Gender and Depot Differences in Adipocyte Insulin Sensitivity and Glucose Metabolism

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ABSTRACT

Objective– To investigate how insulin sensitivity and glucose metabolism differ in adipocytes between different fat depots of male and female mice, and how sex steroids contribute to these differences.

Research Design and Methods– Adipocytes from intraabdominal/perigonadal (PG) and subcutaneous (SC) adipose tissue from normal, castrated or steroid-implanted animals were isolated and analyzed for differences in insulin sensitivity and glucose metabolism.

Results– Adipocytes from both PG and SC depots of females have increased lipogenic rates compared to those from males. In females, Intraabdominal PG adipocytes are more insulin sensitive than SC adipocytes and more insulin sensitive than male adipocytes from either depot. When stimulated by low physiological concentrations of insulin female PG adipocytes show a robust increase in AKT and ERK phosphorylation and lipogenesis, whereas male adipocytes show activation only at higher insulin concentrations. Adipocytes from females have higher mRNA/protein levels of several genes involved in glucose and lipid metabolism. After castration, adipocytes of male mice showed increased insulin sensitivity and increased lipogenic rates, whereas adipocytes of females demonstrate decreased lipid production. Increasing estrogen above physiological levels, however, also reduced lipid synthesis in females, whereas increasing dihydrotestosterone in males had no effect.

Conclusions– There are major gender differences in insulin sensitivity in adipose tissue, particularly in the intraabdominal depot that are regulated by physiological levels of sex steroids. The increased sensitivity to insulin and lipogenesis observed in adipocytes from females may account for their lower level of insulin resistance and diabetes risk despite similar or higher fat content than in males.
Obesity increases dramatically the probability of developing type 2 diabetes, dyslipidemia, cardiovascular disease, fatty liver, and even some types of cancer (1). This occurs in large part due to decreased sensitivity of tissues to insulin, i.e. insulin resistance. Adipocytes in different fat depots appear to have a distinct impact in insulin sensitivity. Accumulation of visceral fat is linked to the development of these metabolic complications, whereas accumulation of subcutaneous fat is not (2-4).

Fat accumulation in different depots is also sexually dimorphic. In humans, men accumulate more visceral fat, whereas women accumulate more subcutaneous fat and have higher percentage of body fat compared to men. There is also evidence indicating that insulin sensitivity differs between males and females (5;6). Thus despite having lower fat mass, the prevalence of diabetes and early abnormalities of glucose metabolism is higher in men than in women (7). Women also have decreased susceptibility to fatty acid-induced peripheral insulin resistance (8). In different rodent models of glucose intolerance, insulin resistance and diabetes, males show a stronger phenotype than females (9-11). These sex-related differences in insulin sensitivity and adipose tissue development and function could be due in part to actions of estrogen and testosterone. For example, decreases in estrogen and increases in testosterone levels which occur during menopause are associated with loss of subcutaneous and gain of visceral fat and increase in insulin resistance (12). However, how insulin action differs between male and female, and how these differences account for a sex-specific regulation of adipose tissue development and function is largely unknown.

In this study, we have investigated how insulin sensitivity and glucose metabolism differ in adipocytes from visceral and subcutaneous depots between male and female mice, and how sex steroids could contribute to these differences. We find gender specific differences in insulin action in adipocytes that may contribute to the sexual dimorphism of insulin resistance and suggest the possibility of more effective gender specific therapies for obesity, diabetes and metabolic syndrome.

**RESEARCH DESIGN AND METHODS**

*Animals and physiological measurements.* C57BL/6 mice obtained from Taconic Laboratories were used between 9 and 15 weeks of age. Mice (9 weeks old) were castrated, sham-operated or implanted with 1.5 mg dihydrotestosterone (5α-DHT) (males) or 0.1 mg 17β-estradiol (females) pellets in a slow (21 days) release form. Glucose tolerance tests and insulin tolerance tests were performed on 9 and 12 weeks old mice respectively. Leptin, Adiponectin, free fatty acid (FFA), estradiol and DHT serum levels were determined using ELISA. All protocols for animal use and euthanasia were approved by the Animal Care Use Committee of the Joslin Diabetes Center in accordance with NIH guidelines.

*Adipocyte isolation and lipogenesis.* Adipocytes from perigonadal and flank inguinal subcutaneous adipose depots from each individual mouse were isolated by collagenase digestion (13). Lipogenesis assays were performed as described in the online appendix. To assess cell number, aliquots of 200 μl adipocytes were fixed with osmic acid and counted in a Coulter counter (14).

*Gene expression.* Total RNA was extracted from 100 μl of isolated adipocytes (Rneasy, QIAGEN). Reverse transcription was performed using 0.5 μg of RNA, and quantitative real-time PCR was performed using a fluorescent temperature cycler (ABI 7900) with SYBR Green.
Western blot – Isolated adipocytes were treated with or without 0.1 or 10 nM insulin for 5-30 minutes. Proteins were extracted from 200 μl of cell suspension homogenized in RIPA buffer with 1% SDS. 30-50 μg of protein were subjected to SDS-PAGE, transferred to PVDF membranes, and blots were probed with the respective antibodies. The antigen-antibody complex was detected by using the horseradish peroxidase (HRP)-coupled secondary antibodies and enhanced chemiluminescence.

Statistical Analysis. Data are reported as mean ± S.E.M. Comparisons for the GTT, ITT and lipogenesis experiments were made using repeated measures ANOVA and the rest of the comparisons were made using Student’s t test; P< 0.05 was considered significant.

RESULTS
Female mice and female adipocytes are more insulin sensitive than males. Previous studies have suggested that female humans and rodents are more insulin sensitive than their male counterparts. When C57BL/6 mice were subjected to intraperitoneal glucose tolerance tests, female mice had lower glucose levels than male mice at every time point (p<0.05) (Fig. 1A) suggesting increased insulin sensitivity. This was confirmed by insulin tolerance tests, which showed a greater fall in blood glucose in response to insulin in female as compared to male mice (Fig. 1B).

To analyze how glucose and lipid metabolism differ between male and female adipocytes, we measured the ability of isolated adipocytes to synthesize lipids via de novo lipogenesis. Under both basal and insulin-stimulated conditions, female adipocytes from perigonadal and inguinal subcutaneous depots showed increased lipid synthesis as compared to male adipocytes whether expressed by lipid content (PG p<0.0001) (Fig. 1C) or by cell number (PG p<0.001, SC p<0.01) (Fig. 1D). The difference between genders for perigonadal fat was 263 % (p<0.005) in the basal state, and 225% at maximal insulin concentration (p<0.001). Female adipocytes from the perigonadal depot also showed increased insulin sensitivity when compared to male adipocytes from the same depot. The insulin concentration causing a half-maximal effect (EC50) on lipogenesis in female adipocytes from the perigonadal depot was 0.19 ± 0.12 nM, whereas in male adipocytes it was 3.5 times higher, 0.67 ± 0.11 nM (p=0.001). Subcutaneous adipocytes from males and females showed a smaller difference in basal and insulin-stimulated effect than the perigonadal fat that was statistically significant when normalized by cell number (p<0.01), however no difference in insulin sensitivity was observed between male and female adipocytes from the SC depot (Fig. 1C, 1D).

Adipocytes from female mice have increased insulin stimulation of AKT and ERK. To determine if the increased insulin sensitivity in female adipocytes observed in lipogenesis was a result of increased insulin signaling, we assessed phosphorylation/activation of two insulin downstream targets, AKT and ERK with two different doses of insulin: a low physiological dose (0.1 nM) and a pharmacological dose (10 nM). This revealed that high insulin concentrations for 5 minutes induced a similar level of AKT and ERK phosphorylation in both female and male adipocytes (Fig. 2A). By contrast, stimulation with the low insulin concentration for 5 or 30 minutes (Fig. 2B, 2C), induced a robust increase in AKT and ERK phosphorylation only in female adipocytes (7.1–11.1 fold for AKT and 3.9–13.9 fold for ERK) (Fig. 2B, 2C), whereas the same physiological concentration of insulin induced only a very modest effect (1.2–1.62 for AKT and 0.9–1.3 fold for ERK) (Fig. 2B, 2C) or no activation in male adipocytes (p <0.05 for both AKT and ERK).
Female adipocytes show higher expression of glucose and lipid metabolism genes. To determine if differences in expression could explain the increased lipogenic activity and the increased insulin signaling in female adipocytes, we analyzed the mRNA expression patterns of genes involved in insulin signaling and in glucose and lipid metabolism using qRT-PCR. We observed that adipocytes from both depots from female mice had higher mRNA levels of the glucose transporters (Glut 1 and Glut 4) and the key lipogenic enzymes fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC) than male adipocytes from the same depots (Fig. 3A). ACC mRNA showed a 4.3- and 2.1-fold difference between male and female cells for perigonadal and subcutaneous fat (p <0.05). Likewise FAS mRNA was 2.7-fold higher in both depots in female versus male adipocytes (p <0.05). Glut 4 was 2.4-fold (PG) and 1.5-fold (SC) higher in females, and Glut 1 was 2.4-fold (PG) and 1.8-fold (SC) higher in female adipocytes (p <0.05). mRNA levels for each of these genes tended to be higher in females PG adipocytes than in SC adipocytes, such that the female perigonadal depot had the highest levels of expression of these genes among all depots compared. In males, the expression levels were similar between the two depots.

These differences in mRNA were paralleled by differences in protein expression as determined by western blot analysis. Thus, protein levels of Glut 4, ACC and FAS were higher in female than in male adipocytes by 2- to 3-fold (p<0.05). By contrast, the insulin receptor and actin levels were not different (Fig. 3B, 3C). Thus, the greater level of lipid synthesis in female adipocytes compared to male adipocytes is due, at least in part, to increased glucose transporters and lipogenic enzymes levels.

Castration reverses lipogenic rates and gene expression. To determine the role of estrogen and testosterone on the sexual dimorphism of lipogenesis and insulin sensitivity, we analyzed the lipogenic capacity of fat cells taken from mice 6 weeks after castration or sham surgery. Adipocytes from castrated females had a decrease in the lipogenic rate (PG p<0.01, SC p<0.005) and in insulin sensitivity (only in PG depot) when compared to adipocytes from the sham controls, more closely resembling those of male adipocytes. PG adipocytes from castrated females, had 47% lower rates of maximal lipogenesis (p<0.05) compared to the control females. The half maximal response occurred at 0.4 nM insulin compared to 0.2 nM in the control females, although this difference was not statistically significant. In SC adipocytes from castrated females, both the basal and the maximal lipogenesis stimulated by insulin was reduced by 50% compared to control females (p<0.05), while the half maximal response did not change and occurred at 0.5 nM of insulin for both castrated and control animals (Fig. 4A and 4C).

Adipocytes from castrated male mice, on the other hand, showed increased lipogenic capacity and increased insulin sensitivity when compared to controls (Fig. 4B and 4D), similar to adipocytes from normal female mice. These effects were observed in both the perigonadal and the subcutaneous depots. For male adipocytes, the half maximal lipogenesis from PG and SC depots occurred at 0.5 and 0.6 nM insulin in controls, whereas in the cells from castrated males the half maximal response occurred at 0.05 nM (p<0.02) and 0.3 nM (n.s) insulin, respectively. Basal lipogenesis was increased 250% (p<0.05) in PG cells and 210% (p<0.05) in SC cells from castrated animals compared to controls, whereas the maximal lipogenesis was increased 200% in PG (p<0.05) and 225% (n.s) in SC cells from castrated males compared to controls.

Quantitative RT-PCR analysis revealed that castration also caused a decrease gene
expression of GLUT1, GLUT 4, FAS and ACC in female adipocytes from both depots when compared to adipocytes from sham operated mice, whereas in male adipocytes the expression of these genes increased in both depots after castration (Fig. 5A). Comparing adipocytes of the PG depot from castrated females to those from sham controls, Glut 4, ACC, FAS and Glut 1 expression were reduced 35% (p<0.05), 55% (p<0.05), 45% (p<0.05) and 44% (p= n.s). Likewise in the SC depot from females, expression of the lipogenic genes ACC and FAS was dramatically reduced, whereas the expression of the glucose transporters 1 and 4 did not change after castration. In the case of the males, PG adipocytes showed a trend to increased expression of Glut 4, ACC and FAS after castration, however these changes were not statistically significant. On the other hand, castration in male mice resulted in a 250-300% increase in Glut 1, FAS and ACC mRNA (p<0.05) in subcutaneous adipocytes (Fig. 5A). Expression of the insulin receptor itself did not change in any of the depots in males nor females after castration (Fig. 5A). Western blot analysis of PG adipocytes from males and females after castration or sham surgery showed changes in agreement with the mRNA levels (Fig. 5B and 5C).

**Effect of castration on body weight, adipose tissue weight, adipocyte size and adipokine levels.** Castration is known to have effects on body weight (15;16). In these experiments, castration was performed at 9 weeks, and the studies done 6 weeks later at 15 weeks of age. At this time, the average weight of the castrated males was 9.8% lower than the sham-operated control males (29.2 ± 2.0 g vs. 26.5 ± 2.9 g) (p=n.s). On the other hand, the average weight of the castrated females was 14.5% higher than the control females (23.4 ± 1.0 g vs. 26.8 ± 2.5 g) (p<0.05). Regarding cell size, we observed that PG adipocytes from male mice were approximately 60% larger that adipocytes from the analogous depot in females (p<0.05). SC adipocytes from males were only 20% bigger than SC adipocytes from females. In females, PG adipocytes were 20% bigger than SC adipocytes, whereas in males, PG adipocytes were 60% bigger than SC adipocytes (p<0.05) (Fig. 6A). After castration, adipocytes in female PG and SC depots increased in size by 70% and 50% respectively (p<0.05). In males, castration did not affect adipocyte size significantly, although there was a trend to decreased cell size in both depots (Fig. 6A).

Similar results were observed in adipose tissue weight. Males PG adipose tissue was double the size of females PG adipose tissue (0.88 ± 0.17g vs. 0.47 ± 0.03 g respectively) (p<0.05). In contrast, males SC adipose tissue was only 25% bigger than females SC adipose tissue (0.63 ± 0.13 g vs. 0.43 ± 0.04 g). In males, the PG fat pad was 33% heavier than the SC inguinal fat pad (p<0.05), whereas in females, these two fat depots were of almost identical size (Fig. 6B). Castration resulted in a doubling of the size of both PG and SC fat pads in females (p<0.05), whereas in males, there was no significant change in either depot weight after castration (Fig. 6B).

Factors that can modulate insulin sensitivity include the adipokines leptin and adiponectin. Serum adiponectin levels were increased in both males and females after castration compared to control mice (p<0.05), with no significant difference between males and females (Fig. 1A supplemental data). Leptin levels tended to be higher in females than in males, and tended to increase after castration in both males and females, but these differences were not statistically significant (Fig. 1B supplemental data).

**Effect of different steroid levels on adipocyte lipogenesis in males and females.** To assess more directly the effect of sex steroids on insulin sensitivity, we measured lipogenic rates on adipocytes from castrated female and male mice (with very low levels
of estrogen or testosterone), control mice (with normal levels of sex steroids) and normal mice implanted for 3 weeks with estrogen or 5α-dihydrotestosterone (DHT) (the most active metabolite of testosterone) pellets. As expected, estradiol and DHT levels were dramatically reduced in castrated animals and increased in implanted ones (Fig. 2 supplemental data). In order to minimize the effects of body weight change after castration, we performed the lipogenesis assays only three weeks after the surgery.

In females, estrogen treatment caused a significant decrease in the lipogenic rates in adipocytes from the PG depot (p<0.005) (Fig. 7A). This was manifest by a 38% decrease in insulin-stimulated lipogenesis in PG adipocytes from estrogen-treated females compared to those from controls (p<0.05). However, the half maximal response occurred at around 0.2 nM insulin, similar to the controls, showing that estrogen treatment does not alter insulin sensitivity. Conversely, short-term castration (STC) decreased lipogenic rates in the perigonadal adipocytes (p<0.01), as seen in the previous experiments. Interestingly, STC did not alter insulin sensitivity in PG adipocytes from females, since half maximal lipogenesis was similar to those from controls, i.e. 0.2 nM insulin (Fig. 7A). In female SC adipocytes, on the other hand, there was no significant change in the lipogenic rates at the high insulin concentrations after castration or estrogen treatment (Fig. 7C). Although castrated females increased their body weight by ~12% (p<0.05) (Fig. 7E), only the PG depot increased in weight (~50%, p<0.05), whereas the size of the SC depot did not change (Fig. 7F). Likewise, females implanted with estrogen pellets showed a significant decrease in weight in the PG fat pad (~35%, p<0.05), but had no change in weight in the SC depot compared to the controls or in total body weight.

In males, short-term castration caused the same effect as the six weeks castration with increased lipogenic rates and increased insulin sensitivity in both depots. In the PG depot, basal and maximal lipogenesis were increased 230% and 171% (p<0.05), and in the SC depot these rates increased by 175% and 153% when compared to controls (p<0.05). Insulin sensitivity also increased in adipocytes from castrated males compared to controls. Half maximal lipogenesis in both depots occurred around 0.1 nM of insulin in adipocytes from castrated males, similar to control female PG fat, whereas in non-castrated controls, half maximal stimulation was at 0.4 (PG) (p<0.001) and 0.5 (SC) (p<0.005) nM insulin (Fig. 7B and 7D). DHT treatment did not cause any change in lipogenic rates or insulin sensitivity in either adipose depot. DHT did cause a non-significant 6% increase in body weight in males with no changes in the adipose tissue weight (Fig. 7E and 7F).

**Effect of sex steroid levels on adiponectin, leptin and free fatty acids.** Changes in the level of sex steroids had variable effects on levels of circulating adipokines. Adiponectin levels tended to increase in castrated females and were decreased by 60% in females implanted with estradiol (p<0.05). In castrated males adiponectin levels were increased (p<0.05), and there was no change in DHT implanted males. Adiponectin levels were similar between males and females. Leptin levels were not changed by any of the treatments in males or females and the levels tended to be higher in females but this difference was not statistically significant. Free fatty acids were not different between males and females and did not change with any of the treatments (Fig. 3 supplemental data).

**DISCUSSION**

Adipose tissue plays a major role in the regulation of glucose homeostasis and insulin
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sensitivity. Interestingly, fat accumulation is sexually dimorphic. Females have a higher percentage of body fat and tend to accumulate more subcutaneous fat than males, whereas males have a lower percentage of body fat and accumulate more visceral fat. Despite the higher level of body fat, female humans and rodents are more insulin sensitive than males. Thus, women have improved glucose tolerance and increased insulin sensitivity compared to men (17;18), and are more resistant to fatty acid-induced insulin resistance (8;19;20). Prevalence of early abnormalities of glucose metabolism are three times higher in men compared to women (7). Likewise, female mice are less prone to diet induced insulin resistance (10;21;22), and many genetically induced forms of insulin resistance have a milder phenotype in females compared to males (11;21;22). Indeed, we find that the increased insulin sensitivity of female versus male mice can be detected on simple intraperitoneal glucose and insulin tolerance testing.

In the present study we have further analyzed gender differences in adipocyte glucose and lipid metabolism as they relate to differences in insulin sensitivity at the cellular level. We find that adipocytes of female mice have increased lipogenic capacity compared to adipocytes from male mice and are also more insulin sensitive, especially those from the intra-abdominal (perigonadal) depot, which are more insulin sensitive than female adipocytes from the SC depot and than male adipocytes from both. This increased lipogenic capacity in female adipocytes is due to at least two factors. One is enhanced insulin sensitivity at intermediate steps in the insulin signaling network such as stimulation of AKT and ERK. Thus, PG adipocytes from female mice show increased phosphorylation/activation of AKT at lower doses of insulin than male adipocytes, and AKT has been shown to be a key intermediate in insulin stimulation of glucose transport (23). The other factor is increased expression of glucose and lipid metabolism genes such as Glut 1, Glut 4, FAS and ACC. Interestingly, despite the increased lipogenic rates, female adipocytes are smaller than male adipocytes, especially those from the PG depot, consistent with studies showing that females adipocytes have increased lipolytic rates compared to those from males (24;25). As a result, in humans females have higher FFA serum levels than males (20), but appear to be protected against insulin resistance induced by elevated FFA (8). However, in mice, we did not detect any significant differences in the FFA serum levels between males and females.

Our results, as well as previous studies (26), demonstrate that female adipocytes have increased insulin sensitivity compared to male adipocytes. This is particularly true for female PG adipocytes, which have a lower EC50 (0.1-0.2 nM insulin) for insulin stimulation of lipogenesis than female SC adipocytes (0.5-0.6 nM) and male adipocytes from either depot (0.4-0.7 nM). Female PG adipocytes have higher lipogenic rate than male adipocytes. Since PG fat in females also has higher lipolytic capacity than in males (25), this would suggest a higher metabolic turnover of PG fat in females leading to decreased fat accumulation in visceral depots in females compared to males.

Sex steroids are known to play a role in the regulation of adipose tissue development and function, as well as whole-body insulin sensitivity. Ovariectomy reduced lipogenic capacity in female adipocytes from both PG and SC depots and reduced insulin sensitivity in female PG adipocytes, indicating a positive role of estrogen in insulin sensitivity and lipogenesis in females. The exact mechanism of this effect is confounded by the fact that castration increases food intake and fat mass in females, and this could contribute indirectly to a reduction in insulin sensitivity.
To differentiate the direct hormonal effects from the indirect effects on body weight, we shortened the period after castration before analysis to three weeks. Under these conditions, gain weight was reduced, and there was no effect of ovariectomy on insulin sensitivity, and a reduced effect on lipogenesis suggesting that body weight is a factor affecting adipocyte insulin sensitivity, although estrogen deficiency may also play a role. Indeed, short-term lack of estrogen following ovariectomy increased PG depot weight and estrogen pellet implantation in normal mice decreased PG depot weight as compared to controls. Interestingly, both of these manipulations affected the SC adipocytes in a similar fashion, while fat pad weight, lipogenic rate and insulin sensitivity remained the same. Thus, an increase in circulating estrogen above the physiological levels preferentially alters PG adipose tissue metabolism, reducing lipogenic rates and depot size. These findings are in agreement with previous reports (27), and consistent with evidence that white adipocytes in different fat depots have fundamentally different properties and may have different developmental lineage (28).

Which estrogen receptor (ER) is mediating these effects is unclear. In females, ER\(\alpha\) expression is similar between depots, whereas we and others found that the levels of ER\(\beta\) are higher in the SC depot (data not shown) (29). Studies with knockout mice of both ER\(\alpha\) or ER\(\beta\) suggest that the effects of estrogen in the adipose tissue are mediated mainly through ER\(\alpha\) (30-32). The preferential effect of estrogen on adipocyte metabolism in the PG depot in females might also involve differences in co-activators, such as the steroid receptor coactivator (SRC) p160 family of proteins (33).

In male mice, androgen receptor expression is higher in PG than SC fat (data not shown). Both short term and long term castration in males resulted in increased insulin sensitivity and increased lipogenesis in both PG and SC depots. This was independent of changes in the adipose tissue weight. Thus, in contrast to the preferential effect of estrogen in females on the PG depot, testosterone exerts an inhibitory effect on lipogenesis and insulin sensitivity in both PG and SC adipocytes. These results are the opposite to what happens in the androgen receptor KO mice, which develop late-onset obesity with reduced energy expenditure (34).

In summary, our results demonstrate an important role for sex steroids and gender in modulating adipose mass and insulin sensitivity. Thus, female adipocytes have increased lipid synthesis compared to male adipocytes in both the PG and SC depots and in the female PG adipocytes also have improved insulin sensitivity to lipogenesis and to insulin signaling. These contribute to whole body insulin sensitivity, allowing female mice to remain insulin sensitive despite similar or higher fat mass.

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Figure 1. Female mice and female adipocytes have increased insulin sensitivity. Glucose tolerance test was performed after and overnight fast. Mice received intraperitoneal injection of 2g/kg body weight glucose. Blood glucose was measured from tail vein samples at the indicated times (A). Insulin tolerance test was performed on random fed mice. Animals received injections of 1U/kg body weight insulin. Blood glucose was measured at the indicated time points (B). Data points are mean + SE with 8 mice in each group. Adipocytes from perigonadal and subcutaneous fat were isolated from male and female mice and stimulated with or without insulin in the presence of $^{14}$C-glucose for 90 minutes as described in Methods. Radioactive glucose incorporated into lipids was measured for each condition in the extracted lipids and normalized to total lipid content (C) or to cell number (D) as determined by counting of osmic acid fixed cells. n=3. **p<0.01 by repeated measures ANOVA. *p<0.05 by student’s t-test for point to point comparisons.
Figure 2. Increased insulin stimulated-AKT and ERK activation in female adipocytes. Adipocytes from perigonadal fat isolated from male and female mice were treated with or without 10 nM (A) or 0.1 nM (B, C) insulin for 5 (A, B, C) or 30 minutes (C). Cell lysates were run on SDS-PAGE and subjected to Western blot analysis using antibodies directed against phosphorylated (pAKT Ser473, pERK Thr202/Tyr204) or total AKT and ERK. Each lane represents a pool of adipocytes from 3-4 mice. The data were also quantified by densitometric scanning of the autoradiographs. (n=2) *P< 0.05 fold stimulation females vs males.
Figure 3. Female adipocytes have increased mRNA expression of lipid and glucose metabolism genes. Adipocytes from perigonadal (PG) and subcutaneous (SC) fat isolated from male and female mice were lysed by homogenization using a denaturing guanidine-thiocyanate containing buffer, mRNA was extracted and quantitative real-time PCR was performed (A). Gene expression for Glut1, Glut4, FAS and ACC mRNAs were normalized against the expression of TATA-binding protein (TBP). (B) Proteins from similar adipocyte isolates were extracted and subjected to SDS-PAGE and Western blotted with antibodies to each of these proteins. Actin was used as a control for protein loading. Each lane represents a pool of adipocytes from 3-4 mice. (C) The data were quantified by densitometry of immunoblots (n=3) and normalized against actin. *P<0.05
Figure 4. Castration reverses lipogenic rates in male and female adipocytes. Male and female mice were castrated or sham operated. Six weeks after surgery, adipocytes from PG and SC fat were isolated and stimulated with or without insulin in the presence of $^{14}C$-glucose for 90 minutes. Radioactivity was measured in the extracted lipids and normalized by total lipid content. n= 6 animals per group. **p<0.05 by repeated measures ANOVA. *p<0.05 by student’s t-test for point to point comparisons sham vs castrated.
Figure 5. Castration reverses gene expression patterns in male and female adipocytes. Male and female mice were castrated (C) or sham (S) operated. Six weeks after surgery, adipocytes from PG and SC fat were isolated, mRNA was extracted and quantitative real time PCR was performed (A). Gene expression for metabolic genes was assessed and normalized against the expression of TATA-binding protein (TBP). (B) Proteins from similar cells were subjected to SDS-PAGE and Western blot with the respective antibodies. Actin was used as a control for protein loading. (C) Quantitation of the proteins was achieved by densitometry normalized by the values for actin. *P< 0.05
Figure 6. Effect of castration on adipocyte size and adipose tissue weight. Male and female mice were castrated (C) or sham operated (S). Six weeks after surgery total body weights and adipose tissue weights from PG and SC fat were assessed, and adipocytes were isolated as described in Methods. Mean cell size was calculated by dividing the total lipid content by the total number of cells on a sample. Graphs show the mean adipocyte size (A) or adipose tissue weight (B) from 6 animals per group *P< 0.05
Figure 7. Effect of short-term castration and steroid treatment on lipogenesis, body weight and adipose tissue weight in males vs females. Male and female mice were castrated, implanted with 17β-estradiol or 5α-DHT pellets or sham operated. 3 weeks after surgery adipocytes from PG (A,B) and SC (C,D) fat were isolated and stimulated with or without insulin in the presence of 14C-glucose for 90 minutes. Radioactivity was measured in the extracted lipids and normalized by total lipid content. (E) Total body weight and (F) adipose tissue weights from PG and SC fat from sham operated (Sham or S) castrated (Cast or C), implanted with estrogen-containing pellets (E) or DHT-containing pellets (T) were assessed. The graphs show the mean from 8 animals per group. **P<0.05 by repeated measures ANOVA. *p<0.05 by student’s t-test for point to point comparisons.