriers was more pronounced in men, though the direction of association was similar for women. Despite these sex-specific observations, the test for interaction showed that the association between the R23K polymorphism and stature was dependent on gender only at 5 years of age. Linear growth of 363S carriers did not differ from non-carriers.

Table 3 shows the adult metabolic profile for the GR genotypes. 23K carriers had lower fasting insulin levels and a lower HOMA-IR than non-carriers. These differences became somewhat larger after correction for perinatal factors (data not shown). In addition, 23K carriers had a lower WHR, but this observation did not reach statistical significance. Interaction between the R23K polymorphism and gender on total and LDL cholesterol levels was observed, which was explained by opposite influences in men and women of the 23K variant on cholesterol levels. The adult metabolic profile of 363S carriers did not differ from non-carriers.

Discussion

In this prospective study in subjects who were born very preterm (i.e., <32 gestational weeks) and followed until 19 years of age, we found that the 23K variant in the GR gene was associated with lower fasting insulin levels and a lower HOMA-IR, as well as with a taller stature departing from the age of 1 year. It was also associated with a smaller waist-to-hip circumference, though this observation was not statistically significant. Carriers of the 23K variant showed complete catch-up growth between the ages of 3 months and 1 year and attained height was similar to the population reference mean. The N363S polymorphism was not associated with any of these outcomes.

In our study, we found that mean adult stature in carriers of the 23K variant was similar to the population reference mean, whereas mean height in the non-carriers was approximately 0.5 SD below this mean. Previous studies in healthy men of different ages found that 23K carriers were on average 4 cm taller than non-carriers (2, 24). This difference in final height was for an important part attributed to the pubertal growth spurt (2). In contrast, we found in our specific population of very preterm subjects that the growth pattern differed significantly between carriers and non-carriers already by the age of 1 year. However, the difference (in SDS) between 23K carriers and non-carriers did not further increase after puberty. Furthermore, a large number of genome-wide linkage scans have been performed aiming at the detection of new chromosomal loci influencing the quantitative trait height (28). Notably, the chromosomal region of the GR gene on chromosome 5q31 is one of the regions that have been implicated in more than one of such studies (29, 30). Possibly, functional genetic variation at the GR gene is explaining these linkages and may be considered a strong positional candidate gene.

In addition, in this specific cohort, we found that the 23K variant was associated with lower fasting insulin levels and a lower HOMA-IR at only 19 years of age, in line with findings from others in elderly people (1). Observations in other cohorts of effects of the R23K polymorphism on body composition and the serum lipid profile (1, 2) were not
confirmed by our data. Furthermore, we found no statistically significant relations with blood pressure.

Experiments in rats have shown that non-handling during early postnatal development permanently increases hypothalamus-pituitary-adrenal (HPA) axis activity (31). Similar effects in offspring were observed with naturally low-grooming mothers (32). Furthermore, it has been indicated that the extent of grooming in rat mothers specifically alters the methylation at the GR gene promoter in the hippocampus, thereby explaining how the effect of maternal care might persist into adulthood (33). During their neonatal course, preterm newborns are to a large extent devoid of maternal care and, instead, are subject to many stressful and sometimes even critical events, including for example respiratory distress, intubation and mechanical ventilation, and frequent blood sampling. Therefore, it could be possible that adverse postnatal circumstances in humans may also result in life-long activation of the HPA axis. This is supported by data from a small study in young adults, showing that basal cortisol levels are elevated after preterm birth (15). The current findings suggest that the 23K variant protects, at least in part, against postnatal growth failure and insulin resistance after preterm birth. We speculate that an extreme stressful event such as preterm birth may induce hypermethylolation of the GR promoter, leading to less GR expression in central feedback regions and, hence, enhanced stress responsiveness. GR expression in carriers of the 23K variant may be less vulnerable to alterations in DNA methylation. Indeed, SNPs have been shown to be associated to methylation of neighbouring CpG sites (34).

The functionality of the studied variants has been elucidated previously. The 23K variant has been associated with higher circulating cortisol levels after overnight dexamethasone suppression (1), whereas the 363S variant has been associated with lower post-dexa cortisol (3). These findings were subsequently confirmed by transfection studies, showing decreased and increased gene expression in response to GR binding, respectively (35).

Because our participants were genotyped at 19 years of age, a survivor effect of GR variation could not be excluded, considering the high neonatal mortality rate in the original cohort (16,17). However, an argument against selective survival of a particular genotype is that the observed genotype frequencies of the studied polymorphisms did not deviate much from the genotype frequencies in the normal Dutch population, implying that gross selective survival of a particular genotype is not very likely to have occurred in our population.

Although sex-specificity of the effects of the R23K polymorphism on linear growth and body composition have been reported by one study (2) and was attributed to a different regulation of HPA axis activity by androgens and estrogens, we did not find much evidence for sexually dimorphic effects of the R23K polymorphism, except for height at 5 years of age and adult cholesterol levels. Clearly, any sex-specific observation must be balanced against the small numbers of subjects carrying the 23K variant (12 men and 12 women).

In conclusion, we found in 19-year-old survivors of very preterm birth that the 23K variant was associated with lower fasting insulin levels and a lower HOMA-IR as well as with a taller stature departing from the age of 1 year. Carriers of the 23K variant showed complete catch-up growth between the ages of 3 months and 1 year and attained height was similar to the population reference mean. Therefore, carriers of the 23K variant are, at least in part, protected against postnatal growth failure and insulin resistance after preterm birth.
References


a national survey of preterm and very-low-birthweight infants in the Netherlands. Lancet 1:55-57


Figure legends

Figures 1A-C
Linear growth according to the R23K polymorphism.

Figure A: Entire population
Figure B: Men
Figure C: Women

Values are means±95% CI.
### Table 1
Perinatal characteristics of participants by GR genotype.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Genotype</th>
<th>23K vs. non-carriers</th>
<th>363S vs. non-carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R23/23K</td>
<td>N363/363S</td>
<td>Non-carriers</td>
</tr>
<tr>
<td>N</td>
<td>24</td>
<td>15</td>
<td>210</td>
</tr>
<tr>
<td>Males (%)</td>
<td>12 (50.0%)</td>
<td>6 (40.0%)</td>
<td>102 (48.6%)</td>
</tr>
<tr>
<td>Obstetric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age (yrs)</td>
<td>28.1±4.5</td>
<td>27.5±3.3</td>
<td>27.2±5.4</td>
</tr>
<tr>
<td>Parity &gt;0 (%)</td>
<td>12 (50.0%)</td>
<td>6 (40.0%)</td>
<td>100 (47.6%)</td>
</tr>
<tr>
<td>Part of multiple pregnancy (%)</td>
<td>2 (8.3%)</td>
<td>2 (13.3%)</td>
<td>54 (25.7%)</td>
</tr>
<tr>
<td>Hypertension during pregnancy (%)</td>
<td>8 (33.3%)</td>
<td>1 (6.7%)</td>
<td>35 (16.7%)</td>
</tr>
<tr>
<td>Drugs and alcohol intoxication (%)</td>
<td>9 (37.5%)</td>
<td>7 (46.7%)</td>
<td>113 (53.8%)</td>
</tr>
<tr>
<td>Prolonged rupture of membranes (%)</td>
<td>2 (8.3%)</td>
<td>4 (26.7%)</td>
<td>51 (24.3%)</td>
</tr>
<tr>
<td>Maternal glucocorticoid treatment (%)</td>
<td>7 (29.2%)</td>
<td>4 (26.7%)</td>
<td>42 (20.0%)</td>
</tr>
<tr>
<td>Body proportions at birth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>30.2±1.4</td>
<td>30.0±1.5</td>
<td>29.9±1.5</td>
</tr>
<tr>
<td>Birth weight:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- g</td>
<td>1,321±268</td>
<td>1,449±411</td>
<td>1,335±327</td>
</tr>
<tr>
<td>- SDS</td>
<td>-0.33±0.93</td>
<td>0.19±0.91</td>
<td>-0.13±1.03</td>
</tr>
<tr>
<td>Postnatal clinical course</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory distress syndrome (%)</td>
<td>10 (41.7%)</td>
<td>7 (46.7%)</td>
<td>102 (48.6%)</td>
</tr>
<tr>
<td>Intracranial hemorrhage (%)</td>
<td>5 (20.8%)</td>
<td>2 (13.3%)</td>
<td>36 (17.1%)</td>
</tr>
<tr>
<td>Sepsis (%)</td>
<td>12 (50.0%)</td>
<td>4 (26.7%)</td>
<td>70 (33.5%)</td>
</tr>
<tr>
<td>Postnatal glucocorticoid treatment (%)</td>
<td>0 (0%)</td>
<td>4 (26.7%)</td>
<td>14 (6.7%)</td>
</tr>
</tbody>
</table>

Values represent mean±SD or percent. Continuous variables were compared with the unpaired t test. Dichotomous variables were compared by the \( \chi^2 \) test, or Fisher’s exact test where appropriate.
Table 2
Length/height SDS by GR genotype.

<table>
<thead>
<tr>
<th>Follow-up visit</th>
<th>Genotype Mean±SD</th>
<th>Comparisons Mean difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R23/23K</td>
<td>N363/363S</td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Birth</td>
<td>-0.45±1.55</td>
<td>-0.12±0.71</td>
</tr>
<tr>
<td>3 mo</td>
<td>-0.74±0.92</td>
<td>-1.21±0.94</td>
</tr>
<tr>
<td>6 mo</td>
<td>-0.01±1.16</td>
<td>-0.57±0.97</td>
</tr>
<tr>
<td>1 yr</td>
<td>0.69±1.10</td>
<td>-0.19±0.72</td>
</tr>
<tr>
<td>2 yrs</td>
<td>0.49±0.81</td>
<td>-0.11±0.68</td>
</tr>
<tr>
<td>5 yrs</td>
<td>0.51±0.81</td>
<td>-0.19±0.62</td>
</tr>
<tr>
<td>19 yrs</td>
<td>0.14±0.64</td>
<td>-0.31±0.60</td>
</tr>
</tbody>
</table>

* P value for gender-genotype interaction <0.05.
Table 3
Metabolic profile at 19 years of age by GR genotype.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Genotype</th>
<th>Mean±SD</th>
<th>Comparisons</th>
<th>Mean difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R23/23K</td>
<td>N363/363S</td>
<td>Non-carriers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>-0.44±1.04</td>
<td>0.05±0.55</td>
<td>0.07±1.26</td>
<td>0.15±1.01</td>
</tr>
<tr>
<td>Waist (SDS)</td>
<td>0.20±1.05</td>
<td>0.71±0.50</td>
<td>0.57±1.14</td>
<td>0.94±0.97</td>
</tr>
<tr>
<td>WHR (SDS)</td>
<td>0.53±0.90</td>
<td>0.57±0.41</td>
<td>0.66±0.67</td>
<td>0.85±0.85</td>
</tr>
<tr>
<td>Absolute fat mass (kg)</td>
<td>11.1±5.3</td>
<td>19.2±3.1</td>
<td>11.8±6.8</td>
<td>20.5±6.7</td>
</tr>
<tr>
<td>Fat percentage (%)</td>
<td>15.1±4.7</td>
<td>31.2±3.7</td>
<td>15.7±6.1</td>
<td>31.2±5.3</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>0.82±0.13</td>
<td>0.91±0.16</td>
<td>0.85±0.17</td>
<td>0.91±0.19</td>
</tr>
<tr>
<td>C-peptide (mmol/l)</td>
<td>0.59±0.15</td>
<td>0.66±0.19</td>
<td>0.48±0.13</td>
<td>0.68±0.16</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td>0.17±0.16</td>
<td>0.24±0.16</td>
<td>0.23±0.19</td>
<td>0.23±0.19</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.61±0.58</td>
<td>4.85±0.58</td>
<td>4.13±0.50</td>
<td>4.59±0.96</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.27±0.25</td>
<td>1.37±0.22</td>
<td>1.40±0.37</td>
<td>1.54±0.35</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.06±0.42</td>
<td>3.02±0.61</td>
<td>2.34±0.67</td>
<td>2.68±0.84</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>126±13</td>
<td>118±12</td>
<td>123±5</td>
<td>122±12</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>63±9</td>
<td>66±9</td>
<td>65±6</td>
<td>69±9</td>
</tr>
<tr>
<td>Cortisol (nmol/l)*</td>
<td>2.62±0.12</td>
<td>2.92±0.12</td>
<td>2.60±0.12</td>
<td>2.88±0.20</td>
</tr>
</tbody>
</table>

* Log-transformed value. † P value for gender-genotype interaction <0.05.