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Programmed Regulation of Rat Offspring Adipogenic Transcription Factor (PPARγ) by Maternal Nutrition

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Abstract

We determined the protein expression of adipogenic transcription factor, peroxisome proliferator-activated receptor gamma (PPARγ) and its co-repressor and co-activator complexes in adipose tissue from the obese offspring of under- and over-nourished dams. Female rats were fed either a high fat (60% kcal) or control (10% kcal) diet prior to mating, and throughout pregnancy and lactation (Mat-OB). Additional dams were 50% food-restricted from pregnancy day 10 to term (intrauterine growth-restricted; IUGR). Adipose tissue protein expression was analyzed in newborn and adult male offspring. Normal birth weight Mat-OB and low birth weight IUGR newborns had upregulated PPARγ with variable changes in co-repressors and co-activators. As obese adults, Mat-OB and IUGR offspring had increased PPARγ with decreased co-repressor and increased co-activator expression. Nutritionally programmed increased PPARγ expression is associated with altered expression of its co-regulators in the newborn and adult offspring. Functional studies of PPARγ co-regulators are necessary to establish their role in PPARγ-mediated programmed obesity.

Keywords

Peroxisome proliferator-activated receptors; co-activator proteins; co-repressor proteins; maternal obesity; maternal under-nutrition

Introduction

The contribution of developmental programming resulting from perturbed maternal nutrition to the obesity epidemic is well recognized. Evidence from epidemiological studies and

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animal models indicate that maternal overnutrition (e.g. obesity and high fat diet) as well as maternal under-nutrition is associated with increased risk of offspring obesity. Notably in humans, both low and high birth weight lead to increased risk for childhood and adult obesity, suggesting increased risk of obesity at both ends of the birth weight spectrum.[1] Animal models have replicated human epidemiologic findings and have identified adipose tissue as one of the principal targets contributing to offspring obesity.[2,3]

Adipogenesis involves adipocyte differentiation, lipogenesis and lipid accumulation within the adipocytes.[4] Adipocyte differentiation is triggered by a set of interacting transcription factors, the peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer binding proteins (C/EBPβ, C/EBPα).[5] In particular, C/EBPs activate the principal adipogenic transcription factor, PPARγ, leading to terminal adipocyte differentiation.[5] Activated PPARγ induces lipogenic transcription factor sterol regulatory element binding protein (SREBP1)[6] and SREBP1, in turn, induces the expression of lipolytic (lipoprotein lipase) and lipogenic (fatty acid synthase) enzymes, which modulate fatty acid uptake and synthesis.[7] In addition, the lipolytic enzyme, hormone-sensitive lipase, hydrolyzes intracellular triglycerides and enables fatty acid release from adipocytes.[8] The transcriptional activity of PPARγ is modulated by select co-repressors, (SIRT1, sirtuin; NCoR, nuclear receptor co-repressor; SMRT, silencing mediator for retinoid and thyroid hormone receptor)[9] and co-activators (p160 family members: SRC1, steroid receptor co-activator 1; TIF2, transcriptional intermediary factor 1).[10]

Adipogenesis occurs in both the prenatal and postnatal states, though there is now convincing evidence that adipogenesis occurs throughout the lifetime. In rodents, white adipose tissue develops mainly after birth. In humans, white adipose tissue development begins early in the second trimester of gestation and by birth is well developed in both the visceral and subcutaneous depots.[11,12]

We have established rat models of maternal obesity and maternal food-restriction that result in programmed offspring obesity.[13,14] Maternal obesity results in normal birth weight newborns that exhibit early (neonatal) onset of obesity (Mat-OB), hypertriglyceridemia and insulin resistance.[13] In contrast, maternal food-restriction results in growth restricted newborns (IUGR) that develop later (adult) obesity as well as hypertriglyceridemia and insulin resistance.[14] In the IUGR offspring, we have demonstrated that the obesity is, in part, a result of increased adipogenesis and lipogenesis with increased expression of the adipogenic transcription factor PPARγ that is evident in newborn and adult offspring.[15,16] Importantly, we have shown functional changes in lipogenesis of increased adipose tissue de novo synthesis and desaturase activity in IUGR and Mat-OB male newborns.[17,18] In addition, using primary cell cultures we have demonstrated increased preadipocyte proliferation, increased adipocyte lipid content and increased adipocyte glucose uptake in IUGR males at ages, 1 day and 3 weeks.[19]

We hypothesized that programmed Mat-OB offspring obesity was similarly associated with increased expression of PPARγ. In view of the increased adipogenic factors in IUGR offspring, we sought to determine if concomitant alterations in PPARγ co-regulator expression accompanied increased PPARγ expression.

Methods

Animals and Diet

Studies were approved by the Animal Research Committee of the Los Angeles BioMedical Research Institute at Harbor-UCLA (LABioMed), and were in accordance with the American Association for Accreditation of Laboratory Care (AALC), and National Institutes of Health (NIH) guidelines. Both rat models have been previously described.[13,14] Briefly, Sprague Dawley rats (Charles River Laboratories, Hollister, California) were housed with a 12 hours day / night cycle with lights on at 6:00 am in a temperature (21±1°C) controlled room. At weaning the rat pups were housed 4 per cage. The animals were separated to two per cage at 125 g and into singular housing when their weight was above 250 g, such that no cage contained more than 500 g of total rat body weight. All rats were housed in polycarbonate cages (Ancar Corp; Bellmore, NY) filled with paperchip bedding (Sherpherd Specialty Papers; Watertown, TN) enriched with red plastic boxes and nylabones for enrichment.

Mat-OB Model

Weanling female rats were fed *ad libitum* diet with either a normal fat (10% kcal fat, Research Purified Diet 12450B58Y2, New Brunswick, NJ; Control n=6) or a high fat (60% kcal, Purified Diet 1249258Y1, New Brunswick, NJ; Mat-OB; n=6) content. At 11 weeks of age, rats were mated and continued on their respective diets during pregnancy and lactation.

IUGR Model

First time pregnant rats (~11 weeks of age) were fed *ad libitum* diet of standard laboratory chow (Lab Diet 5001, Brentwood, MO) until gestation day10, at which time they were either continued on *ad libitum* diet (Control; n=12) or provided 50% food-restricted diet determined by quantification of normal intake in Controls (IUGR; n=6) until term.

In both models, at day 1 after birth, litter size was reduced to 4 males and 4 females to standardize lactation. Individual body weights were recorded. To allow catch-up growth, all IUGR newborns were cross-fostered to Control dams fed *ad libitum* during pregnancy and lactation, and all Control and Mat-OB pups were cross-fostered to dams of the same diet to normalize the study design. All offspring were weaned to the diet consumed by their respective controls (Mat-OB to normal fat diet 12450B58Y2 and IUGR to standard laboratory chow diet 5001) at 3 weeks of age. Since estrogen is known to affect adiposity and lipid metabolism[20], we elected to study male offspring as females would have required estrus assessment.

Body composition

At 9 months of age, 1 male from each of 6 different litters in each group (n=6) were anesthetized using Ketamine (90 mg/kg i.p.) and Xylazine (10 mg/kg, i.p.) and placed in a micro-isolator cage with warm water bottles to avoid hypothermia. Rats underwent a short (1–2 min) non-invasive dual energy X-ray absorptiometry (DEXA) scan (software program for small animals; QDR 4500A; Hologic, Bedford, MA) to obtain an in vivo scan of body composition.

Plasma Analysis

At 9 months of age, one male from each litter was fasted overnight and blood was collected via cardiac puncture in heparinized tubes for analysis. Plasma insulin was measured using rat specific commercial radioimmunoassay kits (insulin RIA kit, LINCO Research Inc., St. Charles, MO). Plasma triglycerides were measured using Raichem Enzymatic Reagents (Cat No. 80008, Raichem, Inc., San Diego, CA) with control serum level 1 (#83082) and control serum level 2 (#83083) and run on an automated Cobas-Mira Chemistry Analyzer (Roche Diagnostic Systems Inc., Sommerville, NJ). Blood glucose determined using Hemocue B-Glucose Analyzer (HemoCue Inc, Mission Viejo, CA).

Adipose Tissue Analysis

At day one after birth, adipose tissue was collected from the pups that were culled to standardize the litter size. Newborns were decapitated and inguinal (subcutaneous) adipose tissue was pooled from 4 males per litter. At 9 months of age, retroperitoneal (visceral) adipose tissue, which is associated with obesity and insulin resistance[21,22] was collected and samples frozen in liquid nitrogen and stored at −80°C till protein analysis. To control for litter effects in the adult studies, one male was studied from each of the litters. Thus when n = 6, it represents one male from each of 6 different litters in Control, IUGR and Mat-OB groups.

Protein was extracted in radioimmuno precipitation assay (RIPA) buffer that contained protease inhibitors (HALT cocktail, Pierce). Supernatant protein concentration was determined by BCA solution (PIERCE, Rockford, IL). Protein expression was determined by Western Blotting, as previously described.[15] Antibodies were obtained from Santa Cruz, CA unless otherwise specified and the band density was analyzed as indicated: PPARγ (57 kDa; Thermo Scientific, Rockford, IL), C/EBPα (42 kDa), SREBP1c (125 kDa), FAS (270 kDa), hormone sensitive lipase (84 kDa), SIRT1 (120 kDa), NCoR (270 kDa), SMRT (160 kDa), SRC1 (160 kDa), TIF2 (158 kDa) and β-actin (Sigma, A-5441; 40 kDa).

Statistical Analysis

Differences between Mat-OB/IUGR and Control groups were compared using unpaired Student's T-test. Protein values were normalized to β-actin, which showed no differences between groups at 1 day or 9 months of age. Values are presented as fold change (mean ± SE).

Results

Body Weight, Body Fat and Food Intake

As previously reported,[13,14] Mat-OB dams had higher body weights at mating, and throughout pregnancy and lactation as compared to Control dams. Further, the total food intake was similar among the two groups of dams with Mat-OB receiving greater percentage of kilocalories via fat. As intended, food-restricted dams had consistently lower body weight.

At 1 day of age, IUGR pups had reduced body weight (6.8 ± 0.2 *vs*. 7.2 ± 0.2 g, P <0.01) whereas Mat-OB offspring had similar body weight (7.4 ± 0.2 *vs*. 7.3 ± 0.1 g) to the Controls. Both Mat-OB and IUGR offspring had increased food intake and corresponding increased weight gain compared with their respective controls, both during lactation and throughout the post-weaning period.[13,14] Subsequently, at 9 months of age, both Mat-OB and IUGR male offspring weighed significantly more than did their respective Controls (Mat-OB: 875 ± 27 *vs*. 660 ± 20 g, P <0.001; IUGR: 742 ± 15 *vs*. 647 ± 18 g, P <0.001). This was reflected by greater percentage of body fat in both Mat-OB (30.7 ± 2.1 *vs*. 15.9 ± 1.7 %, P <0.001) and IUGR (20.3 ± 1.6 *vs*. 12.4 ± 1.5 %, P <0.001) offspring compared with their respective Controls.

Metabolic Profile

At 9 months of age, both Mat-OB and IUGR male offspring had significantly higher levels of blood glucose, plasma insulin and plasma triglycerides as compared to their respective Controls (Table 1).

Protein Expression of Adipogenic and Lipid Factors

Mat-OB newborns at 1 day of age had increased adipose tissue protein expression of adipogenic transcription factors PPARγ (2-fold) and C/EBPβ (~1.5-fold), whereas C/EBPα was decreased compared with Controls (Figure 1A). In addition, Mat-OB newborns demonstrated increased protein expression of lipogenic factors (SREBP1, 1.5-fold; fatty acid synthase, 3-fold) as well as intracellular lipolytic enzyme (hormone-sensitive lipase, 1.8-fold) though with unchanged lipoprotein lipase compared with Controls (Figure 1B). At 9 months of age, Mat-OB males showed persistent increased expression of PPARγ (2.5-fold), SREBP1 (3-fold) and fatty acid synthase (4-fold) and decreased expression of C/EBPα (0.6-fold) and both lipases (~0.5-fold; Figures 2A and B).

IUGR newborns, as previously reported,[14] had increased protein expression of adipogenic transcription factors (PPARγ, 2.3-fold; C/EBPβ, 2.1-fold; C/EBPα,2.3 −fold), however the expression of SREBP1, hormone-sensitive lipase and fatty acid synthase were similar in IUGR and Control newborns.[14] At 9 months of age, IUGR males continued to exhibit increased protein expression of PPARγ (1.8-fold) and C/EBPβ (2.1-fold), but not C/EBPα (1.3-fold) compared with Controls, as well as increased expression of SREBP1 (1.7-fold) and lipid enzymes (hormone sensitive lipase, 6.4-fold; fatty acid synthase, 1.8-fold).[14]

Protein Expression of PPARγ Co-regulators

At 1 day of age, Mat-OB newborns had decreased protein expression of all co-repressors (SIRT1, NCoR, SMRT) and the co-activator SRC1 (Figure 3A). TIF2 expression was unchanged (Figure 3B). At 9 months of age, Mat-OB males continued to show reduced levels of co-repressor protein (SIRT1, NCoR, SMRT), though with increased protein expression of co-activators (SRC1, TIF2; Figures 4A and B).

At 1 day of age, IUGR males exhibited increased expression of co-repressors SIRT1 and SMRT though with decreased expression of NCoR (Figure 3A). The co-activator SRC1 was increased, whereas TIF2 expression was unchanged (Figure 3B). At 9 months of age,

expression of SIRT1 and SMRT was decreased, but NCoR expression was paradoxically increased in IUGR males (Figure 4A). In addition to increased TIF2, SRC1 expression continued to be increased (Figures 3B and 4B).

Discussion

Despite divergent maternal nutrient exposure and intrauterine growth, offspring exposed to maternal obesity and maternal under-nutrition develop obesity, insulin resistance and hypertriglyceridemia. In the current study, even though the Mat-OB newborns were normal birth weight, they had increased protein expression of the adipogenic transcription factor PPARγ, as well as its upstream regulatory transcriptional factor, C/EBPβ. Concomitant with this, Mat-OB newborns exhibited increased lipogenic potential, as indexed by increased SREBP1 and fatty acid synthase that persisted in adult offspring. However, both lipase enzymes were decreased in Mat-OB adult offspring, suggesting decreased adipocyte uptake and release of fatty acids. Although IUGR newborns had lower birth weight, they too exhibited programmed upregulation of adipogenic signaling cascade. We have previously demonstrated that these changes persist in adult offspring who also show evidence of increased lipogenesis and adipose tissue lipases, indicating enhanced fatty acid uptake, synthesis and release from adipocytes.[15]

Unlike IUGR offspring, Mat-OB newborns, as well as adult offspring, had significantly decreased C/EBPα expression compared with Controls. Although C/EBPα is known to promote adipocyte differentiation,[23] it does so only in the presence of PPARγ, whereas PPARγ can induce adipogenesis in absence of C/EBPα .[24] Accordingly, C/EBPα deficient cells are capable of adipocyte differentiation, though these cells are insulin resistant.[25] Consistent with these data, although C/EBPα expression was decreased in Mat-OB offspring, they had increased adipocyte differentiation and insulin resistance.[13]

The protein expression of both hormone-sensitive lipase, which regulates the release of intracellular fatty acids, and lipoprotein lipase, which controls the entry of exogenous fatty acids into adipose tissue, were decreased in the adult Mat-OB offspring, in contrast to increased expression of these lipases that occurred in adult IUGR offspring.[14] Hormone-sensitive lipase levels increase under conditions of starvation[26] and decrease in obese insulin-resistant state.[27] Similarly, increased lipoprotein lipase activity is associated with obesity[28] and reduced/deficient levels lead to hypertriglyceridemia with increased up-regulation of *de novo* fatty acid synthesis.[29] Thus, reduced expression of both lipases may contribute to the insulin resistance, hypertriglyceridemia, decreased release of fatty acids from the adipocytes and increased *de novo* fatty acid synthesis observed in Mat-OB offspring.[13,18]

Despite the marked difference in nutrient environments and differing birth weights, both Mat-OB and IUGR offspring had enhanced adipogenesis and lipogenesis with upregulated PPARγ expression that was evident at birth. Regardless of the increased PPARγ expression, the co-regulator proteins were differentially expressed. Overall, in Mat-OB newborns the co-regulators were suppressed, whereas in IUGR newborns they were increased. Notably, in both Mat-OB and IUGR adults, there was predominantly decreased expression of co-

repressor proteins and increased expression of co-activator proteins, consistent with increased expression of PPARγ and their obese phenotype.

Reduced NCoR is associated with greater insulin sensitivity, as demonstrated by adipocyte specific NCoR knockout mice.[30] In IUGR offspring, the changes in NCoR expression are consistent with their glucose/insulin status – newborns with reduced NCoR and increased insulin sensitivity and adults with increased NCoR and an insulin resistant phenotype.[14,31] In Mat-OB offspring, changes in NCoR expression are more consistent with their obese phenotype rather than their insulin resistant phenotype.

Consumption of a high-fat diet is associated with increased expression of SRC1 and TIF2 in adipose tissue, as well as an increase in the ratio of TIF2/SRC1 expression, which may contribute to weight gain.[32] In the present study, Mat-OB newborns show decreased SRC1 expression, while IUGR newborns had increased SRC1. Accordingly, Mat-OB newborns had an increased ratio of TIF2/SRC1 expression (~2) and we have previously shown that they exhibit accelerated weight gain during the nursing period.[13] Notably, SRC1 also regulates the expression and the transcriptional activity of C/EBPα gene,[33] and C/EBPα in turn, regulates SIRT1 expression during adipogenesis.[34] This is consistent with our findings of decreased expression of SRC1, C/EBPα and SIRT1 in Mat-OB newborns, and increased SRC1, C/EBPα and SIRT1 in IUGR newborns.[15]

In both IUGR and Mat-OB offspring, the co-activator TIF2 was unchanged in the newborns, whereas it was upregulated in adulthood, suggesting that increased TIF2 expression may not be developmentally programmed, but rather may occur secondary to increased fat storage in obese adult offspring. This is consistent with increased lipid accumulation seen in 3T3-L1 cell line that had overexpressed TIF2, and importantly, this occurred only in the presence of PPARγ.[32] Further supporting evidence emerges from TIF2 knockout mice that have lower PPARγ, reduced potential for fat storage and are protected against obesity when fed a high fat diet.[32]

The regulation of PPARγ transcription is not only dependent upon the presence of co-regulators[9,10] but is also modulated by energy status,[35] and the availability of PPARγ ligands.[36,37] In newborn offspring, the variable effects of nutritional programming on PPARγ co-regulator proteins may be attributed to the availability of PPARγ ligands. In the absence of its ligand, PPARγ enlists co-repressors[37] whereas upon ligand binding to PPARγ, the co-repressors are released and co-activators recruited.[38,39] Unsaturated fatty acids, including palmitoleate and oleate are natural ligands for PPARγ,[40,41] and we have previously shown that the plasma palmitoleate and oleate levels are higher in IUGR newborns[17] whereas Mat-OB newborns have lower palmitoleate levels.[18] We speculate that changes in the availability of plasma fatty acid ligands may affect the recruitment of PPARγ co-activator and co-repressor proteins that activate gene expression and drive adipogenesis.

Increased PPARγ levels are seen in adipose tissue of obese animals and humans.[42–44] Further higher levels are associated with high fat diet and decreased levels occur during fasting.[38] Specifically, PPARγ expression is downregulated in obese humans on a low calorie diet[45] and obese rodents undergoing exercise.[46,47] In addition, SIRT1 which

represses PPARγ,[9] is also a nutrient sensor that increases in response to under-nutrition with reduction in fat storage and an increase in lipolysis.[9,48]. The downregulated SIRT1 in Mat-OB offspring born to overnourished dams in the current study is consistent with their exposure to the maternal high-fat diet, and likely contributes to the increased expression of PPARγ in these animals. Although the upregulated SIRT1 in IUGR offspring at birth is also consistent with its role as a nutrient sensor, the mechanism underlying the failure of SIRT1 to suppress PPARγ in these animals is presently unknown. The effect of modulating co-regulators of PPARγ on adipogenesis and lipid storage has been effectively demonstrated using 3T3 cells as stated above, wherein increased expression of co-activators and decreased expression of co-repressors induce fat storage.[32,49]

In conclusion, our studies show that despite the divergent nutritional environment, Mat-OB offspring demonstrate enhanced adipogenesis akin to IUGR newborns. Mat-OB newborns, unlike IUGR, showed early induction of lipogenic factors likely contributing to early onset of adiposity and associated metabolic abnormalities, which exacerbate with age.[13] Thus both under- and over-nutrition programs increased adipogenesis. Although the underpinning contributory factor in both models is attributed to upregulated PPARγ, it may be mediated via different mechanisms. Differential recruitment of co-activators and co-repressors in IUGR and Mat-OB may provide the basis for a transcriptional switch that controls adipocyte differentiation and lipogenesis. However, further functional studies are required to elucidate the relative roles of different co-regulator proteins in the activation of PPARγ in nutritionally programmed obesity.

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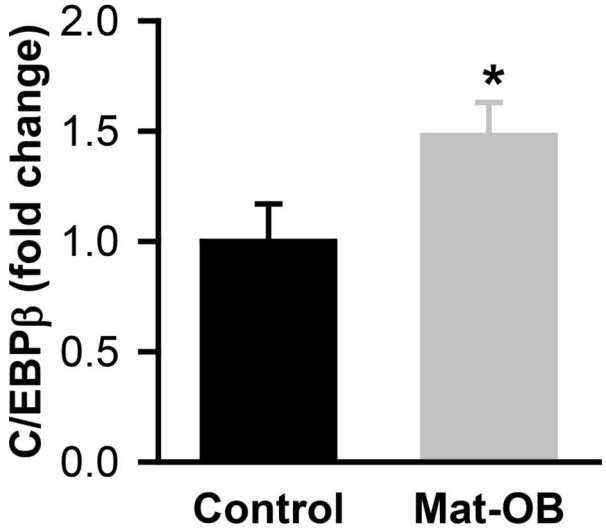
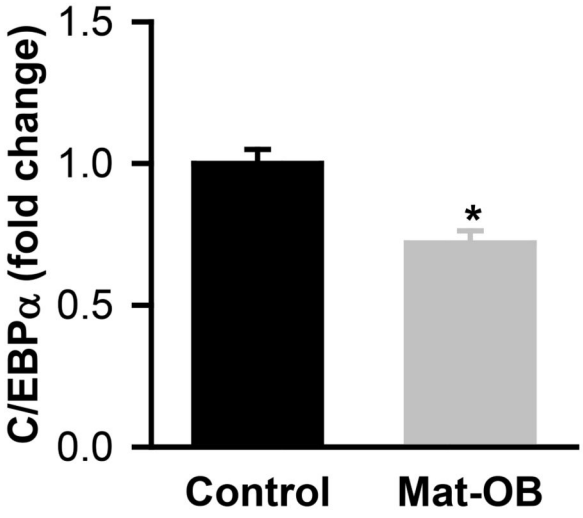
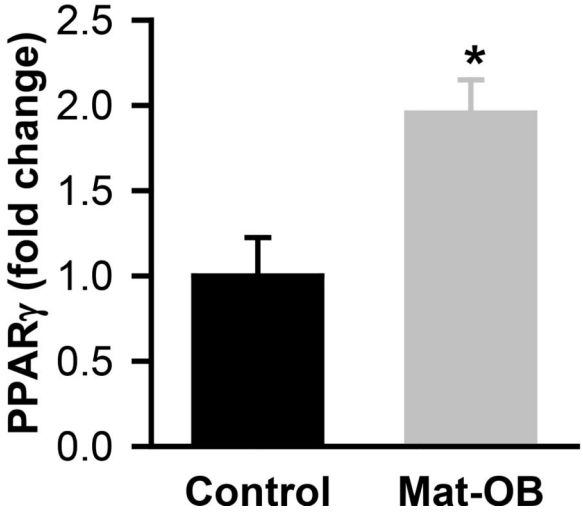
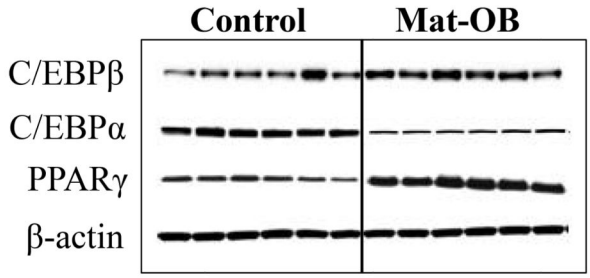
A: Adipose Tissue Adipogenic Factors in Newborn Male Offspring









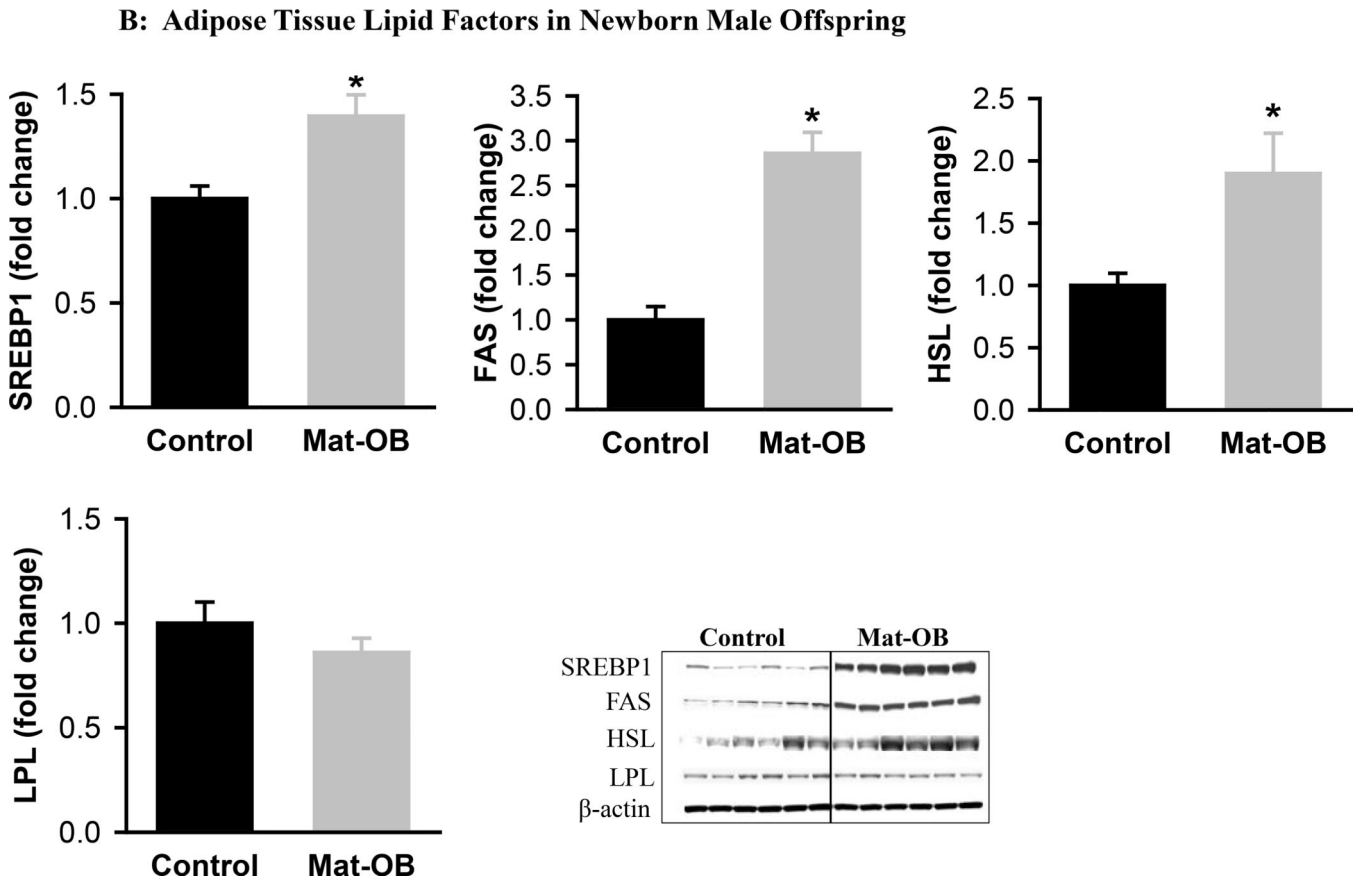
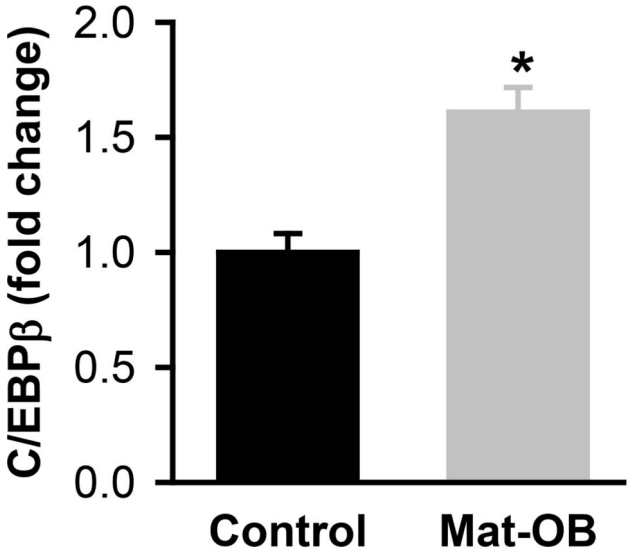
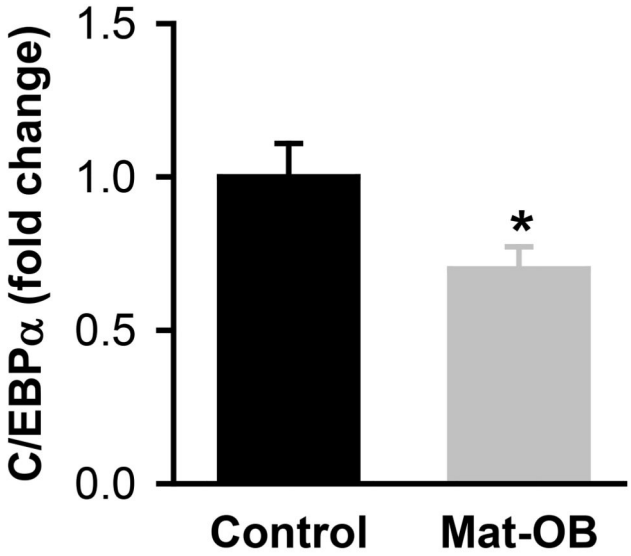


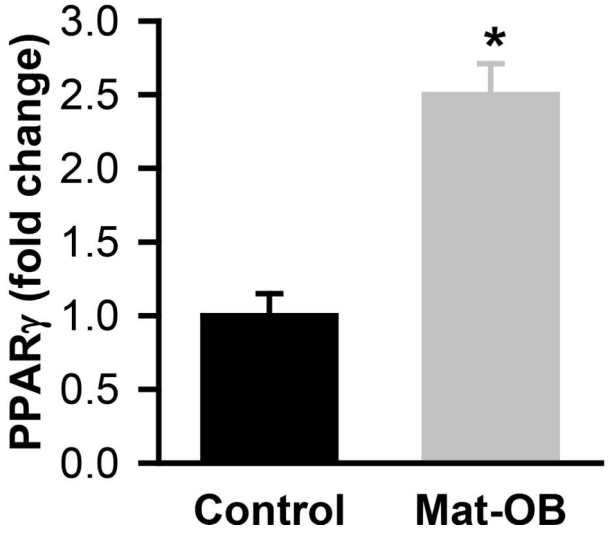
Figure 1. Adipose Tissue Adipogenic and Lipid Factors in Newborn Male Offspring

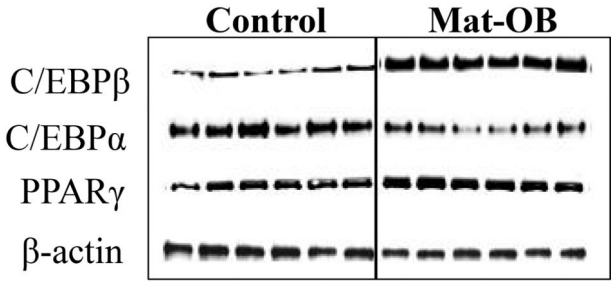
Protein expression of (A) peroxisome proliferator-activated receptor (PPARγ) and CCAAT/enhancer binding protein family members (C/EBPβ and C/EBPα) and (B) sterol regulatory element binding protein (SREBP1), fatty acid synthase (FAS), hormone sensitive lipase (HSL) and lipoprotein lipase (LPL) in Control (■) and Mat-OB (■) newborn males. Data are normalized to β-actin and presented as fold change. Adipose tissue was studied from pooled male samples from each of the 6 litters per group. *P < 0.01 *vs*. Control offspring.

A: Adipose Tissue Adipogenic Factors in Adult Male Offspring









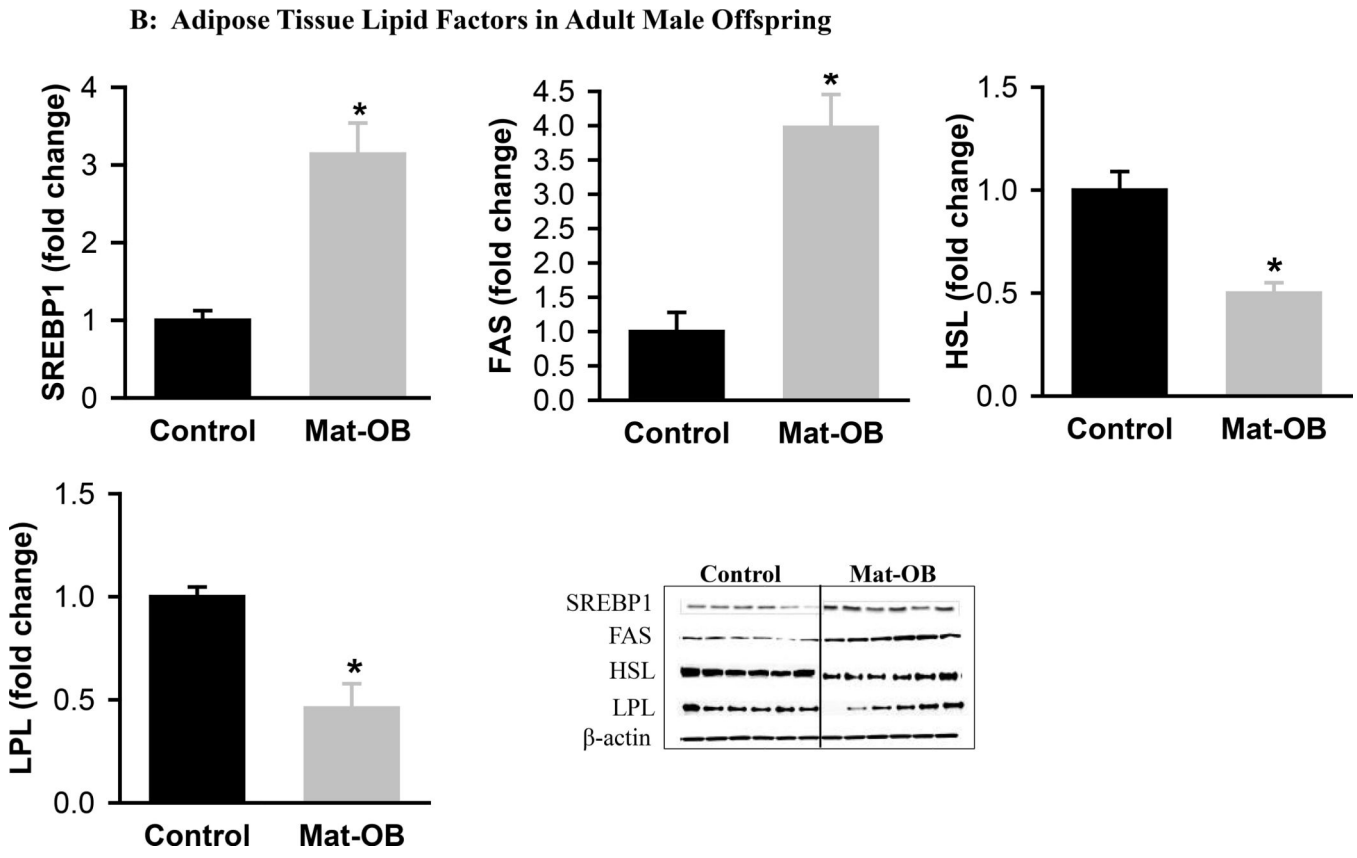
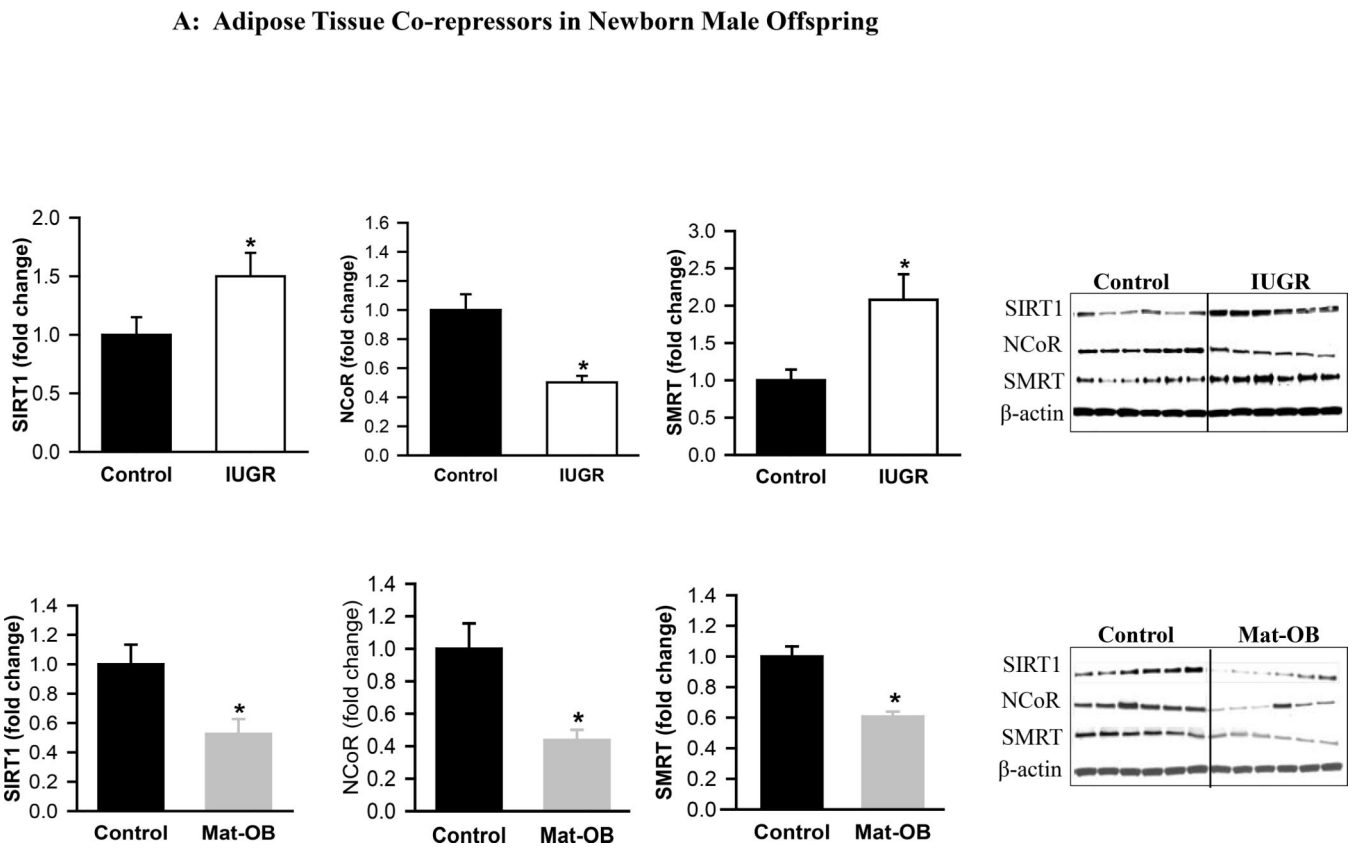


Figure 2. Adipose Tissue Adipogenic and Lipid Factors in Adult Male Offspring

Protein expression of (A) peroxisome proliferator-activated receptor (PPARγ) and CCAAT/enhancer binding protein family members (C/EBPβ and C/EBPα) and (B) sterol regulatory element binding protein (SREBP1), fatty acid synthase (FAS), hormone sensitive lipase (HSL) and lipoprotein lipase (LPL) in Control (■) and Mat-OB (■) adult males. Data are normalized to β-actin and presented as fold change. Number of animals studied was 1 male from each of 6 litters per group. *P < 0.001 *vs*. Control offspring.



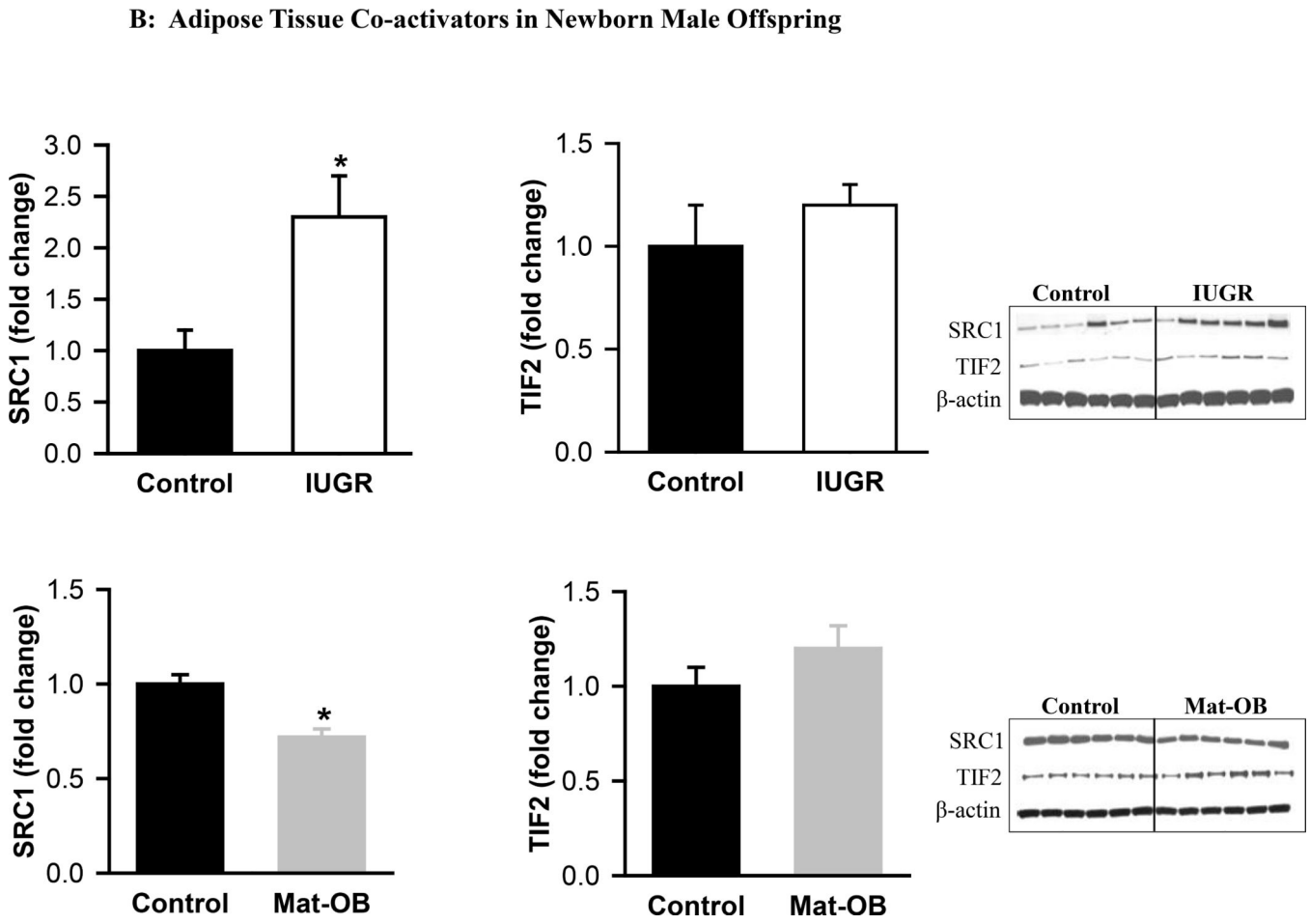
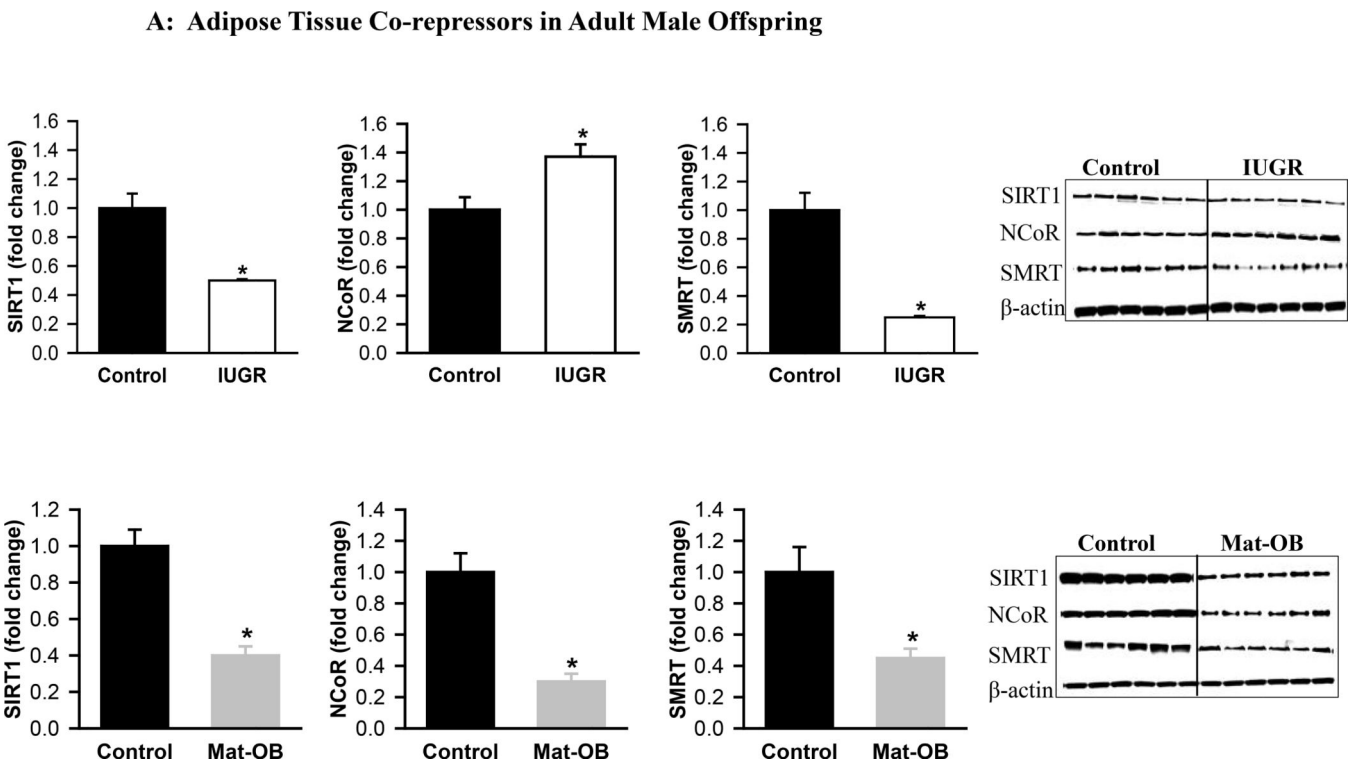


Figure 3. Adipose Tissue Co-repressors and Co-activators in Newborn Male Offspring

Protein expression of (A) co-repressor proteins Sirtuin (SIRT1), NCoR (nuclear receptor co-repressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptor) and (B) co-activator proteins SRC1 (steroid receptor co-activator 1) and TIF2 (transcriptional intermediary factor 1) in Control (■), IUGR (□) and Mat-OB (▩) newborn males. Data are normalized to β-actin and presented as fold change. Adipose tissue was studied from pooled male samples from each of the 6 litters per group. *P < 0.001 *vs*. Control offspring.



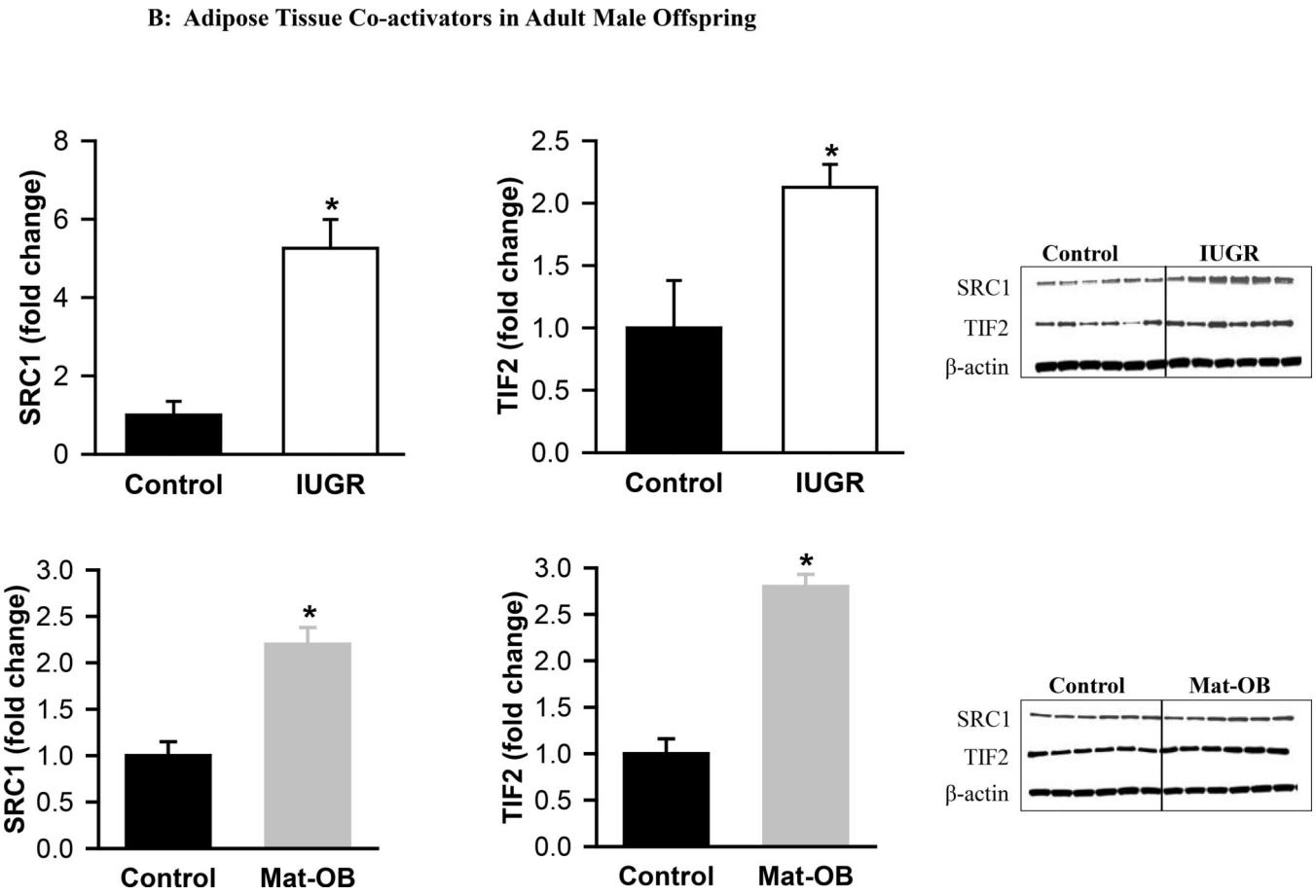


Figure 4. Adipose Tissue Co-repressors and Co-activators in Adult Male Offspring

Protein expression of (A) co-repressor proteins Sirtuin (SIRT1), NCoR (nuclear receptor co-repressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptor) and (B) co-activator proteins SRC1 (steroid receptor co-activator 1) and TIF2 (transcriptional intermediary factor 1) in Control (■), IUGR (□) and Mat-OB (■) adult males. Data are normalized to β-actin and presented as fold change. Number of animals studied was 1 male from each of 6 litters per group. *P < 0.001 *vs*. Control offspring.

Table 1

Metabolic Profile of Adult Males

Adult	Glucose (mg/dl)	Insulin (ng/ml)	Triglyceride (mg/dl)
Control	104 ± 2	0.62 ± 0.05	52 ± 6
IUGR	115 ± 3**	0.83 ± 0.08*	97 ± 8***
Control	97 ± 3	0.69 ± 0.04	85 ± 8
Mat-OB	116 ± 4**	1.90 ± 0.09**	126 ± 12***

Blood glucose and plasma triglyceride and insulin in overnight fasted 9 month old Control, IUGR and Mat-OB males.

Values are Mean ± SE.

* P <0.05;

** P < 0.01;

*** P < 0.001 *vs*. respective Controls.