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Gender-associated Differences in Metabolic Syndrome-related Parameters in Göttingen Minipigs

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Gender-associated differences in pathophysiology and treatment of disease are an evolving area in human medicine that should be addressed in animal models. The aim of this study was to characterize gender differences in metabolic parameters of Göttingen minipigs and to determine which gender has the metabolic profile that is most appropriate as a model for human metabolic syndrome. Blood samples were collected from fasted, lean male and female Göttingen minipigs at 8 wk and 8 mo of age. Samples were analyzed for glucose, fructosamine, insulin, C-peptide, glucagon, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-c), free fatty acids, leptin, testosterone, and 17β-estradiol. Insulin sensitivity and beta cell function were estimated by homeostasis model assessment and degree of obesity by measuring the abdominal circumference. Male minipigs had higher concentrations of both testosterone and estradiol. Female minipigs had a larger abdominal circumference and higher concentrations of C-peptide, insulin, triglyceride, total cholesterol, HDL-c and leptin but a lower concentration of free fatty acids and lower HDL-c:total cholesterol ratio. Compared with male minipigs, female minipigs were more insulin-resistant and had a higher beta-cell function. No gender-associated differences were found in any of the other investigated parameters. In conclusion, female minipigs were more obese and insulin-resistant and had a more atherogenic plasma profile than did their male counterparts and therefore may be better models for metabolic syndrome. Their high concentrations of both testosterone and estradiol may protect male minipigs from obesity and metabolic disturbances.

Abbreviations: ANOVA, analysis of variance; BW, body weight; CV, coefficient of variation; DEXA, dual-energy X-ray absorptiometry; EDTA, ethylenediamine tetraacetic acid; FFA, free fatty acids; FI, food intake; HDL-c, high-density lipoprotein–cholesterol; HOMA-β, homeostasis model-derived index of β cell function; HOMA-S, homeostasis model-derived index of insulin sensitivity; LDL-c, low-density lipoprotein–cholesterol; TG, triglycerides

Gender-associated differences in pathophysiology, diagnosis, and treatment of disease are receiving increasing attention in human medicine[1,57] and should be reflected in the characterization of animal models. Metabolic syndrome, signified by central obesity, insulin resistance, impaired glucose regulation, dyslipidemia with raised triglycerides and low high-density lipid (HDL)–cholesterol, and hypertension,[2] displays significant gender-associated differences in many of the parameters.[5] These differences have been attributed to variations in fat distribution patterns and endocrine profiles, with the sex hormones being of major importance. Although metabolic syndrome generally has been more prevalent in men,[45,30,67] there has been a steep increase in cases in women during the last decade, which is most likely due to a parallel increase in obesity in this gender.[77] The importance of gender-associated differences in relation to prevention, diagnosis, and therapy of metabolic syndrome and relative risk for diabetes and cardiovascular disease has been highlighted in several references.[58,59,68,77]

The Göttingen minipig is a laboratory pig strain that is available across Europe and the United States from 2 barrier units (Dalmose, Denmark and North Rose, NY), with a yearly production of around 10000 minipigs. Minipigs are used widely as a pharmacologic and toxicologic model[8] and is one of few large-animal models described for obesity and diabetes.[41,46,51,52,54,76] This pig strain displays significant gender-associated differences with regard to development of obesity, with female minipigs having a significantly higher fat accumulation potential than their male counterparts.[9] This gender-associated difference has not been examined in depth in relation to other components of the metabolic syndrome nor relative to concentrations of sex hormones.

The aim of this study was to evaluate possible gender-associated differences in metabolic syndrome-related parameters in normal-weight Göttingen minipigs to compare with those in human patients and to identify the gender with the metabolic profile most suitable as a model for human metabolic syndrome.

Materials and Methods

Animals. Animals were Göttingen minipigs from Ellegaard Göttingen Minipigs ApS (Dalmose, Denmark). Genetically this pig strain is a combination of 33% Minnesota minipig, 59% Vietnamese potbelly pig, and 8% German Landrace pig,[29] and the inbreeding coefficient is reported to be below 10%.[11] Pathogen status is monitored according to guidelines by Federation of Eu-

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ropean Laboratory Animal Science Associations, and the latest health-monitoring report, together with historical data, can be found on the vendor's website (http://www.minipigs.dk). The Danish population consists of 8 breeding families and a varying number of breeding boars. All pigs have a unique 5-digit number, and all information regarding each animal is stored in a database under the corresponding number. Male and female minipigs 8 wk and 8 mo (28 to 32 wk) old (n = 30 to 36) were studied before and after sexual maturity, which is reached at 3 to 4 mo of age in male minipigs and 4 to 5 mo of age in female pigs. Animals were selected from all 8 families to identify possible influence of breeding family on the investigated parameters. Each animal was studied only once. The animals were group-housed in pens under controlled conditions with a 12 h-light:dark cycle (lights on, 0600 to 1800), relative humidity of 50% to 70%, and a temperature of 22 to 24 °C for the 8-wk-old animals and 20 °C for the 8-mo-old animals. Trained personnel cared for the animals. The study was approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

Diet and feeding regimen. Animals had ad libitum access to drinking water and were fed restrictedly twice daily with a standard minipig diet (Special Diets Services, Essex, UK; Table 1). This diet is a natural-ingredient diet composed of oat hulls and bran, barley, wheat, soybean hulls, wheatfeed, dehulled extracted toasted soya, molasses, sunflower extract meal, macrominerals, vitamins, and microminerals. The degree of restriction corresponded to around 50% of ad libitum food intake.[10] Minipigs 8 wk of age were fed in groups an amount equal to 220 g daily per pig, whereas minipigs 8 mo of age were fed an average of 360 (females) or 380 (males) g daily per pig. We assumed that the pigs ate equal amounts. The gender-differentiated feeding started at 3 to 4 mo of age and is necessary to avoid obesity in the female minipigs due to the different growth potentials in the 2 genders.[10]

Birth weight and body weight (BW). All animals were weighed on the day of blood sampling, and data on birth weight were retrieved from the database at Ellegaard Göttingen Minipigs ApS.

Abdominal circumference. Abdominal circumference was measured with a tape measure at the umbilical level on the day of blood sampling. This biometric measure has been shown to be predictive for the amount of retroperitoneal, visceral and the remaining carcass fat (the latter representing primarily subcutaneous fat) in female Ossabaw pigs 7 to 8 mo of age[23] and for visceral and the remaining carcass fat in male Yucatan pigs 8 to 15 mo of age.[90] The predictive nature of the abdominal circumference measure remained valid for Göttingen minipigs of both genders at 30 wk of age ($R^2 = 0.91$ for total body fat mass obtained by dual-energy X-ray absorptiometry [DEXA] scanning; $R^2 = 0.82$ for visceral fat mass and $R^2 = 0.85$ for retroperitoneal fat mass, both obtained by quantitative dissection; $P < 0.0001$ for all). In Göttingen minipigs 10 wk of age, the total body fat mass obtained by DEXA scanning is also predictable by the abdominal circumference ($R^2 = 0.85$, $P < 0.0001$).[15]

Sampling and handling of blood. Animals were fasted overnight (approximately 18 h) with free access to water. Blood samples were taken aseptically from the cranial vena cava by using vacuum phlebotomy tubes in conscious animals restrained in dorsal recumbency. All blood samples were taken between 0830 and 1000, and a total of 10 ml heparinized and 10 ml unstabilized blood was taken. From the unstabilized blood, 210 µl immediately was pipetted into a tube coated with ethylenediamine tetraacetic acid (EDTA) and containing 25 µl NaF (catalog no. S7920, Sigma-Aldrich, 42 mg NaF/ml NaCl, 5 mg NaF/ml blood) for analysis of free fatty acids (FFA) and glycerol. In addition, 2 ml was pipetted into an EDTA-coated tube containing 50 µl protease inhibitor aprotinin (Trasylol 10000 kIE/ml, Bayer HealthCare, Lyngby, Denmark) for analysis of insulin, C peptide, and glucagon. The remainder of the unstabilized blood was left to clot for 1 h at room temperature before centrifugation. All other tubes were kept on ice until centrifugation, which was done within 1.5 h after blood sampling. The EDTA-treated tubes were centrifuged at $5600 \times g$ for 2 min, whereas the heparin-containing and uncoated tubes were centrifuged at $3500 \times g$ for 10 min, all at room temperature. Plasma and serum was pipetted on dry ice immediately after centrifugation and stored at either –80 °C (plasma for FFA and glycerol) or –20 °C (all other analyses).

Analyses of endocrine parameters. ***Sex hormones.*** Testosterone and estradiol 17β concentrations in serum were measured by using commercial enzyme-linked immunosorbent assay kits for porcine samples (catalog nos. pT-96 and pE2-96, ELISA Development, Euskirchen, Germany). Estradiol was extracted from the serum by using diethyl ether and reconstituted in assay buffer before measurement. For the testosterone assay, the intra- and interassay coefficients of variation (CVs) were 4.9% and 11.3%, respectively, and the minimal detectable concentration was 0.1 ng/ml. For the estradiol assay the intra- and interassay CVs were 3.6% and 2.1%, respectively, and the minimal detectable concentration was 6.2 pg/ml. CV% for the extraction was 5.3%. Crossreactivity in the estradiol assay was: estrone, <2; estradiol benzoate, <1.2%; estriol, <0.3%; 17α-estradiol, <0.03%, testosterone, <0.004%, progesterone, <0.0001%; and cortisol <0.0001%. Crossreactivity in the testosterone assay was: 5α-dihydroxytestosterone, 96%; androstendione 3%; progesterone, <0.01%, 17β-estradiol, 0.01%; cortiosol, 0.02%; and corticosterone, 0.001%.

Parameters related to glucose metabolism. Insulin, C-peptide, and glucagon concentrations were analyzed in EDTA–plasma with trasylol, whereas glucose and fructosamine concentrations were analyzed in heparin–plasma. Glucose and fructosamine concentrations were measured on an analyzer (Hitachi 912, Roche A/S Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Insulin concentration was analyzed using an inhouse 2-site immunometric assay with monoclonal antibodies as catching and detecting antibodies and using purified porcine insulin for calibration of the assay. The minimal detectable concentration was 3.6 pM, and the upper limit (with no sample dilution) was 1785 pM. Inter- and intra-assay variations at 3 concentration levels were 12.2% and 2% (at 8.4 pM), 7.0% and 2% (at 81 pM), and 5.3% and 1.5% (at 680 pM), respectively. C-peptide and glucagon concentrations were measured by using commercial radioimmunoassay kits (Porcine C-peptide RIA Kit, catalog no. PCP-22K, and Glucagon RIA Kit, cat. no. GL-32K, Linco Research, St Charles, MO).

Parameters related to lipid metabolism. FFA and glycerol concentrations were analyzed in EDTA–plasma with NaF, and triglyceride (TG), total cholesterol and high-density lipoprotein–cholesterol (HDL-c) concentrations were analyzed in heparin–plasma. All parameters were measured on a Hitachi 912 analyzer (Roche A/S Diagnostics) according to the manufacturer's instructions.

Leptin. Leptin concentration was analyzed in heparin–plasma by using a modified version of a commercial kit (Active Porcine

Leptin IRMA, catalog no. DSL-82100, Diagnostic Systems Laboratories, Webster, TX). To improve sensitivity of the assay, standards A through E were diluted 1:1 with the 0-ng/ml standard and 100 µl instead of 50 µl of both standards and unknown samples were used.

Calculations and statistical analysis. ***Calculations.*** Homeostasis model-derived indexes of insulin sensitivity (HOMA-S) and β-cell function (HOMA-β) were calculated as follows:[57]

$$\text{HOMA-S} = 22.5 \div (\text{fasting plasma glucose [mM]} \times \text{fasting plasma insulin [pM]})$$

$$\text{HOMA-}\beta = (20 \times \text{insulin [pM]}) \div (\text{glucose [mM]} - 3.5)$$

HDL-c ratio was calculated as HDL-c ÷ total cholesterol.

Statistical analyses. Data are presented as means ± SEM. Statistical analysis of the results was performed by using SAS software (version 9.1 for Windows, SAS Institute, Cary, NC) for combined analysis of variance (ANOVA) and multiple regression analysis (Proc GLM in SAS). Mathematical transformation of the response was done when required. For all statistical analyses, a *P* value of less than or equal to 0.05 was considered significant. Two statistical analyses were performed: standard 2-way ANOVA, including age, gender, and the interaction between age and gender as explanatory variables (model 1), and a more detailed model including age, gender, interaction between age and gender, birth weight, and breeding family as explanatory variables and BW and FI as covariates for all metabolic plasma parameters (model 2). In addition, glucose was added to model 2 for fructosamine and glucagon, insulin to the model for FFA and TG, and C-peptide to the model for insulin to confirm known relationships between these parameters. For birth weight, only the difference between males and females was examined. For abdominal circumference, statistical analysis was done separately for each age group, because the relation between abdominal circumference and body fat mass differs depending on the age of the animals [15]. Statistical analysis of leptin was done only for female minipigs and of testosterone only for male minipigs, because the values for the opposite gender were at or below assay sensitivity for these 2 parameters. The models were reduced by backward stepwise reduction, in which we removed nonsignificant interactions first and thereafter the variable with the highest *P* value greater than 0.05. Because BW and mean food intake (FI, g/kg) were included as covariates in model 2, these 2 parameters were retained in the model regardless of significance, and all results from this model 2 thus are corrected for effects of BW and FI. When significant interaction was detected between gender and age, pairwise comparisons between relevant groups were done. Relevant groups with respect to gender were females 8 wk versus males 8 wk and females 8 mo versus males 8 mo, and relevant groups with respect to age were females 8 wk versus females 8 mo and males 8 wk and males 8 mo. Statistical outliers and influential observations, identified as observations with a standard residual greater than 3.0 or a Cook D value greater than 1, respectively, were excluded from the analysis and from the graphic presentation of the data.

Results

BW, abdominal circumference, sex hormone values, and parameters related to lipid and glucose metabolism are shown in Figures 1 through 5. Statistical results from the 2-way ANOVA (model 1) are shown on the figures, whereas statistical results from model 2 are described below. When comparing the results from the 2 statistical analyses, it should be borne in mind that in model 2 the age and gender effects reported for the metabolic plasma parameters are corrected for BW, FI, and other significant variables in the model.

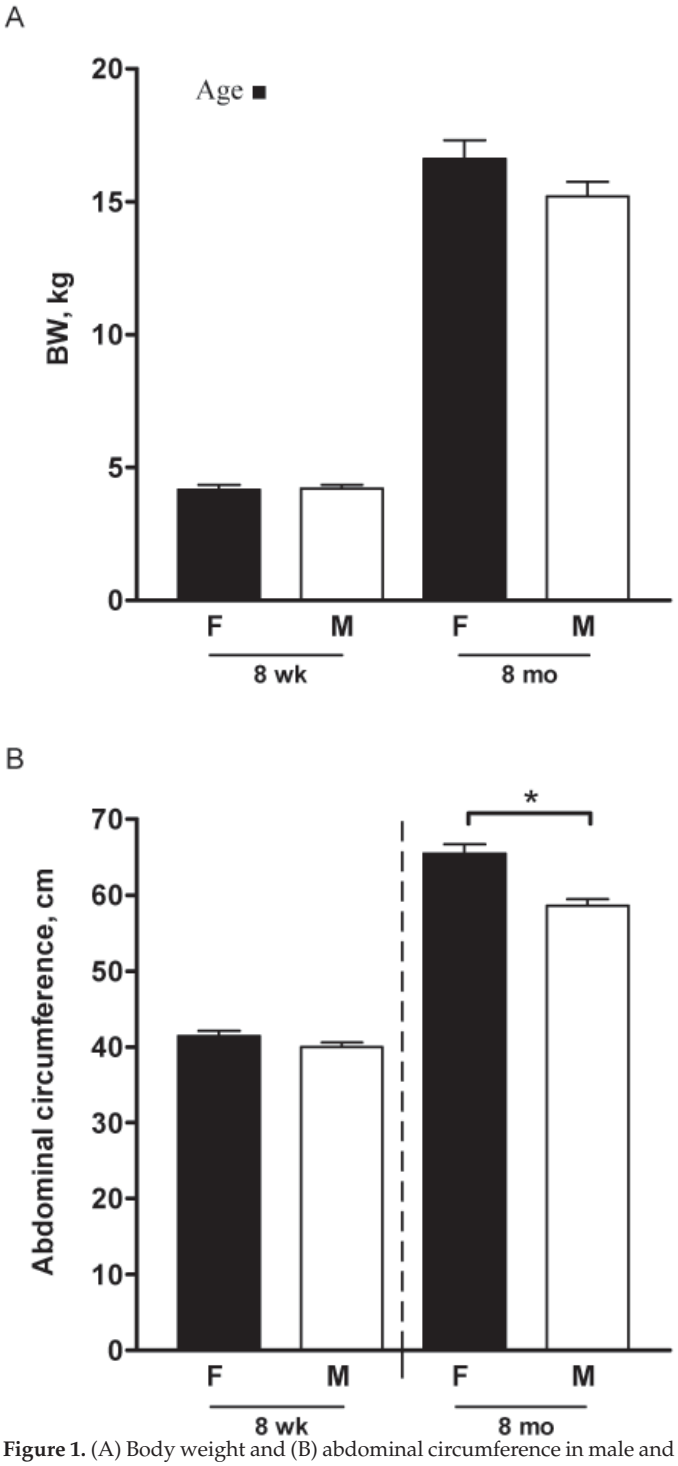

Figure 1. (A) Body weight and (B) abdominal circumference in male and female Göttingen minipigs 8 wk and 8 mo of age. Mean ± standard error of the mean, n = 29 to 36. Results of 2-way ANOVA are noted. Statistical analysis for abdominal circumference was done separately for each age group. *, $P \leq 0.001$ between genders, ■, $P \leq 0.001$ between age groups.

Birth weight, BW, and FI. No difference in birth weight (mean ± standard error of the mean) was found between male and female

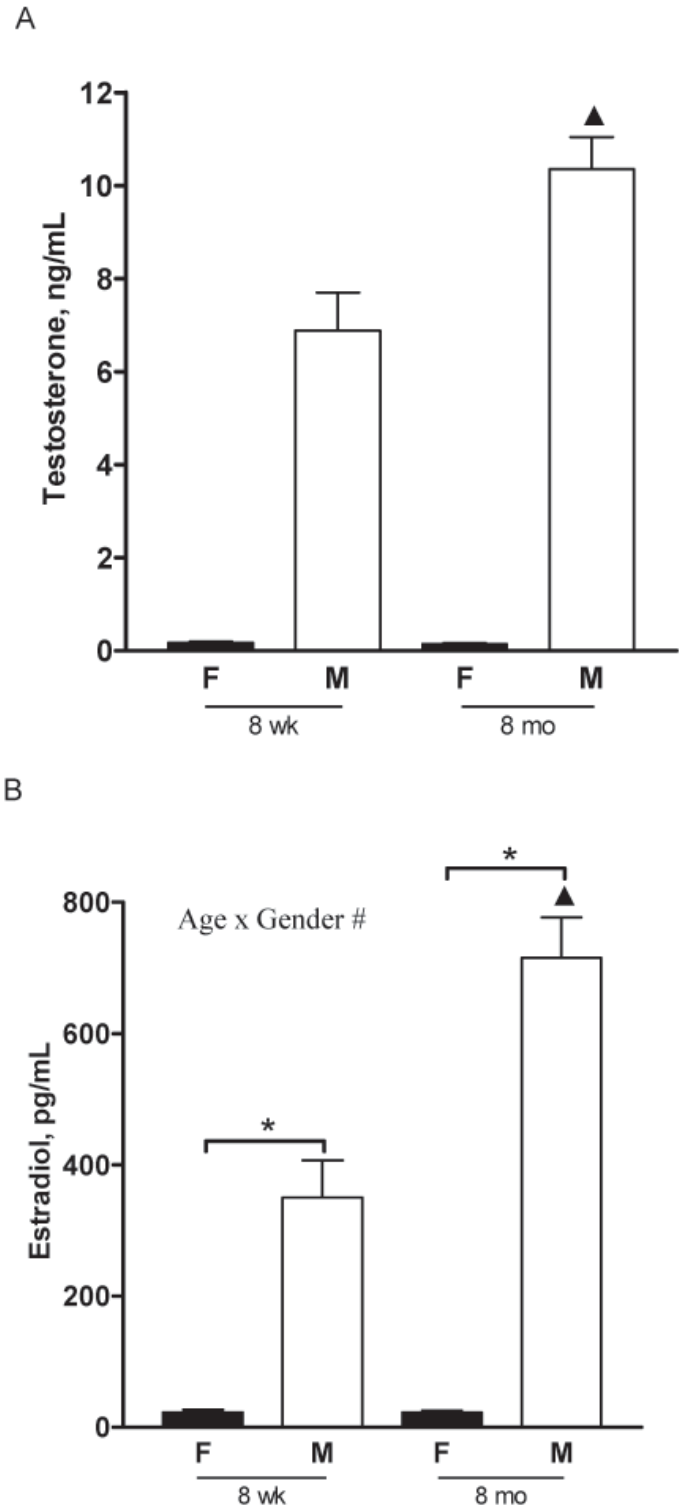

Figure 2. Fasting serum levels of (A) testosterone and (B) estradiol in male and female Göttingen minipigs 8 wk and 8 mo of age. Mean ± standard error of the mean, n = 29 to 36. Results of 2-way ANOVA are noted. Statistical analysis for testosterone was done for males only because all values from females were at or below assay sensitivity. #, $P \leq 0.01$ for interaction between age and gender; *, $P \leq 0.001$ between genders; ▲, $P < 0.001$ between males 8 wk and males 8 mo.

minipigs (461.5 ± 12.4 versus 455.1 ± 14.6 g). BW (Figure 1 A) did not differ between the genders either at 8 wk or 8 mo of age but increased with age ($P < 0.0001$) and was positively correlated with birth weight ($P < 0.01$, $R^2 = 0.90$). All 8-wk-old animals received 220 g food daily, corresponding to 56 ± 3 g/kg in the females and 54 ± 2 g/kg in the males (no significant difference), but to obtain the same BW in the 2 genders, the 8-mo-old males received 20 g more food daily than did the females (380 g versus 360 g, corresponding to 23 ± 1 g/kg in the females and 26 ± 1 g/kg in the males, $P < 0.05$).

Abdominal circumference. Statistical analysis of this parameter was done separately for each age group, because the relation between abdominal circumference and body fat mass differs depending on the age of the animal. Females had a higher abdominal circumference than males only at 8 mo of age (Figure 1 B). When corrected for the effect of BW, females had a higher abdominal circumference than males both at 8 wk ($P < 0.05$) and 8 mo of age ($P < 0.0001$), and there was a positive association between BW and abdominal circumference in both age groups ($P < 0.0001$ for both, $R^2 = 0.52$ for age 8 wk and $R^2 = 0.76$ for age 8 mo).

Sex hormones (Figure 2). Testosterone concentration was at or below assay sensitivity (0.1 pg/ml) in most female minipigs of both age groups, and therefore only results from the male animals were used for statistical analysis. There was an increase in testosterone concentration from 8 wk of age to 8 mo of age (6.9 ± 0.8 versus 10.4 ± 0.7 ng/ml, respectively; Figure 2 A). With model 2 BW was the only significant explanatory variable positively related to testosterone ($P < 0.01$, $R^2 = 0.11$), and the age-dependent increase therefore seemed to be partly explained by the concurrent increase in BW.

Estradiol concentration showed a statistically significant interaction between gender and age ($P < 0.001$) with both models, and model 2 furthermore yielded a positive association with BW ($P < 0.05$, $R^2 = 0.89$). The estradiol concentration was higher in males compared with females at both ages, but significantly more so at 8 mo of age. The increase in estradiol concentration with age in the males seen with model 1 (Figure 2 B) was not significant when corrected for the effect of BW.

Metabolic parameters related to glucose metabolism (Figure 3). No gender-associated differences were found for glucose, fructosamine, and glucagon concentrations. Glucose concentration increased with age ($P < 0.001$) and was further inversely related to BW ($P < 0.01$, $R^2 = 0.19$). Fructosamine levels increased with age ($P < 0.001$), were positively related to glucose ($P < 0.001$), and were dependent on breeding family ($P < 0.01$), with the highest fructosamine concentrations found in families 3 and 8 ($R^2 = 0.57$). Glucagon was negatively associated with glucose ($P < 0.05$, $R^2 = 0.07$). For C-peptide concentration the interaction between gender and age (Figure 3 C) was no longer significant in the more detailed model, and there was only an effect of gender, with C-peptide concentrations being higher in the female minipigs ($P < 0.001$, $R^2 = 0.17$).

For insulin, gender was again the only significant explanatory variable ($P < 0.001$, $R^2 = 0.13$), with female minipigs having significantly higher insulin concentrations than males. When C-peptide was included in the model for insulin, this was the only significant explanatory variable ($R^2 = 0.76$), supporting the known correlation between C-peptide and insulin concentrations.

HOMA indexes (Figure 4). The HOMA-S index was significantly higher in male minipigs ($P < 0.01$, $R^2 = 0.12$), whereas the

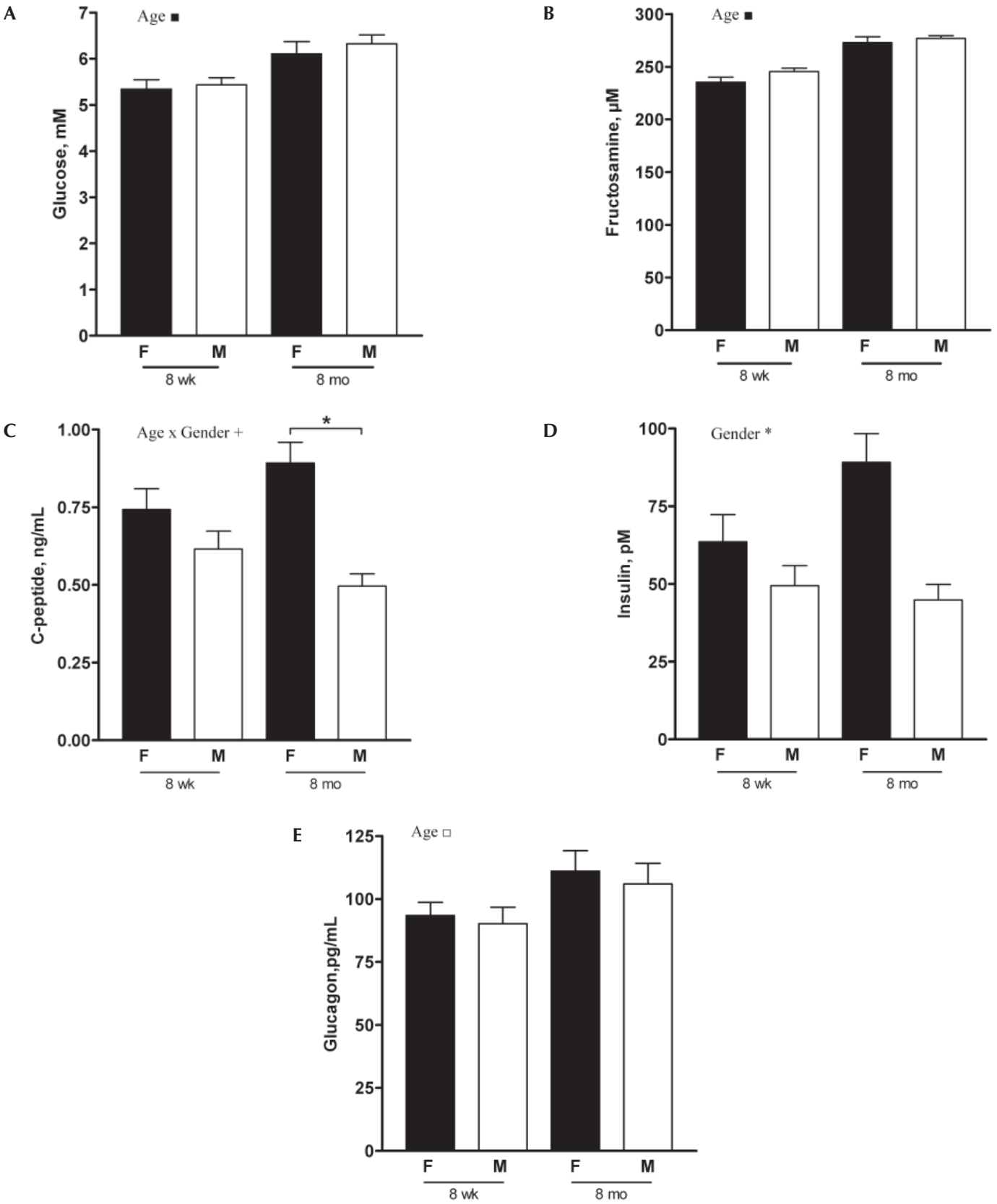

Figure 3. Fasting plasma levels of parameters related to glucose metabolism in male and female Göttingen minipigs 8 wk and 8 mo of age. (A) Glucose, (B) fructosamine, (C) C-peptide, (D) insulin, and (E) glucagon. Mean ± standard error of the mean, n = 29 to 36. Results of 2-way ANOVA are noted. +, $P \leq 0.05$ for interaction between age and gender; *, $P \leq 0.001$ between genders; □, $P \leq 0.05$ between age groups; ■, $P \leq 0.001$ between age groups.

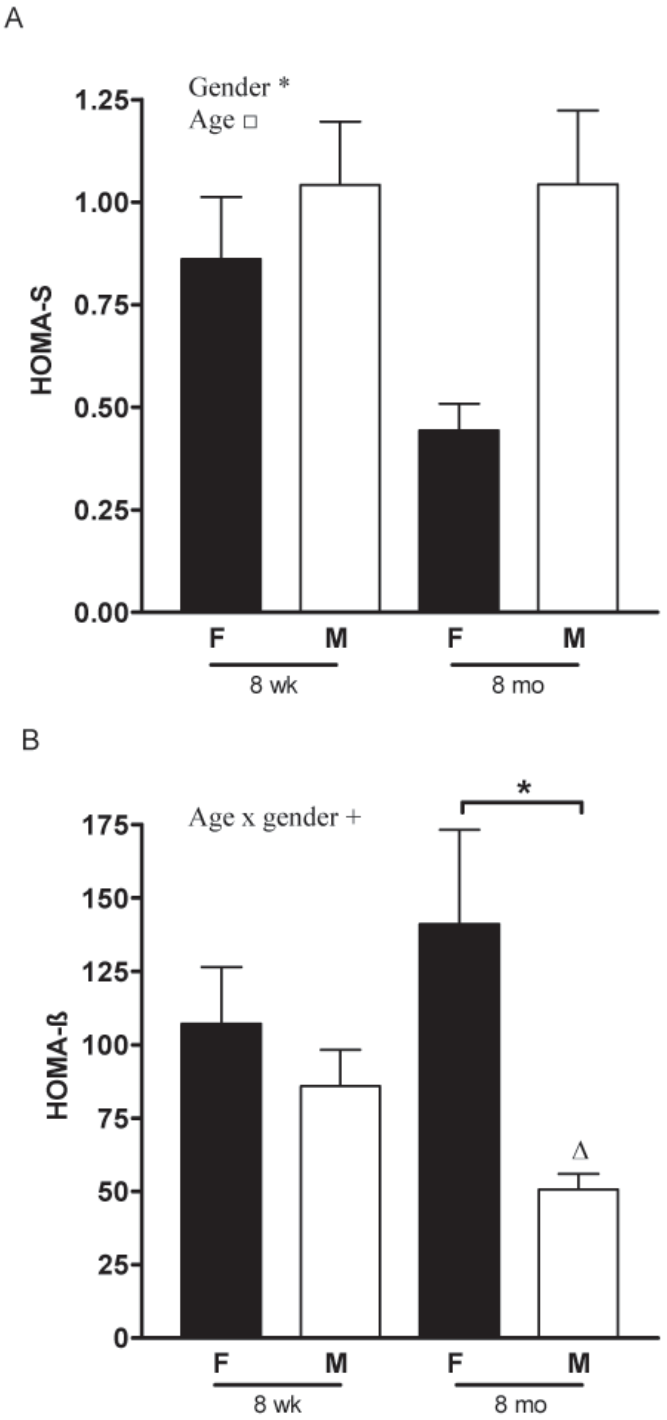

Figure 4. Homeostasis model-derived indexes of (A) insulin sensitivity (HOMA-S) and (B) β cell function (HOMA-β) in male and female Göttingen minipigs 8 wk and 8 mo of age. Mean ± standard error of the mean, n = 29 to 36. Results of 2-way ANOVA are noted. +, $P \leq 0.05$ for interaction between age and gender; *, $P \leq 0.001$ between genders; □, $P < 0.05$ between age groups; △, $P < 0.05$ between males 8 wk and males 8 mo.

HOMA-β index was higher in females ($P < 0.001$), decreased with age ($P < 0.001$), and was positively correlated with BW ($P < 0.001$, $R^2 = 0.22$). As for C-peptide, the interaction between gender and age for HOMA-β was not significant in the more detailed model.

Metabolic parameters related to lipid metabolism (Figure 5). For TG, there was a statistical interaction between gender and age ($P < 0.05$) and a positive association with insulin ($P < 0.01$). The TG concentration was higher in females of both ages and decreased significantly with age in males only. In addition, breeding family influenced the TG concentration ($P = 0.05$), which was highest in families 3, 4, and 9 ($R^2 = 0.51$). For cholesterol, too, there was interaction between gender and age ($P < 0.01$), an effect of breeding family ($P < 0.05$), and a positive association with BW ($P < 0.05$, $R^2 = 0.59$). Both juvenile and sexually mature female minipigs had higher cholesterol concentrations than did males of the corresponding age, and although the cholesterol concentration decreased significantly with age in both genders, the decrease was more pronounced in males. The cholesterol concentration was highest in families 4 and 7.

For HDL-c, gender ($P < 0.001$), age ($P < 0.001$), and BW ($P < 0.01$) were significant explanatory variables ($R^2 = 0.49$). Female minipigs had a higher HDL-c concentration than did males, and the HDL-c decreased similarly with age in both genders and was further positively associated with BW. The HDL-c concentration was lowest in families 2 and 8, although the effect of family did not reach statistical significance ($P = 0.06$). For the HDL-c ratio, only gender ($P < 0.001$) and family ($P < 0.001$) were significant explanatory variables ($R^2 = 0.34$). Females had lower HDL-c ratio than did males, and the HDL-c ratio were lowest in families 3 and 4.

For the FFA concentration, the interaction became insignificant in the more detailed model 2; instead there was a significant effect of gender ($P < 0.001$), insulin concentration ($P < 0.01$), and FI ($P < 0.05$) ($R^2 = 0.30$). Females had lower FFA concentration at both ages, insulin was inversely correlated with FFA, and FI was positively related to the FFA concentration. Glycerol was only related to breeding family ($P = 0.05$) with the highest glycerol concentrations found in families 3 and 9 ($R^2 = 0.2$).

Leptin. The fact that many of the leptin values were below assay sensitivity in male minipigs indicates that the leptin concentration is lower in male minipigs compared with female minipigs. In female minipigs, statistical analysis showed that leptin concentrations increased from 8 wk of age to 8 mo of age (0.4 ± 0.05 versus 0.9 ± 0.1 ng/ml, $P < 0.001$). In model 2, age was not significant, but leptin was positively associated with birth weight ($P < 0.05$) and breeding family ($P < 0.01$), with the highest concentrations found in family 3 ($R^2 = 0.61$).

Discussion

We investigated parameters related to human metabolic syndrome in juvenile and young sexually mature, normal-weight Göttingen minipigs, with a focus on gender-associated differences. We detected significant gender-related differences in many of the measured variables, with the female minipigs being more obese, more insulin resistant, and having higher plasma concentrations of lipids.

In humans, low birth weight predisposes to development of central obesity, metabolic syndrome, and type 2 diabetes later in life.[6,35,56] The metabolic impairments develop over time and are hypothesized to primarily arise when food is readily available and rich in energy.[34] The lack of influence of birth weight on the central parameters related to glucose and fat metabolism in the present study could thus be attributed to the young age of the pigs, the restricted feeding regimen, or the fact that none

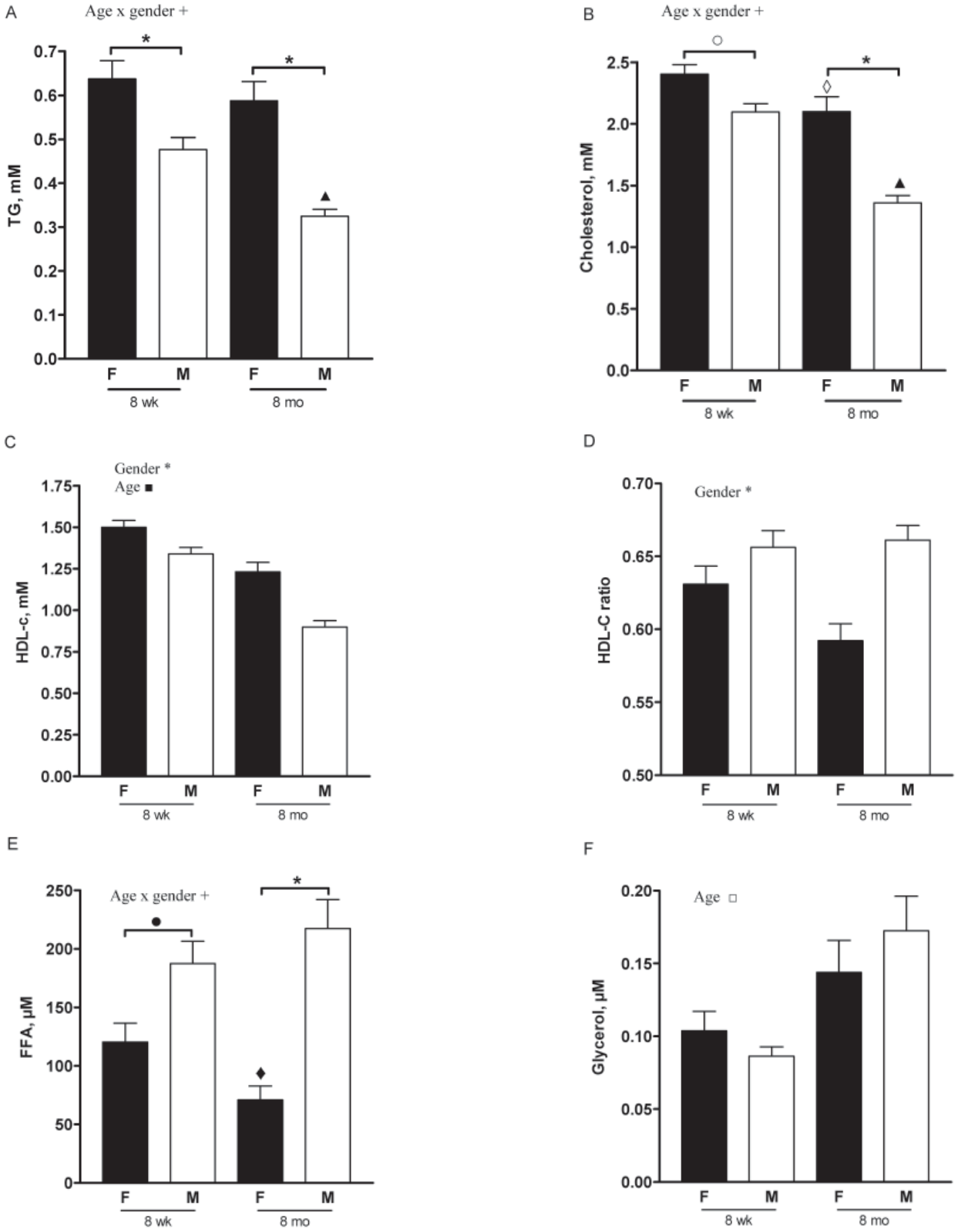

Figure 5. Fasting plasma levels of parameters related to lipid metabolism in male and female Göttingen minipigs 8 wk and 8 mo of age. (A) Triglycerides, (B) total cholesterol, (C) HDL-c, (D) HDL-c ratio, (E) FFA, and (F) glycerol. Mean ± standard error of the mean, n = 27 to 36. Results of 2-way ANOVA are noted. +, $P \leq 0.05$ for interaction between age and gender, ○, $P \leq 0.05$ between genders; ●, $P \leq 0.01$ between genders; *, $P \leq 0.001$ between genders; □, $P < 0.05$ between age groups; ■, $P < 0.001$ between age groups; ▲, $P < 0.001$ between males 8 wk and males 8 mo; ◇, $P \leq 0.05$ between females 8 wk and females 8 mo; ◆, $P \leq 0.01$ between females 8 wk and females 8 mo.

of the pigs had a birth weight low enough to induce metabolic changes.

Due to the restricted feeding regimen aiming at producing similar body weights between the 2 genders, gender-associated difference in BW were not noted in the 2 investigated age groups. However, the female minipigs of 8 mo age needed a significantly smaller food ration to achieve this BW, indicating better food conversion efficiency or lower energy expenditure (or both). Since it cannot be excluded that differences in calorie intake and BW may influence the metabolic parameters studied, the more advanced statistical model (model 2) were corrected for these 2 parameters.

As expected, the testosterone concentration was higher in male minipigs than in females, and the sexually mature males had a higher testosterone concentration than juvenile males. These findings are similar to the gender-associated differences found in testosterone concentration in humans and to the increase in testosterone concentration seen around puberty in boys.[83] In addition, the male minipigs had a 15- and 30-fold higher estradiol concentration than did female minipigs before and after sexual maturity, respectively. This difference has been noted previously, although to a lesser extent, in crossbred pigs[16] and is thought to be due to pronounced testicular estradiol production in boars.[87] This hypothesis has been confirmed through castration studies in both Göttingen[15] and Yucatan minipigs,[66] in which castration leads to an almost complete elimination of circulating estradiol. This contrasts markedly with the situation in humans, in which the estradiol concentration is approximately the same in prepubertal boys and girls but significantly higher in women than men both during and after puberty.[28,83] Testosterone and estradiol concentrations in our male minipigs were higher than those in men and premenopausal women, respectively, whereas the female minipigs lacked testosterone, and their estradiol concentrations were lower than those of premenopausal women and more in the range of those of men and postmenopausal women.[28,83] The very high concentrations of both testosterone and estradiol in male minipigs may well influence their metabolism and protect this gender against obesity and metabolic disturbances.

Abdominal circumference showed a significant gender difference, with female minipigs having a significantly larger abdominal circumference than males, especially at 8 mo of age. Because this biometric measure is predictive for the amount of visceral fat, retroperitoneal fat and the remaining subcutaneous carcass fat in pigs of different strains and ages[15,23,90] the results indicate not only a higher body fat content but also a larger amount of visceral fat in the female compared with the male pigs. The higher total body fat content in the female pigs is comparable to the situation in humans, in which the female gender has a higher body fat percentage.[3,28,78] In humans, however, men usually have a larger amount of visceral fat than do women.[28] Because this pattern seems to be opposite in minipigs, female minipigs might be more prone to the metabolic impairments associated with central obesity.[72,75,80]

Regarding glucose metabolism, minipigs showed no gender-associated differences in either glucose or fructosamine concentrations, which data are in contrast to many human studies, in which men have a slightly higher glucose concentration than do women.[17,33,67,89] Surprisingly, glucose concentration in minipigs was negatively related to BW, a finding that may be due to the fact that in these normal-weight lean pigs any increase in body weight is primarily an increase in lean body mass which has a high degree of glucose utilization.[26,93] High fat feeding leads to increased BW and increased fasting glucose concentration in male minipigs,[52] therefore a positive correlation between BW and glucose might occur in a group of pigs in which obesity varied more widely. Glucose and fructosamine both increased with age in the 2 genders, which is in accordance with earlier results from male minipigs.[53] Furthermore, fasting glucose concentration and breeding family independently influenced the fructosamine concentration, in parallel to the situation in humans, in which both genetics and fasting glucose concentration independently influence the concentration of glycosylated hemoglobin.[82]

Fasting insulin and C-peptide concentrations both were higher in female minipigs than male, and especially so after sexual maturity. A higher fasting insulin concentration with no differences in fasting glucose concentration suggests a higher degree of insulin resistance in female minipigs. This situation is also apparent when looking at the HOMA-S parameter, which is lower in female minipigs than male, the difference again being more pronounced after sexual maturity. Similar gender-associated differences in fasting insulin concentration and insulin resistance have been reported in several studies in humans,[28,44,88,89] although others find no difference.[67,92] The relatively higher insulin resistance in female minipigs might be explained by their greater adiposity or lower estradiol concentration, given that obesity increases insulin resistance[25,43] whereas estradiol seems to have the opposite response.[13,48,81] However the beneficial effects of estradiol on glucose metabolism in men remain controversial, because some studies link high estradiol concentration with high fasting insulin concentration and insulin resistance.[72,74]

Similar to humans,[89] β cell function was higher in female minipigs, as signified by their higher HOMA-β, than in male minipigs. Most likely the higher β cell function in the females represents an adaptation of insulin secretion to the higher insulin resistance seen in this gender, rather than indicating impaired β cell function in the males. The fact that there was no difference in fasting glucose between the genders indicates that the fasting insulin production in both cases was appropriate for the degree of insulin resistance. This finding corresponds to the situation in humans, in which insulin sensitivity and insulin secretion show a curvilinear relationship.[43]

The lipid parameter that provides the best measure of risk for coronary heart disease has been the focus of debate, and TG, total cholesterol, HDL-c, and low-density lipoprotein–cholesterol (LDL-c) have all been proposed as candidates.[4,12,40,69,73,84] In the present study, the HDL-c ratio was chosen as the preferred risk measure together with absolute concentrations of TG and total cholesterol, because several studies have shown that the ratio between total and HDL-c is equal or superior to measures of total cholesterol, LDL-c, and LDL-c:HDL-c ratio.[12,45,65]

Female minipigs had higher TG and total and HDL-c concentrations and lower HDL-c ratio compared with males, the difference being most obvious after sexual maturity. These data indicate a more adverse lipid profile in the females than males, unlike the situation in humans, where men in most populations have a higher TG concentration, lower HDL-c concentration, and lower HDL-c ratio than do women, while there is rarely any gender-associated difference in the concentration of total cholesterol.[4,47,60,67,89] The male-type lipid profile in humans is thought to increase the risk of dyslipidemia and cardiovascular mortality,[42] and because this

gender-associated difference essentially is reversed in Göttingen minipigs, perhaps female minipigs would be more suitable as an animal model for human dyslipidemia. Indeed, female Göttingen minipigs develop more extensive hypercholesterolemia and early atherosclerotic lesions than do males, when fed a high cholesterol diet.[39] A similar gender tendency with regard to diet-induced dyslipidemia has been found in Ossabaw and Yucatan minipigs, in which females seem to develop greater changes in plasma lipids than do males when both genders are fed a high-fat diet.[23,37,86]

Estradiol, testosterone, obesity, and insulin resistance all influence the lipid profile, but because the 4 factors are highly intercorrelated, their effects are difficult to separate. In addition, the influence of sex hormones on lipid parameters remains somewhat controversial. In many studies estradiol has been shown to have beneficial effects on lipid parameters in both men and women,[13,27,63,85] whereas other research has related hyperestrogenemia in men to coronary heart disease.[50,71] Testosterone has been shown to be both negatively[31,63] and positively[32] related to HDL-c, whereas obesity[38,63,79] and insulin resistance,[49] in comparison, seem to have clear adverse effects on the lipoprotein profile. Therefore, the more favorable lipid profile in male minipigs may be explained by the lower degree of adiposity and insulin resistance together with the higher estradiol and testosterone concentrations in this gender.

In addition, breeding family influenced the lipid parameters TG, total cholesterol, and HDL-c ratio, and this genetic effect is in accordance with studies in humans.[18,27,55,70] Therefore, breeding family should be taken into consideration when choosing animals for studies with dyslipidemia.

Female minipigs had a lower FFA concentration, indicating decreased lipolysis, increased lipogenesis, or increased oxidation of FFA than in males. Both decreased lipolysis and increased lipogenesis lead to increased fat deposition in the females, as seen in a previous study[9] in which female minipigs accumulated fat more readily than did males. However, because there was no effect of gender on glycerol concentration, marked gender-associated differences in lipolysis are unlikely. Insulin and sex hormones influence lipid metabolism, with insulin increasing and sex hormones decreasing lipogenesis.[7,20-22,62,94] The higher concentration of insulin and lower concentration of both estradiol and testosterone in female minipigs could thus be part of the explanation for the lower FFA concentration in this gender, which is supported by the inverse relation between FFA and insulin concentrations.

The leptin concentration seemed to be lower in male minipigs than female at both ages, which pattern is similar to the situation in humans, where women have a higher leptin concentration compared with that in men.[24,36,64] This difference has been attributed to different concentrations of sex hormones, degrees of obesity, and fat distribution patterns in the 2 genders,[14,24,64,78] all of which could be true in minipigs also. Leptin concentration correlates well with degree of obesity in humans,[14,19,64] and the higher leptin concentration in the female minipigs thus might indicate a higher level of obesity in this gender, which is consistent with their increased abdominal circumference measure.

The discussed gender- and age-associated effects on metabolic parameters in Göttingen minipigs are restricted to the investigated age groups and might be related to sexual maturation rather than to increased age per se. Furthermore, no conclusions can be drawn from the present study with regard to the effects of different diets and degrees of obesity on the development of the metabolic syndrome in the 2 genders; these effects will be important aspects to consider in future model development. Although both male and female[23,41,52,86,91] minipigs develop components of the metabolic syndrome when fed a high-energy diet, their high testosterone and estradiol concentrations may protect male minipigs against diet-induced obesity and metabolic impairments, thus making this gender less attractive as a model for metabolic syndrome. The fact that fasting glucose concentration and insulin resistance increase with age indicate a slight deterioration of glucose metabolism in the 8-mo-old animals compared to the 8-wk-old ones. Since it may be more difficult to induce insulin resistance, glucose intolerance and other characteristics of the metabolic syndrome in the juvenile, faster growing animals with a more efficient glucose metabolism, we hypothesize that sexually mature female minipigs are preferable as models for the human metabolic syndrome.

The present study shows that young, normal-weight Göttingen minipigs manifest significant gender differences in sex hormones and parameters related to carbohydrate and lipid metabolism. Male minipigs have higher concentrations of both estradiol and testosterone, whereas female minipigs are more obese, more insulin resistant and seem to have the most atherogenic plasma profile. These characteristics suggest that female Göttingen minipigs are better models for human metabolic syndrome than are male minipigs. Furthermore, the effects of age and breeding family should be taken into consideration in model development.

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