Hyperandrogenemia in Early Adulthood Is an Independent Risk Factor for Abnormal Glucose Metabolism in Middle Age

Katri Tuorila¹, Meri-Maija Ollila¹, Marjo-Riitta Järvelin²,³,⁴,⁵, Juha S. Tapanainen¹,⁶, Stephen Franks⁷, Katri Puukka⁸, Terhi T. Piltonen¹, Laure Morin-Papunen¹

¹Department of Obstetrics and Gynecology, University of Oulu and Oulu University Hospital, Medical Research Center, PEDEGO Research Unit, Oulu, Finland
²MRC-PHE Centre for Environment and Health, Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom
³Center for Life Course Health Research, Faculty of Medicine, University of Oulu, Oulu, Finland
⁴Unit of Primary Health Care, Oulu University Hospital, OYS, Oulu, Finland
⁵Department of Life Sciences, College of Health and Life Sciences, Brunel University London, London, United Kingdom
⁶Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
⁷Institute of Reproductive and Developmental Biology, Imperial College London, London, United Kingdom
⁸NordLab Oulu, Department of Clinical Chemistry, University of Oulu and Oulu University Hospital, Medical Research Center Oulu, Oulu, Finland

Corresponding author and person to whom reprint requests should be addressed:
Laure Morin-Papunen, MD., Ph.D., Adjunct Professor

© The Author(s) 2021. Published by Oxford University Press on behalf of the Endocrine Society. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com
Grants and funding: NFBC1966 received financial support from University of Oulu Grant no. 65354 and no. 24000692, Oulu University Hospital Grant no. 2/97, 8/97 and no. 24301140, Ministry of Health and Social Affairs Grant no. 23/251/97, 160/97, 190/97, National Institute for Health and Welfare, Helsinki Grant no. 54121, Regional Institute of Occupational Health, Oulu, Finland Grant no. 50621, 54231, ERDF European Regional Development Fund Grant no. 539/2010 A31592, National Institute for Health Research (UK), Medical Research Council (UK) (Program Grant G0802782) and Genesis Research Trust (UK) Grant no. P58199. This work was also supported by grants from the Finnish Medical Foundation, the North Ostrobothnia Regional Fund, Academy of Finland (315921, 321763, 104781, 120315, 129269, 1114194, 24300796, 295760), the Sigrid Juselius Foundation and Medical Research Center Oulu. The study is not supported or sponsored by any commercial organization, grant, or fund.

Disclosure statement: The authors have nothing to disclose.
Abstract

Context: The role of androgen excess as a contributing factor to abnormal glucose metabolism (AGM) and insulin resistance in women remains controversial.

Objective: To investigate whether hyperandrogenemia (HA) estimated by serum testosterone (T) level and free androgen index (FAI) at ages 31 and 46 is associated with insulin resistance, insulin secretion and AGM by age 46.

Design: Prospective study including 5,889 females followed at ages 31 and 46.

Setting: General community.

Participants: Women with HA were compared with normoandrogenic women at ages 31 and 46.

Intervention: None.

Main outcome measurements: AGM, including pre-diabetes and T2DM, homeostatic model assessments of insulin resistance (HOMA–IR) and of pancreatic β-cell function (HOMA–B).

Results: At age 31, HA women displayed increased HOMA–IR (P=0.05), HOMA–B (P=0.006), and higher fasting insulin (P=0.034) than normoandrogenic women after adjusting for body mass index (BMI). At age 46, there was a nonsignificant trend towards higher fasting glucose (P=0.07) and glycated hemoglobin A1 (P=0.067) levels in HA women.

Women in the highest T quartile (odds ratio [OR]= 1.80;95%CI, 1.15–2.82) at age 31 and in
the two highest FAI quartiles at ages 31 (Q4: OR=3.76; 95% CI, 2.24–6.32) and 46 (Q4: OR=2.79; 95% CI, 1.74–4.46) had increased risk for AGM, independently of BMI, when compared with women in Q1. Sex hormone-binding globulin (SHBG) was inversely associated with AGM (at age 31: Q4: OR=0.37; 95% CI, 0.23–0.60, at age 46: Q4: OR=0.28; 95% CI, 0.17–0.44).

Conclusion: Hyperandrogenemia and low SHBG in early and middle aged associates with AGM independently of BMI.

Keywords: Hyperandrogenemia, Abnormal glucose metabolism, free androgen index, insulin resistance
Introduction

Considering that type 2 diabetes (T2DM) is a major global health issue (1) predisposing to cardiovascular diseases, the most important cause of death worldwide, it is important to identify risk factors predisposing to abnormal glucose metabolism (AGM, including both pre-diabetes and T2DM). Among fertile aged women, hyperandrogenemia (HA) is a common endocrine disorder (2, 3) that has been associated with an increased risk for T2DM, metabolic syndrome (MetS) and non-alcoholic fatty liver disease in some studies (4-7), although not all studies agree (8-11).

In women, androgen excess, hyperglycemia and insulin resistance are intertwined through mechanisms that are not yet well understood. However, studies suggest this mechanism could be partially explained by the unfavorable effect of androgens on glucose uptake in women by promoting hepatic insulin resistance, reducing skeletal glucose uptake and inducing oxidative stress (12-15). In addition, it is commonly recognized that insulin resistance induces compensatory hyperinsulinemia which promotes ovarian androgen secretion (16). More specifically, insulin acts synergistically with luteinizing hormone, increasing testosterone (T) production from ovarian theca cells and inhibiting hepatic synthesis of sex hormone-binding globulin (SHBG), which leads to increased amounts of unbound, biologically active T (17).

Recent studies using rodent models have also suggested T excess causes prolonged activation of androgen receptor in pancreatic islet β-cells, inducing insulin hypersecretion and eventually secondary β-cell failure, thus predisposing to T2DM (18-20). However, in the former literature, the association between androgens, insulin resistance and T2DM in women is still not fully clarified (4, 9, 21-27). This may be due, at least partly, to the limitations of many published studies, such as cross-sectional design, unrepresentative clinic populations,
different definitions of HA (use of serum levels of T vs. FAI, use of cut-off values vs. quartiles) and variable methods of steroid hormone measurement.

The aim of the present study was to investigate, for the first time to our knowledge, in a longitudinal population-based prospective data set, the association of HA in early adulthood (at age 31) and in middle age (at age 46), with AGM in middle age. In addition, we had access to a wide range of additional data allowing adjustment for several confounding factors.

Materials and Methods

Study population

The current study population arises from the Northern Finland Birth Cohort 1966 (NFBC1966) which is a large, longitudinal, prospective, population-based birth cohort comprising all individuals expected to be born in 1966, in the two Northernmost provinces in Finland (Oulu and Lapland) (28). During that year 5,889 females were born alive, and thereafter the data has been collected at the ages 1, 14, 31 and 46 (Figure 1), also linking to multiple other data sources, including national registers. At age 31, a comprehensive postal questionnaire about reproductive health, work and social background was sent to 5,608 women, of whom 4,523 (81%) responded. In addition, clinical examination, including anthropometric measurements and blood sampling, was performed in 3,127 (76%) women. At age 46, another comprehensive postal questionnaire was carried out and 3,706 (72%) of the contacted 5,123 women responded. Clinical examination including measurements of height, weight, waist and hip circumference, together with blood sampling for hormonal and metabolic parameters, were performed in 3,280 (64%) women. Body mass index (BMI) was calculated (kg/m²) by using measured height (average of two measurements) and weight (28).
Laboratory methods

At ages 31 and 46, the serum levels of total T were measured using Agilent triple quadrupole 6410 LC/MS equipment with an electrospray ionization source operating in positive-ion mode (Agilent Technologies, Wilmington, DE, USA). Multiple reaction monitoring was used to quantify T, using d3-testosterone, with the following transitions: m/z 289.2 to 97 and 289.2 to 109 for T and 292.2 to 97 and 292.2 to 109 for d3-testosterone.

At age 31, SHBG was assayed as previously described (29), and at age 46 by chemiluminometric immunoassay (Immulite 2000, Siemens Healthcare, Lanberis, UK). As the SHBG analysis method changed over the course of the study, the SHBG values from age 31 were transformed to be comparable with the SHBG values analyzed at age 46 using this formula: 0.7615 x old method 31yr SHBG + 0.7088, and the results are reported according to this method (30).

At age 31 fasting plasma glucose were determined by glucose dehydrogenase method (Granutest 250, Diagnostica Merck, Darmstadt, Germany) and serum insulin levels by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden), and at age 46 by an enzymatic dehydrogenase method (glucose) (Advia 1800, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) and by a chemiluminometric immunoassay (insulin) (Advia Centaur XP, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA), respectively. To quantify the degree of insulin resistance, the homeostatic model assessment of insulin resistance (HOMA–IR) values were calculated using the validated calculator available at http://www.dtu.ox.ac.uk. In the regression analyses, we used HOMA–IR as a categorial variable with a cut-off value of 2.5 for normal vs. abnormal (31, 32). To quantify pancreatic β-cell insulin secretion, the homeostatic model assessment of insulin of β-cell function (HOMA–B, continuous variable) was calculated using the equation 20x
Insulin(µU/mL)/[Glucose(mmol/L) – 3.5]. Concentrations of HbA1c and total hemoglobin were measured by an immunochemical assay method (Advia 1800; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) and their ratios were reported as mmol/mol. In the regression analyses, we used HbA1c as a categorial variable with a cut-off value of 48.0 mmol/mol for normal vs. abnormal (33). Free androgen index (FAI) was calculated using the equation 100 x T(nmol/l)/SHBG(nmol/l). Follicle stimulating hormone (FSH) concentrations were determined using an immunochemiluminometric method (Advia Centaur; both Siemens Healthcare Diagnostics, Tarrytown, NY).

**Definition of elevated T level**

Elevated T level at ages 31 and 46 was defined according to the normal upper limit for T at these respective ages based on the 97.5% percentile calculated in this population (2.3 nmol/l at age 31 and 1.7 nmol/l at age 46). This definition was based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and Clinical and Laboratory Standards Institute (CLSI) guidelines regarding the formation of the reference values (34, 35).

**Definition of abnormal glucose metabolism**

At age 46 a two-hour OGTT test was performed after an overnight (12h) fast in 2,780 women. Plasma glucose levels were measured at the baseline and at 30, 60 and 120 minutes after the 75g glucose load. Analyzed glucose levels were further classified according to WHO standards into normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and T2DM (36). Also, information about previously diagnosed T2DM was gathered from the postal questionnaire sent at age 46. All self-reported T2DM diagnoses were verified and completed from the hospital discharge registers and the national drug registers from the Social Insurance Institution of Finland. Presence of IFG, IGT or T2DM was classified as abnormal glucose metabolism (AGM).
**Statistical methods**

Pregnant women and women using hormonal contraceptive pills or having a hormonal intrauterine device were excluded from the analyses at ages 31 and 46. In addition, women using hormonal replacement therapy (N=101, 1.7%) or statins were excluded from the analyses at age 46.

Continuous data were presented as means ± SD or as medians for skewed distributions [25th percentile; 75th percentile]. The differences between continuous baseline parameters were analyzed using Student’s t-test or Mann-Whitney U-test, when appropriate. In the baseline analyses, the effect of BMI was estimated using general linear modeling (ANCOVA). Categorial data were analyzed using cross-tabulation and Pearson’s Chi-squared test.

We investigated the association between T and AGM, HOMA–IR and HOMA–B using T as the categorial variable. SHBG and FAI were used as continuous variables. In addition, we divided T, SHBG and FAI into quartiles at both ages 31 and 46 and used the 1st (lowest) quartile as a reference group to study the associations with AGM and HOMA–IR at age 46.

Binary logistic regression models were used to estimate the factors associated with AGM and HOMA–IR and linear regression models to study factors associated with HOMA–B. The results of logistic models were reported as odd ratios (ORs) with 95% confidence intervals (95% CIs) and results of linear regression models as beta coefficients (Bs) with 95% confidence intervals and P-values. Model I included adjustment for BMI at age 46 and model II adjustments for BMI, smoking (never a smoker, former smoker for > 6 months, former smoker for < 6 months and current smoker), education (basic, secondary, tertiary) and consumption of alcohol (classified as: not use, light use, moderate, heavy use) at age 46.

A P-value < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 22.0 (SPSS, Inc., 1989, 2013, IBM Corp.).
**Ethical approval**

The study followed the principles of the Declaration of Helsinki. The Ethics Committee of the Northern Ostrobothnia Hospital District approved the study (decision number 94/2011 and 12/2003). All subjects have received written and oral information and gave their written consent to use all data.

**Results**

**Baseline characteristics**

*Comparison of women with elevated and normal T levels at age 31*

At age 31, women with elevated T levels (T > 2.3 nmol/l) tended to have higher BMIs (P=0.07) and waist circumferences (P=0.09) and were more often overweight or obese (BMI ≥ 25 kg/m²) than the women with normal T levels (48.8% [n=21] vs. 33.5% [n=628], P=0.04) (Table 1). Women with elevated T levels had also significantly higher levels of fasting insulin (P=0.02), HOMA–IR (P=0.03) and HOMA–B (P=0.001) compared with women with normal T levels, independently of BMI (Table 1). As the presence of T2DM is well-known to associate with impaired insulin secretion, we run analyses after excluding the women with T2DM from the results concerning HOMA-B and HOMA-IR.

Women with elevated T levels reported more often oligoamenorrhea (51.9% vs. 12.3%, P <0.001), hirsutism (31.6% vs. 12.8%, P=0.02) and both symptoms (43.5% vs. 4.6%, P<0.001) than women with normal T levels.
Comparison of women with elevated and normal T levels at age 46

At age 46, women with elevated T levels (T > 1.7 nmol/l) did not significantly differ regarding their baseline anthropometric parameters or glucose metabolic indices from the women with normal T levels although there was a nonsignificant trend towards higher fasting glucose (P=0.07) and HbA1c (P=0.07) levels in women with elevated T levels (Table 1). The prevalence of overweight/obesity did not significantly differ between women with elevated and normal T levels (50.9% [n=27] vs. 52.5% [n=1015], P=0.81). By age 46, 20.8% (n=10) of the women with elevated T reported having experienced infertility problems compared to 18.6% (n=368, P=0.84) of the women with normal levels. Of note, by age 46, women with elevated and normal T had a comparable prevalence of menopause (estimated by a follicle-stimulating hormone (FSH) level >30 IU (37, 38); 9.8% [n=5] vs. 13.2% [n=278], P=0.67).

The occurrence of type 1 diabetes mellitus (T1DM) and T2DM in the first-degree relatives was asked in the postal questionnaire at age 46. At that age, only 1.9% (n=1) of the women with elevated T had a 1st degree relative with T1DM compared to 3.9% (n=84) of the women with normal T (P=0.72). Similarly, 42.3% (n=22) of the women with elevated T had a 1st degree relative with T2DM compared to 31.5% (n=680) of the women with normal T (P=0.13).

Testosterone, SHBG and FAI levels in women with NGT and AGM at ages 31 and 46 (Table 2)

Women with AGM at age 46 had significantly higher levels of T at age 31 (P=0.03) but the difference vanished after adjusting for BMI (P=0.15). Also, these women had significantly lower SHBG levels and higher FAI than the women with NGT both at ages 31 and 46, independently of BMI (at age 31 P<0.001 for both; at age 46 P=0.02 and P=0.001, respectively).
Association of elevated testosterone with AGM, HOMA–IR and HOMA–B

Elevated T at age 31 or at age 46 was not associated with AGM at age 46 (OR at age 31=2.09; 95% CI, 0.85 – 5.14; OR at age 46=0.60; 95% CI, 0.21 – 1.70), but at age 31 elevated T was significantly associated with increased HOMA–IR at age 46 in the unadjusted model (OR= 2.68; 95% CI, 1.19 – 6.02).

Elevated T at age 31, but not at age 46, was positively associated with HOMA–B at age 46, also after adjusting for BMI (Model I: B at age 31=0.21; 95% CI, 0.02 – 0.40, P < 0.03) and with higher HbA1c levels at age 46 in unadjusted model (OR at age 31=5.35; 95% CI, 1.18 – 24.16).

Association of sex-hormone binding globulin with AGM, HOMA–IR and HOMA–B

Sex hormone-binding globulin at age 31 was inversely associated with AGM at age 46 after adjusting for BMI (Model I: OR=0.99; 95% CI, 0.98 – 0.99) and at age 46, also after other adjustments (Model II: OR = 0.98; 95% CI, 0.98 – 0.99). Both at ages 31 and 46, SHBG was inversely associated with HOMA–IR at age 46, also after adjustments (Model II: at age 31 OR = 0.98; 95% CI, 0.98 – 0.99; Model II: at age 46 OR =0.98; 95% CI, 0.97 – 0.99).

At ages 31 and 46 SHBG associated inversely with HOMA–B at age 46 after adjusting for BMI (Model I: B at age 31= -0.09; 95% CI, -0.15 – -0.04, P=0.001; Model I: B at age 46= -0.12; 95% CI, -0.16 – -0.07, P<0.001). Moreover, serum levels of SHBG at ages 31 and 46 associated inversely with HbA1c levels at age 46 in unadjusted models (at age 31 OR= 0.97; 95% CI, 0.94 – 0.99; at age 46 OR=0.97; 95% CI, 0.96 – 0.99).
**Association of free androgen index with AGM, HOMA–IR and HOMA–B**

Free androgen index at ages 31 and 46 associated positively with AGM at age 46 after adjustments (Model II: at age 31 OR =1.31; 95% CI, 1.19 – 1.43; Model II: at age 46 OR =1.07; 95% CI, 1.00 – 1.15). Similarly, FAI at ages 31 and 46 was positively associated with HOMA–IR after adjustments (Model II: at age 31 OR =1.24; 95% CI, 1.14 – 1.35; Model II: at age 46 OR =1.07; 95% CI, 1.00 – 1.15). 

Free androgen index at age 31 and 46 was associated positively with HOMA–B at age 46 also after adjusting for BMI (Model I: B at age 31 = 0.06; 95% CI, 0.01 – 0.10, P=0.010; Model I: B at age 46 = 0.11; 95% CI, 0.02 – 0.08, P=0.001). In addition, FAI at age 31 associated significantly with HbA1c levels at age 46 also after adjustments (Model II: OR=1.22; 95% CI, 1.01 – 1.47).

**Association of quartiles of T, SHBG and FAI with AGM and HOMA-IR**

We found an independent positive association between the highest T quartile at age 31 and AGM at age 46. Furthermore, after adjustments, all three top quartiles of SHBG at ages 31 and 46 associated inversely and third and fourth quartiles of FAI at ages 31 and 46 positively with AGM at age 46 (Tables 3 and 4).

The second quartile of T at age 31 associated inversely with HOMA-IR at age 46 after adjustments. In addition, all three top quartiles of SHBG both at ages 31 and 46 associated inversely with HOMA-IR at age 46. Finally, at age 31, third and fourth quartiles of FAI and at age 46, all three top quartiles of FAI associated positively with HOMA-IR at age 46 (Tables 5 and 6).
Persisting HA and the risk of AGM

The prevalence of AGM was not increased in women who were in the highest T quartile, either at ages 31 or 46 (n=84), compared to those women who were in the lowest T quartile at both ages (n=80) (OR = 1.13; 95% CI, 0.46 – 2.75). However, being in the highest FAI quartile at both ages (n=110) was significantly associated with AGM (Model II: OR = 5.83; 95% CI, 1.43 – 23.76) when compared to those who were in the lowest FAI quartile at both ages (n=92). Moreover, women in the lowest SHBG quartile at both ages (n=133) had increased risk for AGM (Model II: OR = 4.97; 95% CI, 1.67 – 14.77) when compared to women in the highest SHBG quartile, at both ages (n=129).

When performing the analyses, by substituting BMI with waist circumference (WC, with a cut-off value of 80cm), the results did not substantially change.

Discussion

To our knowledge, this is the first and largest population-based, follow-up study investigating the association between hyperandrogenemia (expressed as elevated T levels and/or FAI) and glucose metabolism, as we included not only T2DM, but also prediabetes, insulin resistance (HOMA–IR), insulin secretion (HOMA–B) and HbA1c levels. We were able to show significant associations between measured HA and SHBG at age 31 with AGM at age 46. Further analysis revealed that the associations were not only driven by BMI but also by hyperandrogenemia per se. Interestingly, elevated levels of T at age 31 and FAI, at ages 31 and 46, were associated with increased insulin resistance and secretion, mostly independent of BMI.

The present results indicate that high T levels at age 31 measured by LC-MS/MS, are associated with abnormal glucose metabolism in later life; compared to women in the lowest
quartile at age 31, women in the highest T quartile at age 31 had almost twice the risk for AGM at age 46. In line with this, our group has previously shown, in the same cohort, a positive association between elevated T and FAI levels at age 31 with gestational diabetes (39). So far, previous studies have explored the relationship between sex steroid hormone levels and T2DM with conflicting results (4, 9, 21-27). Moreover, they have not considered pre-diabetic states. A former meta-analysis, including mainly cross-sectional studies, reported that high levels of T were associated with an increased risk of T2DM in women (40). Similarly, a recent, large, retrospective study reported that the risk of T2DM started to increase significantly when serum T exceeded 1.5 nmol/L, with the highest risk in women with serum testosterone ≥3.5 nmol/L (4). However, later studies with follow-up periods of 5 to 11 years, using the LC-MS/MS, could not confirm this finding (9, 27) and a previous systematic meta-analysis, including thirteen prospective studies, did not show any association between total T and T2DM (9). Of note, most of the studies included in the meta-analysis did not use LC-MS/MS. Although there is still a debate (41) about the most appropriate method of measurement, the current consensus is that T measurement in women should be performed using LC-MS/MS (42). This issue might explain the discrepancy between our results and those of the aforementioned meta-analysis. Another important difference is that the present study included a wider spectrum of glucose metabolism abnormalities, as it also included prediabetes.

Our study showed a positive association of FAI with HOMA–IR, HbA1c levels and AGM, even after BMI adjustment, and an even tighter association for the women with persistently raised androgen levels (i.e. being in the highest FAI quartile both at ages 31 and 46). In previous studies, the link between FAI and T2DM has been controversial. O’Reilly et al. reported a strong positive relation (4), and Muka et al. reported a positive association that vanished after adjustments for age, fasting status, insulin, glucose and BMI (9). Even though
FAI is not fully trouble-free in the evaluation of HA in women, it is commonly used and considered as a reliable proxy for bioavailable T (43, 44). All in all, these findings suggest that HA, at least as evaluated by FAI, is associated with AGM. Moreover, the present results strengthen the case for a pivotal effect of weight as a risk factor as well as the existence of a complex synergistic interrelationship between HA, obesity, and the risk of AGM through life.

In the present study and in line with previous literature (45, 46), SHBG was associated inversely with insulin resistance, insulin secretion and AGM, independently of BMI. The synthesis of SHBG is influenced by several hormonal and metabolic factors, including insulin which inhibits hepatic secretion of SHBG (47, 48). In line with our results, a prospective six-year follow-up analysis from three Finnish population-based cohorts (including the NFBC1966) indicates that circulating SHBG is predictive of the degree of insulin resistance and glycemia and that elevated SHBG has protective role on T2DM risk (49). These findings suggest that SHBG may not be only a passive carrier protein but may also have an active function and play an independent role in the pathogenesis of T2DM. Further studies are needed to investigate whether the level of SHBG could be used as a predictive factor to identify subjects at risk for T2DM.

Interestingly, at age 31, the women with elevated T displayed significantly increased insulin resistance and secretion (expressed as greater HOMA–B, HOMA-IR, and insulin values) compared with the normoandrogenic women, independently of BMI. Also, at age 31, the glucose levels remained in the normal range and did not differ from those in normoandrogenic women, suggesting that the increase in insulin secretion was able to compensate worsened insulin resistance. Further, the two highest FAI quartiles at age 31 associated with increased insulin resistance (HOMA-IR) and risk of AGM at age 46. Last, compared to the women with NGT, the women with AGM at age 46 were more hyperandrogenic at ages 31 and 46 as they had higher levels of T at age 31 and higher FAI.
both at age 31 and 46. This finding fits well with the results of previous studies about the possible mechanism linking HA and abnormal glucose metabolism. In mice, T excess has been shown to cause a chronic androgen receptor activation in pancreatic $\beta$-cells, producing insulin hypersecretion and eventually secondary $\beta$-cell failure, which may predispose to T2DM (18, 19). In line with this hypothesis, at age 46, the women with excess androgen seemed to have limited capacity to increase insulin secretion as their HOMA-B levels were comparable to those of normoandrogenic women at age 46 and their fasting glucose and HbA1c levels tended to be higher than those found in normoandrogenic women. This could be seen as the onset of impaired $\beta$-cell insulin secretion. However, we did not detect significant difference in the 0-hour and 2-hour glucose levels during the 2h-OGTT between normoandrogenic and hyperandrogenic women at age 46, which might be due to the relatively small number of the subjects in the analysis.

Another interesting observation was that the correlation between HA and glucose metabolism disorders observed at age of 31 disappeared at age 46. One explanation might be that the higher prevalence of overweight/obesity observed in women with HA at age 31 was not present any longer at age 46. An additional explanation could also be the decline in androgen levels with time, decreasing also its adverse effect on glucose metabolism (50). Last, there might be also some unknown explanatory factors which could not be clarified in the present study setting. All in all, although the design of our study does not allow us to draw conclusions about causality or mechanism, these results are of interest regarding an early identification of women at risk for glucose metabolism disorders.

This study enhances our knowledge of the involvement of HA in disordered glucose metabolism by providing a large unique prospective dataset with unselected, homogenous study population. Other strengths of this study are the long follow-up period as well as high participation and response rates and low dropout rate. Furthermore, anthropometric
parameters were, mostly, directly clinically measured. In addition, T measurements were performed using LC-MS/MS, the gold standard method (42). The OGTT was performed in a large subgroup of the study population at age 46, allowing the diagnosis of both prediabetes and previously undiagnosed T2DM. The inclusion of both prediabetes and T2DM as main outcomes is clinically important, as between 70 and 90 % of prediabetic people will develop T2DM (51, 52). We were also able to analyze the effect of many confounding factors, such as the occurrence of family history of T1DM and T2DM which did not differ between women with normal and elevated T levels at age 46. Last, our results allow us to consider possible mechanisms driving disordered glucose metabolism in women.

The limitations of this study include the use of only serum T as a marker of HA, even though other androgens, such as adrenal androgens have a place in the evaluation of androgenicity in women. Over the course of the cohort’s follow up, the laboratory method used in the evaluation of SHBG changed, and we had to use a conversion formula to make the FAI results from age 31 and 46 comparable. However, the current method using conversion formula calculated by linear regression analysis was the least biased way to make the FAI results at ages 31 and 46 comparable. Also, a recent use of contraceptive pills might have affected the SHBG level, as we do not have information about the time when the women came off the contraceptive pill. As some of the confidential intervals in the analyses were rather wide, some analyses may have been underpowered. Further, larger studies are therefore needed to confirm our results.
Conclusion

The present study showed a positive association between early adulthood HA and abnormal glucose metabolism in middle age. In addition, there was a significant inverse association between SHBG and AGM, independently of BMI, suggesting that levels of SHBG could help identifying women at risk of disordered glucose metabolism. Our results also underline the complexity of the interrelationship of androgen excess with weight, insulin resistance and insulin secretion. The present results also emphasize the importance of weight management early on, particularly in hyperandrogenic women, to reduce the risk for glucose metabolism disorders. Young women with HA and overweight or obesity should also be assessed regularly for glucose tolerance. However, whether HA remains a metabolic risk later in adulthood remains elusive. In the future, new studies should be designed to estimate whether therapeutic reduction of T excess in early adolescence is beneficial in decreasing the risk of glucose metabolism disorders later in life.

Acknowledgments

We thank all cohort members and researchers who participated in the 31- and 46-year study. We also wish to acknowledge the work of the NFBC project center.

Data Availability Statement

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request. The cohort center grants the final study permit.
References

1. Zimmet PZ, Magliano DJ, Herman WH, Shaw JE. Diabetes: a 21st century challenge. *Lancet Diabetes Endocrinol.* 2014;2(1):56-64.

2. Azziz R, Sanchez LA, Knochenhauer ES, Moran C, Lazenby J, Stephens KC, Taylor K, Boots LR. Androgen excess in women: experience with over 1000 consecutive patients. *J Clin Endocrinol Metab.* 2004;89(2):453-62.

3. Sanchón R, Gambineri A, Alpañés M, Martínez-García MÁ, Pasquali R, Escobar-Morreale HF. Prevalence of functional disorders of androgen excess in unselected premenopausal women: a study in blood donors. *Hum Reprod.* 2012;27(4):1209-16.

4. O’Reilly MW, Glisic M, Kumarevou B, Subramanian A, Manolopoulos KN. Tahrani AA, Keerthy D, Muka T, Toulis KA, Hanif W, Thomas GN, Franco OH, Arlt W, Niranharakumar K. Serum testosterone, sex hormone-binding globulin and sex-specific risk of incident type 2 diabetes in a retrospective primary care cohort. *Clin Endocrinol (Oxf).* 2019;90(1):145-54.

5. Brand JS, van der Tweel I, Grobbee DE, Emmelot-Vonk MH, van der Schouw YT. Testosterone, sex hormone-binding globulin and the metabolic syndrome: a systematic review and meta-analysis of observational studies. *Int J Epidemiol.* 2011;40(1):189-207.

6. Kische H, Gross S, Wallaschofski H, Völzke H, Dörr M, Nauck M, Haring R. Clinical correlates of sex hormones in women: The study of health in Pomerania. *Metab Clin Exp.* 2016;65(9):1286-96.

7. Sarkar M, Wellons M, Cedars ME, VanWagner L, Gunderson EP, Ajmera V, Torchen L, Siscovick D, Carr JJ, Terry JG, Rinella M, Lewis CE, Terrault N. Testosterone Levels in Pre-Menopausal Women are Associated With Nonalcoholic Fatty Liver Disease in Midlife. *Am J Gastroenterol.* 2017;112(5):755-62.

8. Soriguer F, Rubio-Martín E, Fernández D, Valdés S, García-Escobar E, Martín-Núñez GM, Esteva I, Almaraz MC, Rojo-Martínez G. Testosterone, SHBG and risk of type 2 diabetes in the second evaluation of the Pizarra cohort study. *Eur J Clin Invest.* 2012;42(1):79-85.

9. Muka T, Nano J, Jaspers L, Meun C, Bramer WM, Hofman A, Dehghan A, Kavours M, Laven JSE, Franco OH. Associations of Steroid Sex Hormones and Sex Hormone-Binding Globulin With the Risk of Type 2 Diabetes in Women: A Population-Based Cohort Study and Meta-analysis. *Diabetes.* 2017;66(3):577-86.

10. Goto A, Morita A, Goto M, Sasaki S, Miyachi M, Aiba N, Terauchi Y, Noda M, Watanabe S. Associations of sex hormone-binding globulin and testosterone with diabetes among men and women (the Saku Diabetes study): a case control study. *Cardiovasc Diabetol.* 2012;11:130.

11. Mather KJ, Kim C, Christophi CA, Aroda VR, Knowler WC, Edelstein SE, Florez JC, Labrie F, Kahn SE, Goldberg RB, Barrett-Connor E. Steroid Sex Hormones, Sex Hormone-
12. Zhang B, Wang J, Shen S, et al. Association of androgen excess with glucose intolerance in women with polycystic ovary syndrome. Biomed Res Int. 2018;2018:6869705.

13. Yu I-C, Lin H-Y, Liu N-C, Sparks JD, Yeh S, Fang L-Y, Chen L, Chang C. Neuronal androgen receptor regulates insulin sensitivity via suppression of hypothalamic NF-κB-mediated PTP1B expression. Diabetes. 2013;62(2):411-423.

14. Inada A, Fujii NL, Inada O, Higaki Y, Furuichi Y, Nabeshima Y-I. Effects of 17β-estradiol and androgen on glucose metabolism in skeletal muscle. Endocrinology. 2016;157(12):4691-4705.

15. Liu S, Navarro G, Mauvais-Jarvis F. Androgen excess produces systemic oxidative stress and predisposes to β-cell failure in female mice. PLoS ONE. 2010;5(6):e11302.

16. Barber TM, Dimitriadis GK, Andreou A, Franks S. Polycystic ovary syndrome: insight into pathogenesis and a common association with insulin resistance. Clin Med (Lond). 2016;16(3):262-6.

17. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med. 2005;352(12):1223-36.

18. Navarro G, Allard C, Morford JJ, Xu W, Liu S, Molinas AJ, Butcher SM, Fine NF, Blandino-Rosano M, Sure VN, Yu S, Zhang R, Münzberg H, Jacobson DA, Katakam PV, Hodson DJ, Bernal-Mizrachi E, Zsombok A, Mauvais-Jarvis F. Androgen excess in pancreatic β cells and neurons predisposes female mice to type 2 diabetes. JCI Insight. 2018;3(12):12.

19. Mishra JS, More AS, Kumar S. Elevated androgen levels induce hyperinsulinemia through increase in Ins1 transcription in pancreatic beta cells in female rats. Biol Reprod. 2018;98(4):520-31.

20. Xu W, Morford J, Mauvais-Jarvis F. Emerging role of testosterone in pancreatic β-cell function and insulin secretion. J Endocrinol. 2019(Jan 1. pii: JOE-18-0573.R1).

21. Andersson B, Márin P, Lissner L, Vermeulen A, Björntorp P. Testosterone concentrations in women and men with NIDDM. Diabetes Care. 1994;17(5):405-11.

22. Haffner SM, Katz MS, Stern MP, Dunn JF. The relationship of sex hormones to hyperinsulinemia and hyperglycemia. Metab Clin Exp. 1988;37(7):683-8.

23. Khaw KT, Barrett-Connor E. Fasting plasma glucose levels and endogenous androgens in non-diabetic postmenopausal women. Clin Sci. 1991;80(3):199-203.

24. Moghetti P, Tosi F, Castello R, Magnani CM, Negri C, Brun E, Furlani L, Caputo M, Muggio M. The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women. J Clin Endocrinol Metab. 1996;81(3):952-60.
25. Golden SH, Dobs AS, Vaidya D, Szklo M, Gapstur S, Kopp P, Liu K, Ouyang P. Endogenous sex hormones and glucose tolerance status in postmenopausal women. *J Clin Endocrinol Metab.* 2007;92(4):1289-95.

26. Ding EL, Song Y, Manson JE, Rifai N, Buring JE, Liu S. Plasma sex steroid hormones and risk of developing type 2 diabetes in women: a prospective study. *Diabetologia.* 2007;50(10):2076-84.

27. Fenske B, Kische H, Gross S, Wallaschofski H, Völzke H, Dörr M, Nauck M, Keevil BG, Brabant G, Haring R. Endogenous Androgens and Sex Hormone-Binding Globulin in Women and Risk of Metabolic Syndrome and Type 2 Diabetes. *J Clin Endocrinol Metab.* 2015;100(12):4595-603.

28. University of Oulu: Northern Finland Birth Cohort 1966. [Internet]; 1966 [. Available from: https://etsin.fairdata.fi/dataset/716939c3-7a2a-4b6a-91f3-92aca09bc52d.

29. Taponen S, Martikainen H, Järvelin M, Laitinen J, Pouta A, Hartikainen A, Sovio U, McCarthy MI, Franks S, Ruokonen A. Hormonal profile of women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab.* 2003;88(1):141-7.

30. Ollila MM, West S, Keinanen-Kiukaanniemi S, Jokelainen J, Auvinen J, Puukka K, Ruokonen A, Järvelin M, Tapanainen JS, Franks S, Piltonen TT, Morin-Papunen LC. Overweight and obese but not normal weight women with PCOS are at increased risk of Type 2 diabetes mellitus—a prospective population-based cohort study. *Human Reproduction.* 2017;32(4):968.

31. Atabek ME, Pirgon O. Assessment of insulin sensitivity from measurements in fasting state and during an oral glucose tolerance test in obese children. *J Pediatr Endocrinol Metab.* 2007;20(2):187-95.

32. Singh Y, Garg MK, Tandon N, Marwaha RK. A study of insulin resistance by HOMA-IR and its cut-off value to identify metabolic syndrome in urban Indian adolescents. *J Clin Res Pediatr Endocrinol.* 2013;5(4):245-51.

33. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva: World Health Organization; 2011.

34. Solberg HE, Gräsbeck R. Reference values. Adv CLin Chem. 1989;27:1-79.

35. Horowitz GL Establishment and use of reference values. In: Burtis CA, Ashwood ER, Bruns DE Tietz textbook of clinical chemistry and molecular diagnostics. 5th ed. St. Louis, MO: Elsevier Saunders;2012:95–118.

36. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med.* 1998;15(7):539-53.
37. Scaglia H, Medina M, Pinto-Ferreira AL, Vazques G, Gual C, Perez-Palacios G. Pituitary LH and FSH secretion and responsiveness in women of old age. Acta Endocrinol (Copenh). 1976;81(4):673-9.

38. Gass, M, Rebar, R. Glob. libr. women's med., (ISSN: 1756-2228) 2008; DOI 10.3843/GLOWM.10079.

39. West S, Ollila MM, Franks S, Piltonen T, Jokelainen J, Nevalainen J, Puukka K, Ruokonen A, Järvelin M, Auvinen J, Tapanainen J, Morin-Papunen LC. Overweight, obesity and hyperandrogenemia are associated with gestational diabetes mellitus: A follow-up cohort study. Acta Obstet Gynecol Scand. 2020;99(19):1311-1319.

40. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2006;295(11):1288-99.

41. Legro RS, Schlaff WD, Diamond MP, Coutifaris C, Casson PR, Brazyki RG, Christman GM, Trussell JC, Krawetz SA, Snyder PJ, Ohl D, Carson SA, Steinkamp MP, Carr BR, McGovern PG, Cataldo NA, Gosman GG, Nestler JE, Myers ER, Santoro N, Eisenberg E, Zhang M, Zhang H. Total testosterone assays in women with polycystic ovary syndrome: precision and correlation with hirsutism. J Clin Endocrinol Metab. 2010;95(12):5305-13.

42. Handelsman DJ, Wartofsky L. Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. J Clin Endocrinol Metab. 2013;98(10):3971-3.

43. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018;110(3):364-79.

44. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab. 1999;84(10):3666-72.

45. Le TN, Nestler JE, Strauss JF, Wickham EP. Sex hormone-binding globulin and type 2 diabetes mellitus. Trends Endocrinol Metab. 2012;23(1):32-40.

46. Wallace IR, McKinley MC, Bell PM, Hunter SJ. Sex hormone binding globulin and insulin resistance. Clin Endocrinol (Oxf). 2013;78(3):321-9.

47. Plymate SR, Jones RE, Matej LA, Friedl KE. Regulation of sex hormone binding globulin (SHBG) production in Hep G2 cells by insulin. Steroids. 1988;52(4):339-40.

48. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, Clore JN, Blackard WG. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1991;72(1):83-9.

49. Wang Q, Kangas AJ, Soininen P, Tiainen M, Tynkkynen T, Puukka K, Ruokonen A, Viikari J, Kahonen M, Lehtimäki T, Salomaa V, Perola M, Smith GD, Raitakari OT, Järvelin M, Wurtz P, Kettunen J, Ala-Korpela M. Sex hormone-binding globulin associations with
circulating lipids and metabolites and the risk for type 2 diabetes: observational and causal effect estimates. *Int J Epidemiol*. 2015;44(2):623-37.

50. Piltonen T, Koivunen R, Perheentupa A, Morin-Papunen LC, Ruokonen A, Tapanainen J. Ovarian age-related responsiveness to human chorionic gonadotropin in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2004;89(8):3769-3775.

51. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. *Lancet*. 2012;379(9833):2279-90.

52. Li G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, Li H, Li H, Jiang Y, An Y, Shuai Y, Zhang B, Zhang J, Thompson TJ, Gerzoff RB, Roglic G, Hu Y, Bennett PH. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study: a 20-year follow-up study. *Lancet*. 2008;371(9626):1783-9.
Legends for Figure and Tables

Figure 1. Flow chart of the study. T1DM: Type 1 diabetes; T2DM: Type 2 diabetes; NGT: Normal glucose tolerance; Pre-DM: Pre-diabetes; AGM: Abnormal glucose metabolism. Clinical examinations included for example blood sampling. * Women using hormonal contraceptive pills, having hormonal intrauterine device or pregnant at age 31 excluded. ** Women using hormonal contraceptive pills, statins, hormonal replacement therapy or having hormonal intrauterine device at age 46 excluded.

Table 1. The results are reported as mean ± SD or as median [25th percentile; 75th percentile]. The differences between the two study groups were analyzed by Student’s t-test or Mann-Whitney U-test when appropriate, and with cross-tabulation and χ² test. The effect of BMI was estimated using general linear modelling (ANCOVA).

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-B, homeostatic model assessment of pancreatic β-cell function; HbA1c, glycated hemoglobin A1.

*We assessed HOMA-IR and HOMA-B after excluding women with diagnosis of type 2 diabetes.

Table 2. The results are reported as mean ± SD or as median [25th percentile; 75th percentile]. The differences between the two study groups were analyzed by Student’s t-test. The effect of BMI was estimated using general linear modelling (ANCOVA).
Abbreviations: SHBG, sex hormone-binding globulin; FAI, free androgen index; NGT, normal glucose tolerance; AGM, abnormal glucose metabolism, including pre-diabetes or type 2 diabetes; BMI, body mass index.

**Table 3.** Results are shown as ORs with 95% CIs in quartiles, when quartile 1 was used as the reference category.

Model I: Adjusted for BMI as a binary variable (cut-off at 25.0 kg/m²) at age 46.

Model II: Adjusted for smoking, education, consumption of alcohol and BMI as a binary variable (cut-off at 25.0 kg/m²) at age 46.

Abbreviations: SHBG, sex hormone-binding globulin; FAI, free androgen index; AGM, abnormal glucose metabolism, including pre-diabetes or type 2 diabetes.

**Table 4.** Results are shown as ORs with 95% CIs in quartiles, when quartile 1 was used as the reference category.

Model I: Adjusted for BMI as a binary variable (cut-off at 25.0 kg/m²) at age 46.

Model II: Adjusted for smoking, education, consumption of alcohol and BMI as a binary variable (cut-off at 25.0 kg/m²) at age 46.

Abbreviations: SHBG, sex hormone-binding globulin; FAI, free androgen index; AGM, abnormal glucose metabolism, including pre-diabetes or type 2 diabetes.
Table 5. Results are shown as ORs with 95% CIs in quartiles, when quartile 1 was used as the reference category.

Model I: Adjusted for BMI as a binary variable (cut-off at 25.0 kg/m²) at age 46.

Model II: Adjusted for smoking, education, consumption of alcohol and BMI as a binary variable (cut-off at 25.0 kg/m²) at age 46.

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; SHBG, sex hormone-binding globulin; FAI, free androgen index; AGM, abnormal glucose metabolism, including pre-diabetes or type 2 diabetes.

Table 6. Results are shown as ORs with 95% CIs in quartiles, when quartile 1 was used as the reference category.

Model I: Adjusted for BMI as a binary variable (cut-off at 25.0 kg/m²) at age 46.

Model II: Adjusted for smoking, education, consumption of alcohol and BMI as a binary variable (cut-off at 25.0 kg/m²) at age 46.

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; SHBG, sex hormone-binding globulin; FAI, free androgen index; AGM, abnormal glucose metabolism, including pre-diabetes or type 2 diabetes.
Northern Finland Birth Cohort 1966
Females born alive N=5889

31-year postal questionnaire was sent to N=5608 women
- Responded to the postal questionnaire N=4523 women
- Participated to the clinical examinations N=3127

46-year postal questionnaire was sent to N=5123 women
- Responded to the postal questionnaire N=3708 women
- Participated to the clinical examination N=3280 women

Previously diagnosed T2DM N=123
- T1DM N=46 (excluded)
- Unknown DM type N=38 (excluded)

Oral glucose tolerance test was performed to N=2780

Information about glucose tolerance status N=2841 women

NGT N=2354
- N=1596*
- N=1464**

Pre-DM N=318
- N=221*
- N=184**

T2DM N=169
- N=123*
- N=95**

AGM N=487
- N=344*
- N=279**
Table 1. Baseline characteristics in normoandrogenic and hyperandrogenic women at ages 31 and 46.

At age 31

|                      | T ≤ 2.30 nmol/l (n=1795-1877) | T > 2.30 nmol/l (n=39-43) | Crude P-value | BMI-adjusted P-value |
|----------------------|-----------------------------|--------------------------|---------------|----------------------|
| BMI (kg/m²)          | 23.21 [21.16; 26.24]        | 24.96 [21.17; 29.73]     | 0.07          |                      |
| Waist (cm)           | 79.22 ± 12.11               | 85.03 ± 18.84            | 0.09          |                      |
| BMI ≥ 25 (kg/m²) n (%)| 628 (33.5)                  | 21 (48.8)                | 0.04          |                      |
| fP-Glucose (mmol/l)  | 4.93 ± 0.48                 | 4.92 ± 0.51              | 0.95          | 0.47                 |
| fS-Insulin (mU/l)    | 7.93 ± 3.42                 | 10.48 ± 7.54             | 0.02          | 0.03                 |
| HOMA-IR*             | 0.93 [0.76; 1.16]            | 1.00 [0.84; 1.40]        | 0.03          | 0.002                |
| HOMA-B*              | 95.90 [83.30; 112.00]        | 106.50 [95.20; 124.00]   | 0.001         | 0.007                |

At age 46

|                      | T ≤ 1.70 nmol/l (n=1676-1933) | T > 1.70 nmol/l (n=42-53) | Crude P-value | BMI-adjusted P-value |
|----------------------|-----------------------------|--------------------------|---------------|----------------------|
| BMI (kg/m²)          | 25.22 [22.73; 29.04]        | 25.35 [21.74; 28.80]     | 0.81          |                      |
| Waist (cm)           | 86.95 ± 13.00               | 87.34 ± 16.15            | 0.83          |                      |
| BMI ≥ 25 (kg/m²) n (%)| 1015 (52.5)                 | 27 (50.9)                | 0.82          |                      |
| fP-Glucose (mmol/l)  | 5.37 ± 0.77                 | 5.57 ± 1.06              | 0.06          | 0.07                 |
| fS-Insulin (mU/l)    | 7.30 [5.10; 10.60]          | 7.00 [5.20; 10.70]       | 0.75          | 0.41                 |
| HOMA-IR*             | 1.60 [1.13; 2.27]           | 1.72 [1.17; 2.40]        | 0.71          | 0.56                 |
| HOMA-B*              | 81.58 [60.99; 111.47]        | 81.11 [58.33; 108.00]    | 0.84          | 0.91                 |
| HbA1c (mmol/mol)     | 36.18 ± 4.99                | 37.47 ± 7.83             | 0.37          | 0.07                 |
| 2-hour OGTT (mmol/l) |                             |                          |               |                      |
| 0h                   | 5.35 ± 0.57                 | 5.26 ± 0.39              | 0.30          | 0.45                 |
| 2h                   | 5.77 ± 1.50                 | 5.63 ± 1.65              | 0.56          | 0.77                 |
Table 2. Levels of testosterone (T), SHBG and FAI at ages 31 and 46 in women with NGT or AGM at age 46.

|                  | NGT at age 46 (n=994-1018) | AGM at age 46 (n=205-215) | Crude P-value | BMI-adjusted P-value |
|------------------|-----------------------------|---------------------------|---------------|----------------------|
| **T (nmol/l)**   | 1.05 ± 0.45                 | 1.14 ± 0.54               | 0.03          | 0.15                 |
| **SHBG (nmol/l)**| 51.45 ± 28.28               | 42.14 ± 39.56             | <0.001        | <0.001               |
| **FAI**          | 2.39 ± 1.44                 | 3.51 ± 2.18               | <0.001        | <0.001               |

|                  | NGT at age 46 (n=1457)      | AGM at age 46 (n=246)     | Crude P-value | BMI-adjusted P-value |
|------------------|-----------------------------|---------------------------|---------------|----------------------|
| **T (nmol/l)**   | 0.90 ± 0.46                 | 0.86 ± 0.34               | 0.24          | 0.50                 |
| **SHBG (nmol/l)**| 63.99 ± 29.12               | 48.81 ± 34.88             | <0.001        | 0.02                 |
| **FAI**          | 1.44 [1.05; 2.00]           | 1.93 [1.40; 2.78]         | <0.001        | 0.001                |
Table 3. Associations of testosterone, SHBG and FAI quartiles at age 31 with AGM at age 46 in women in Northern Finland Birth Cohort 1966.

|                | Testosterone |           |           |           |
|----------------|--------------|-----------|-----------|-----------|
|                | Quartile 1   | Quartile 2| Quartile 3| Quartile 4|
| N              | 331          | 307       | 286       | 282       |
| Crude          | 1.00         | 1.43 (0.95 – 2.17) | 0.80 (0.50 – 1.27) | 1.95 (1.29 – 2.93) |
| Model I        | 1.00         | 1.39 (0.90 – 2.14) | 0.78 (0.48 – 1.26) | 1.83 (1.20 – 2.81) |
| Model II       | 1.00         | 1.36 (0.87 – 2.13) | 0.83 (0.51 – 1.37) | 1.80 (1.15 – 2.82) |

|                | SHBG         |           |           |           |
|                | Quartile 1   | Quartile 2| Quartile 3| Quartile 4|
| N              | 321          | 300       | 285       | 278       |
| Crude          | 1.00         | 0.44 (0.30 – 0.64) | 0.26 (0.17 – 0.40) | 0.23 (0.15 – 0.37) |
| Model I        | 1.00         | 0.54 (0.36 – 0.79) | 0.38 (0.25 – 0.60) | 0.37 (0.23 – 0.60) |
| Model II       | 1.00         | 0.52 (0.35 – 0.78) | 0.36 (0.22 – 0.57) | 0.37 (0.23 – 0.60) |

|                | FAI          |           |           |           |
|                | Quartile 1   | Quartile 2| Quartile 3| Quartile 4|
| N              | 306          | 310       | 315       | 302       |
| Crude          | 1.00         | 1.49 (0.88 – 2.51) | 2.49 (1.52 – 4.08) | 5.13 (3.21 – 8.19) |
| Model I        | 1.00         | 1.40 (0.81 – 2.39) | 2.06 (1.23 – 3.43) | 3.48 (2.13 – 5.69) |
| Model II       | 1.00         | 1.47 (0.83 – 2.59) | 2.16 (1.27 – 3.70) | 3.76 (2.24 – 6.32) |
Table 4. Associations of testosterone, SHBG and FAI quartiles at age 46 with AGM at age 46 in women in Northern Finland Birth Cohort 1966.

|                  | Testosterone |               |               |               |
|------------------|--------------|---------------|---------------|---------------|
|                  | Quartile 1   | Quartile 2    | Quartile 3    | Quartile 4    |
| N                | 431          | 415           | 434           | 423           |
| Crude            | 1.00         | 0.82 (0.56 – 1.18) | 0.73 (0.50 – 1.06) | 0.72 (0.49 – 1.05) |
| Model I          | 1.00         | 0.84 (0.57 – 1.23) | 0.78 (0.53 – 1.14) | 0.78 (0.53 – 1.15) |
| Model II         | 1.00         | 0.83 (0.56 – 1.24) | 0.70 (0.47 – 1.06) | 0.78 (0.52 – 1.17) |

|                  | SHBG |               |               |               |
|------------------|------|---------------|---------------|---------------|
|                  | Quartile 1   | Quartile 2    | Quartile 3    | Quartile 4    |
| N                | 429          | 430           | 428           | 416           |
| Crude            | 1.00         | 0.47 (0.33 – 0.65) | 0.15 (0.10 – 0.24) | 0.18 (0.12 – 0.28) |
| Model I          | 1.00         | 0.56 (0.40 – 0.79) | 0.21 (0.13 – 0.33) | 0.30 (0.19 – 0.47) |
| Model II         | 1.00         | 0.57 (0.40 – 0.82) | 0.23 (0.14 – 0.37) | 0.28 (0.17 – 0.44) |

|                  | FAI |               |               |               |
|------------------|-----|---------------|---------------|---------------|
|                  | Quartile 1   | Quartile 2    | Quartile 3    | Quartile 4    |
| N                | 421          | 421           | 428           | 433           |
| Crude            | 1.00         | 1.50 (0.93 – 2.42) | 2.30 (1.47 – 3.61) | 4.34 (2.83 – 6.65) |
| Model I          | 1.00         | 1.29 (0.80 – 2.11) | 1.80 (1.14 – 2.88) | 2.73 (1.75 – 4.25) |
| Model II         | 1.00         | 1.39 (0.83 – 2.30) | 1.79 (1.10 – 2.90) | 2.79 (1.74 – 4.46) |
Table 5. Associations of testosterone, SHBG and FAI quartiles at age 31 with HOMA-IR at age 46 in women in Northern Finland Birth Cohort 1966.

|          | Testosterone |          |          |          |
|----------|--------------|----------|----------|----------|
|          | Quartile 1   | Quartile 2 | Quartile 3 | Quartile 4 |
| N        | 360          | 345      | 334      | 303      |
| Crude    | 1.00         | 0.69 (0.49 – 0.97) | 0.97 (0.70 – 1.34) | 1.03 (0.74 – 1.44) |
| Model I  | 1.00         | 0.62 (0.43 – 0.90)  | 0.86 (0.60 – 1.23)  | 0.95 (0.66 – 1.37)  |
| Model II | 1.00         | 0.61 (0.42 – 0.90)  | 0.84 (0.58 – 1.22)  | 0.94 (0.64 – 1.38)  |

|          | SHBG         |          |          |          |
|----------|--------------|----------|----------|----------|
|          | Quartile 1   | Quartile 2 | Quartile 3 | Quartile 4 |
| N        | 334          | 346      | 357      | 339      |
| Crude    | 1.00         | 0.45 (0.33 – 0.62)  | 0.24 (0.17 – 0.34)  | 0.17 (0.12 – 0.25)  |
| Model I  | 1.00         | 0.57 (0.40 – 0.79)  | 0.39 (0.27 – 0.57)  | 0.30 (0.20 – 0.45)  |
| Model II | 1.00         | 0.59 (0.42 – 0.83)  | 0.39 (0.27 – 0.57)  | 0.30 (0.20 – 0.45)  |

|          | FAI          |          |          |          |
|----------|--------------|----------|----------|----------|
|          | Quartile 1   | Quartile 2 | Quartile 3 | Quartile 4 |
| N        | 355          | 333      | 317      | 315      |
| Crude    | 1.00         | 1.22 (0.82 – 1.81) | 2.52 (1.74 – 3.64) | 4.18 (2.92 – 6.00) |
| Model I  | 1.00         | 1.05 (0.69 – 1.60) | 1.81 (1.22 – 2.69) | 2.35 (1.59 – 3.46) |
| Model II | 1.00         | 0.98 (0.64 – 1.52) | 1.78 (1.19 – 2.67) | 2.27 (1.52 – 3.40) |
Table 6. Associations of testosterone, FAI and SHBG quartiles at age 46 with HOMA-IR at age 46 in women in Northern Finland Birth Cohort 1966.

|          | Testosterone |          |          |          |
|----------|--------------|----------|----------|----------|
|          | Quartile 1   | Quartile 2 | Quartile 3 | Quartile 4 |
| N        | 486          | 481      | 485      | 482      |
| Crude    | 1.00         | 0.88 (0.66 – 1.16) | 0.81 (0.61 – 1.08) | 0.85 (0.64 – 1.14) |
| Model I  | 1.00         | 0.89 (0.65 – 1.21) | 0.88 (0.65 – 1.20) | 0.93 (0.68 – 1.27) |
| Model II | 1.00         | 0.93 (0.67 – 1.29) | 0.91 (0.66 – 1.27) | 0.99 (0.72 – 1.38) |

|          | SHBG         |          |          |          |
|----------|--------------|----------|----------|----------|
|          | Quartile 1   | Quartile 2 | Quartile 3 | Quartile 4 |
| N        | 488          | 480      | 483      | 484      |
| Crude    | 1.00         | 0.30 (0.23 – 0.39) | 0.20 (0.15 – 0.27) | 0.10 (0.07 – 0.15) |
| Model I  | 1.00         | 0.37 (0.28 – 0.50) | 0.33 (0.24 – 0.45) | 0.20 (0.14 – 0.29) |
| Model II | 1.00         | 0.36 (0.27 – 0.49) | 0.34 (0.24 – 0.47) | 0.21 (0.14 – 0.30) |

|          | FAI          |          |          |          |
|----------|--------------|----------|----------|----------|
|          | Quartile 1   | Quartile 2 | Quartile 3 | Quartile 4 |
| N        | 479          | 488      | 483      | 484      |
| Crude    | 1.00         | 1.83 (1.27 – 2.63) | 2.81 (1.98 – 3.98) | 6.84 (4.89 – 9.56) |
| Model I  | 1.00         | 1.50 (1.03 – 2.20) | 2.07 (1.43 – 2.99) | 3.82 (2.67 – 5.45) |
| Model II | 1.00         | 1.64 (1.10 – 2.45) | 2.06 (1.40 – 3.05) | 4.31 (2.96 – 6.27) |