Sexual Differentiation, Pregnancy, Calorie Restriction, and Aging Affect the Adipocyte-Specific Secretory Protein Adiponectin

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Adiponectin or adipocyte complement–related protein of 30 kDa (Acrp30) is a circulating protein produced exclusively in adipocytes. Circulating Acrp30 levels have been associated with insulin sensitivity in adult mice and humans, yet the Acrp30 profile over the lifespan and its hormonal regulation in vivo have not been previously described. Hence, we set forth to determine whether hormonal and metabolic changes associated with sexual maturation, reproduction, aging, and calorie restriction affect Acrp30. In mice, Acrp30 levels increase during sexual maturation by 4-fold in males and 10-fold in females. Neonatal castration (CX) allows Acrp30 of adults to reach female levels. CX in adults does not lead to female Acrp30 levels unless glucocorticoid exposure is elevated simultaneously by implant. Ovariectomy of infant mice does not interfere with the pubertal rise of Acrp30. However, ovariectomy in adults increases Acrp30. Estrogen suppressed Acrp30 in mice and 3T3-L1 adipocytes. In parallel to changes in estrogen action, Acrp30 decreased in late gestation but increased in both calorie-restricted and old (anovulatory) mice. The reduction of Acrp30 in lactating dams is consistent with a suppressive effect of prolactin and a stimulating effect of bromocriptine. In summary, Acrp30 levels in serum are under complex hormonal control and may play a key role in determining systemic insulin sensitivity under the respective conditions. Diabetes 52:268–276, 2003

A adipose tissue secretes several circulating proteins that appear to effect carbohydrate and lipid metabolism (1). Elevated levels of some of these factors, including tumor necrosis factor-α (2), plasminogen-activator inhibitor-1 (3), angiotensinogen (4), and the recently identified protein resistin (5), are associated with insulin resistance. On the other hand, replacement of fat-specific proteins like leptin or adipocyte complement–related protein of 30 kDa (Acrp30)/adiponectin is associated with increased insulin sensitivity (6–9). In contrast to leptin levels, which are generally proportional to overall adipose mass, Arita et al. (10) found that plasma Acrp30 levels are higher in women and lean individuals. Subsequent studies confirmed the relationship between Acrp30 levels and insulin sensitivity. For example, type 2 diabetic patients had lower Acrp30 levels than nondiabetic control subjects, and a calorie restriction (CR) program that lowered BMI by 10% increased serum Acrp30 (11). We also found significant elevations in food-restricted mice (6). In rhesus monkeys disposed to type 2 diabetes, circulating Acrp30 levels decrease before the onset of hyperglycemia (12).

Circulating Acrp30 levels may play a critical role in basal metabolism, and we therefore investigated the endocrine regulation of Acrp30 in vivo. Similar to humans, female mice have elevated plasma Acrp30 compared with males. We studied the basis for the sexually dimorphic expression of Acrp30 by assessing the gonadal contribution at various stages of development. We also studied the effects of puberty, gestation, lactation, chronic CR, and aging. The response to elevated glucocorticoid, estrogen, and prolactin exposure was studied as a first step in elucidating the endocrine mechanisms that regulate Acrp30.

RESEARCH DESIGN AND METHODS

Laboratory animals and surgical procedures. The effects of puberty, pregnancy, lactation, ovariectomy (OVX), and estrogen, prolactin, and bromocriptine implants were studied in FVB mice (Charles River Laboratories) housed in groups of two to five in filter-top cages in a controlled environment (22–25°C, 40–50% humidity, and 12-h light-dark cycle with lights on from 0600–1800). The mice had free access to water and Teklad Rodent Diet no. 8604 (Harlan Teklad) at the Association for Assessment of Laboratory Animal Care–accredited facility of the Albert Einstein College of Medicine. Avertin 1.2% (Sigma-Aldrich) was used for general anesthesia (0.2 ml/10 g body wt) to perform bilateral OVX and introduce implants. Silastic capsules of 15 mm length, 1.47 mm internal diameter, and 1.96 mm outer diameter were packed...
with either 17β-estradiol (E2) and cholesterol (at a 1:9 ratio) or cholesterol (Steraloids). Model 1007D osmotic pumps (Alzet) delivered pro lactin (Dr. A.F. Parlow, National Institute of Diabetes and Digestive and Kidney Diseases) and 2-bromo-5-cholestosterone (Sigma). The animal research review committee at the Albert Einstein College of Medicine approved the procedures. The effects of neonatal and adult castration (CX) were studied in Long-Evans rats (Charles River Laboratories) housed individually in polycarbonate cages covered with polyester filter bonnets in an accredited animal facility at Johns Hopkins University. The animals were maintained on a constant 16:8 h light-dark cycle with lights on at 0600 h Eastern standard time. Food and water were available ad libitum. Male rats were bred in the laboratory, and gestational day 1 was based on the presence of a sperm plug. Pregnancy was monitored, and the day of birth constituted postnatal day (PND) 1. At PNDs 2–4, all pups were removed from the dams and held on a heating pad (with towels layered on top, to avoid overheating) to maintain body temperature. During this time, male pups assigned to have their gonads removed were anesthetized by hypothermia and castrated bilaterally, and incisions were closed with adhesive. Control males received sham operations. The procedures were approved by the Johns Hopkins animal care and use committee.

The effects of adult CX in the context of glucocorticoid treatment were studied in Swiss Webster mice (Charles River Laboratories) electronically tagged (Biomedic Data System) in plastic cages (one animal per cage) under a 12:12 h light-dark cycle with lights on at 0600 h Eastern standard time. Food and water were available ad libitum. Female rats were bred in the laboratory, and gestational day 1 was based on the presence of a sperm plug. Pregnancy was monitored, and the day of birth constituted postnatal day (PND) 1. At PNDs 2–4, all pups were removed from the dams and held on a heating pad (with towels layered on top, to avoid overheating) to maintain body temperature. During this time, male pups assigned to have their gonads removed were anesthetized by hypothermia and castrated bilaterally, and incisions were closed with adhesive. Control males received sham operations. The procedures were approved by the Johns Hopkins animal care and use committee.

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Blood collection. Blood samples were obtained from the tail vain or the orbital sinus by heparinized capillary tubes (Fisher) between 0900 and 1100 and stored at −20 °C until measurement of Acrp30.

125Iodine (125I) labeling of Acrp30 and secondary antibody. The chloramine T method (14) with one mCi of 125I (New England Biolabs) was used to radiolabel full-length murine recombinant Acrp30 (6) for clearance studies and rabbit IgGs used for Western blots. Unlabeled antibody was removed by gel filtration on a PD-10 column (Amersham Pharmacia Biotech). The radiolabeled product used in the clearance studies was detected as a single band on SDS-PAGE at 30 kDa.

Cell culture. 3T3-L1 murine fibroblasts were propagated and differentiated as previously described (15). Dulbecco’s modified Eagle’s medium was purchased from Cellgro. E2 and ICI 182,780 were purchased from Steraloids and ICI Pharmaceuticals, respectively, whereas all other chemicals were purchased from Fisher.

Preparation of cell lysates from 3T3-L1 cells. 3T3-L1 cells were washed twice with cold PBS and lysed in TNE buffer (1% Triton X-100, 150 mmol/l NaCl, 5 mmol/l EDTA, and 50 mmol/l Tris, pH 8.0) containing 60 mmol/l prednisolone pellet implants were performed at the same time. Slow-release and standard Agway RMH 3000 rodent diet (Agway). Orchidectomy and tagged (Biomedic Data System) in plastic cages (one animal per cage) under a 12:12 h light-dark cycle with lights on at 0600 h Eastern standard time. Food and water were available ad libitum. Female rats were bred in the laboratory, and gestational day 1 was based on the presence of a sperm plug. Pregnancy was monitored, and the day of birth constituted postnatal day (PND) 1. At PNDs 2–4, all pups were removed from the dams and held on a heating pad (with towels layered on top, to avoid overheating) to maintain body temperature. During this time, male pups assigned to have their gonads removed were anesthetized by hypothermia and castrated bilaterally, and incisions were closed with adhesive. Control males received sham operations. The procedures were approved by the Johns Hopkins animal care and use committee.

RESULTS

At 1 week of age, circulating Acrp30 values in male and female FVB mice were 3.0 ± 0.8 μg/ml. By 6 weeks, Acrp30 reached 10.2 ± 2.0 and 30.1 ± 4.2 μg/ml in males and females, respectively (P < 0.001) (Fig. 1A). The clearance of intravenous [125I]Acrp30 did not differ between adult males and females (Fig. 1B). A number of studies have linked elevated Acrp30 levels with improved insulin sensitivity (6). Elevated serum Acrp30 levels in females are consistent with the improved insulin sensitivity of female FVB mice compared with their age-matched male counterparts, as judged by decreased glucose and insulin levels (Fig. 2A and B), as well as an improved insulin tolerance test (Fig. 2C). We measured both Acrp30 mRNA and protein levels in various fat pads isolated from male and female mice. Surprisingly, Northern blot analysis did not reveal significant sex differences in Acrp30 mRNA of gonadal white and interscapular brown adipose tissue (Fig. 3A). In agreement with Western blot analyses showing that Acrp30 protein levels within the fat depots are significantly lower in males than females, suggesting sexually dimorphic protein production and secretion in adipocytes (Fig. 3B).

Elevated neonatal androgen exposure is associated with sex-specific imprinting. Adult Acrp30 levels were therefore measured in Long-Evans rats that had undergone CX 2–4 days after birth. Neonatal CX allowed plasma Acrp30 in adult males to rise above controls (P < 0.001) and reach female values. CX in adult rats had no affect (Fig. 4A). Acrp30 levels in Swiss Webster and FVB mice were also unaffected by adult CX (Fig. 4B and data not shown). However, Acrp30 was significantly upregulated (P < 0.001) in an osteoporosis model that combined CX with the synthetic glucocorticoid analog prednisolone (13,18) (Fig. 4B). Secondary hypogonadism and increased glucocorticoid activity are also found under other physiological conditions, such as chronic food restriction. Accordingly, Acrp30 levels were higher in both young and old male F1 (C57BLxBALB) mice under chronic calorie-restriction than in animals with ad libidum access to food (28.2 ± 2.0 and 30.0 ± 2.0 μg/ml vs. 17.0 ± 2.0 and 18.0 ± 2.0 μg/ml) (Fig. 4C). In agreement with observations in Fig. 3, changes in serum Acrp30 are brought about post-transcriptionally, since mRNA levels in gonadal white adipose tissue remain unchanged (Fig. 4D).

To determine whether ovarian maturation during puberty plays a role in the sex-specific expression of Acrp30...
in females, the ovaries were surgically removed in FVB mice at 1 week of age. OVX before puberty (1 week) did not prevent Acrp30 from reaching normal female levels (Fig. 5A). In fact, OVX in adults allowed plasma Acrp30 to increase 10 days after surgery above sham-OVX controls (42.1 ± 3.2 vs. 30.5 ± 4.2 μg/ml, respectively, in OVX and sham-OVX; P < 0.05). The effect of estrogen withdrawal associated with OVX was reflected in the uterine weights (18 ± 4 and 98 ± 10 mg, respectively, in OVX and sham-OVX; P < 0.001). Mice that received E2 implants and OVX were examined to determine whether estrogen might suppress Acrp30. In agreement with a suppressive effect of estrogen, Acrp30 levels decreased after 10 days of continuous exposure to supraphysiological levels of E2 (250–350 pg/ml) (Fig. 5A). Once again, uterine weights reflected the bioactivity of the E2 implants (92.4 ± 8.3 and 21.9 ± 4.0 mg, respectively, in E2- and vehicle-implanted mice).

This suggests that estrogen exerts a negative impact on Acrp30 levels in vivo. Because estrogen levels drop dramatically after ovarian cessation, we predicted that levels of Acrp30 should increase in old anovulatory mice. To test this hypothesis, we performed a cross-sectional study, looking at age-related differences in serum Acrp30 levels in young (4–5 months) and old (24–30 months) F1 (C57BLxBALB) mice (Fig. 5B). Acrp30 levels are ~80% higher in old mice. We cannot, of course, exclude other age-related influences on Acrp30 expression. However, if the increase in Acrp30 under food restriction is a consequence of secondary hypogonadism, we would expect to see a rise of Acrp30 in response to food restriction in young mice (reduced estrogen in response to reduced caloric intake) but not in old anovulatory mice (low estrogen under all conditions), and this is indeed the case (Fig. 5B). Similar to the observations in Figs. 3 and 4, changes in serum Acrp30 are brought about posttranscriptionally, since mRNA levels in gonadal white adipose tissue remain unchanged (Fig. 5C). This observation emphasizes that depending on the physiological state, tissue mRNA and serum protein levels do not always correlate.
FIG. 3. A: Acrp30 mRNA levels in adipose tissue in adult male (●; n = 3) and female (□; n = 3) FVB mice. Total RNA was extracted from dorsal subcutaneous white adipose tissue (WAT), interscapular brown adipose tissue (BAT), and epididymal or perimetrial/ovarian white adipose tissue and analyzed for Acrp30 mRNA by Northern blot. Two-way ANOVA using fat depot and sex as independent variables identified a significant (P < 0.05) effect of fat depot in males and females but no effect of sex. Representative 1.2-kb Northern bands are shown below the bar graph. Subcut., subcutaneous. *Significant (P < 0.05) effect of fat depot. B: Intracellular Acrp30 levels in adipose tissue from adult (●; n = 3) and female (□; n = 3) FVB mice. Equal amounts of protein (50 μg) from various fat depots were analyzed for Acrp30 by Western blot analysis using rabbit antisera against murine Acrp30 and [1-125]-labeled anti-rabbit IgGs. Each gel contained four standards of purified mouse Acrp30 at four different concentrations to generate absolute values and ensure linearity and reproducibility of the signal. Two-way ANOVA using sex and fat depots as independent variables identified significant (P < 0.05) effects of sex in each depot as well as a significant effect of depot in the case of gonadal WAT. A representative Western blot is shown below the bar graph. Subcut., subcutaneous. †Significant (P < 0.05) effect of sex; ‡significant (P < 0.05) effect of depot.

The suppression of Acrp30 in vivo may, however, be an indirect effect of elevated E2 caused by a number of downstream effects, such as the estrogen-mediated suppression of pituitary leutening hormone and follicle-stimulating hormone. Hence, 3T3-L1 adipocytes were used to determine whether the effects of estrogen in vivo were direct or indirect. FCS (10%) used to maintain adipocyte cultures is already rich in gestational estrogens, but the addition of increasing amounts of E2 suppressed Acrp30 levels (Fig. 6A; left panel), and the effect was blocked by the potent ER antagonist ICI 182,780 (19). Secretion of the protein into the extracellular medium was blocked as well (Fig. 6A; right panel). The specificity of estrogen action is supported by the presence of ER in differentiated 3T3-L1 adipocytes detected with a monoclonal antibody against murine ERα (Fig. 6B). A similar 66-kDa band was also detected in uterine extracts without immunoprecipitation. Interestingly, treatment of 3T3-L1 adipocytes with E2 suppressed Acrp30 mRNA levels, suggesting that in this isolated in vitro system, mRNA and protein go hand in hand (Fig. 6C). This effect reflects direct action of the ER on the Acrp30 gene, since similar effects can be observed in the presence of the protein biosynthesis inhibitor cycloheximide. Pregnancy is associated with a remarkable increase of various estrogens (20), and consistent with the E2 effects described herein, Acrp30 in mice decreased in the second and third week of gestation (P < 0.05) (Fig. 7A). Food intake increases by nearly 100% late in gestation, but the suppression of plasma Acrp30 late in gestation was evident even after 1 week of 50% CR (P < 0.05) (Fig. 7B). Despite the postpartum drop in estrogen, Acrp30 levels remained suppressed during lactation (P < 0.05) (Fig. 7A).

The effect of elevated lactogens seen during lactation and late gestation was assessed with osmotic minipumps releasing 50–100 μg of prolactin per day. Consistent with a suppressive effect of prolactin, circulating Acrp30 levels decreased after 72 h of elevated prolactin exposure (P < 0.05) (Fig. 8A). In contrast, Acrp30 levels increased with bromocriptine implants, presumably under dopaminergic inhibition of prolactin synthesis and secretion (P < 0.05) (Fig. 8A).

The correlation between low body weight, low prolactin, elevated insulin sensitivity, and high Acrp30 levels seen with CR is also evident in the Snell dwarf mouse (Fig. 8B). This model is homozygous for a mutation in pituitary transcription factor-1 (Pit-1), which plays a key role in the pituitary’s expression of prolactin, growth hormone, and thyroid-stimulating hormone (21).

DISCUSSION

We observed that circulating Acrp30 levels in mice increased abruptly by the third week of postnatal life but were relatively stable thereafter. In contrast to Acrp30, circulating levels of another adipose-specific marker, leptin, are relatively constant from 1 to 6 weeks of age and only begin to rise by the seventh week (22). What triggers the rise of Acrp30 early in puberty is not known. Our results, however, indicate that it does not involve the maturation of a gonadal feedback loop because surgical excision of the testes or ovaries before puberty did not interfere with the rise of Acrp30 in adults. Marked differences between the peripubertal secretions of male and female gonads support the likelihood that the pubertal Acrp30 surge is mediated by nongonadal mechanisms, and the similar timing in the male and female Acrp30 surge suggests a common mechanism. Previous studies suggest that the peripubertal surges of growth hormone, IFG-I, prolactin, and lutetinizing hormone are also triggered by nongonadal mechanisms (23–25).

Adult female mice have higher Acrp30 levels than males, in agreement with the sexual dimorphism previously de-
A: Effects of neonatal CX on Acrp30 levels in adult male Long-Evans rats. Blood was sampled from adult male rats (100 days old) that were either intact (Male; body weight = 488 ± 20, n = 10), castrated at 2–4 days after birth (CX-Neonate; body weight = 379 ± 9, n = 10), or castrated at 60–70 days after birth (CX-Adult; body weight = 436 ± 19, n = 10). Plasma samples from sham-operated animals (S-CX-Neonate and S-CX-Adult) and ovariectomized female (Female) rats were included for comparison. Plasma was analyzed for Acrp30 by Western blot analysis as previously described. Plasma leptin samples were obtained for Acrp30 measurement at 5 months (YOUNG), and after 24 months (OLD). Two-way ANOVA using effects of CR and age as the independent variables identified a significant (P < 0.05) effect of CR on Acrp30. Significant (P < 0.05) effects of prednisolone treatment and CX. *Significant (P < 0.05) effects of prednisolone treatment as the independent variable identified significant (P < 0.05) effects of prednisolone treatment as well as the combination of CR and prednisolone treatment.

B: Effects of CR and aging in male mice. Blood samples were obtained for Acrp30 measurement at 5 months (YOUNG; n = 5) and after 24–30 months (OLD; n = 5). F1 mice (C3HBLA/BLB) used here were maintained at The Jackson Laboratories. At 2 months after birth, calorie-restricted mice (CR) started receiving 60% of their individual baseline intake. Blood samples were obtained for Acrp30 measurement at 5 months (YOUNG) and after 24–30 months (OLD). Two-way ANOVA using effects of CR and age as the independent variables identified a significant (P < 0.05) effect of calorie-restriction. *Significant (P < 0.05) effect of calorie-restriction.

C: Effects of CR and aging in male mice. Blood samples were obtained for Acrp30 measurement at 5 months (YOUNG; n = 5) and after 24–30 months (OLD; n = 5). F1 mice (C3HBLA/BLB) used here were maintained at The Jackson Laboratories. At 2 months after birth, calorie-restricted mice (CR) started receiving 60% of their individual baseline intake. Blood samples were obtained for Acrp30 measurement at 5 months (YOUNG) and after 24–30 months (OLD). Two-way ANOVA using effects of CR and age as the independent variables identified a significant (P < 0.05) effect of calorie-restriction. *Significant (P < 0.05) effect of calorie-restriction.

FIG. 4. A: The effects of neonatal CX on Acrp30 levels in adult male Long-Evans rats. Blood was sampled from adult male rats (100 days old) that were either intact (Male; body weight = 488 ± 20, n = 10), castrated at 2–4 days after birth (CX-Neonate; body weight = 379 ± 9, n = 10), or castrated at 60–70 days after birth (CX-Adult; body weight = 436 ± 19, n = 10). Plasma samples from sham-operated animals (S-CX-Neonate and S-CX-Adult) and ovariectomized female (Female) rats were included for comparison. Plasma was analyzed for Acrp30 by Western blot analysis as previously described. Plasma leptin samples were obtained for Acrp30 measurement at 5 months (YOUNG), and after 24 months (OLD). Two-way ANOVA using effects of CR and age as the independent variables identified a significant (P < 0.05) effect of CR on Acrp30. Significant (P < 0.05) effects of prednisolone treatment and CX. *Significant (P < 0.05) effects of prednisolone treatment as the independent variable identified significant (P < 0.05) effects of prednisolone treatment as well as the combination of CR and prednisolone treatment.

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correlative data that illustrate the relationship between insulin resistance and low plasma Acrp30. Changes in insulin action during puberty have been poorly described in rodent models. We showed that in young adult mice, the fasting glucose, insulin, and insulin tolerance tests indicate that females are more insulin sensitive than their male littermates. Early studies in adult rats showed that 95%pancreatectomy resulted in a higher incidence of diabetes in males than females even after CX or OVX (32). The development of diabetes after pancreatectomy did not differ between female rats that were masculinized by neonatal exposure to elevated testosterone and male rats (33). A sexual dimorphism has also been described for...
non–insulin-dependent diabetes in the Wistar fatty rat on a high-sucrose diet (34).

Both humans and rodents develop insulin resistance during pregnancy (35,36). Here, we show for the first time that Acrp30 levels are suppressed from mid-gestation until weaning. Of all the endocrine changes that peak around pregnancy, placental lactogens and prolactin best fit the time course for the suppression of Acrp30. Placental prolactin-like molecules appear in the maternal circulation around mid-gestation, whereas pituitary prolactin levels increase progressively during gestation and remain elevated until suckling ceases (37). Isolated hyperprolactinemia is associated with insulin resistance, which is sometimes treated with various dopamine receptor agonists like bromocriptine (38,39). Indeed, the expected effects of prolactin and bromocriptine implants were observed on plasma Acrp30 in adult mice. Once again, the parallel rise of prolactin and Acrp30 in plasma seen during puberty should be distinguished from their apparently inverse relationship in adulthood.

It is well known that chronic CR (not malnutrition) increases insulin sensitivity in adult rodents compared with ad libitum feeding (40). Based on the consistent correlation between plasma Acrp30 and insulin sensitivity, it was not surprising to see that CR is associated with elevated plasma Acrp30 in young male and female mice.

FIG. 7. A: Circulating Acrp30 levels during gestation and lactation. Serial blood samples were obtained from young female FVB (n = 5) mice before mating; during the first, second, or third trimesters of pregnancy; and after 1 week of lactation. One-way ANOVA using the effect of the individual stage of reproduction as the independent variable identified significant (P < 0.05) effects in the second and third trimesters of pregnancy. *Significant (P < 0.05) effects. **Significant effect evident with 1 week of lactating compared with the last trimester of pregnancy. B: Circulating Acrp30 levels during gestation in response to CR. Samples were obtained from tail vein in the morning of days 12.5 and 18.5 of pregnancy in C57BL mice. On day 12.5, animals were separated into two groups, with one group receiving 274 DIABETES, VOL. 52, FEBRUARY 2003

FIG. 8. A: Effects of elevated prolactin and bromocriptine exposure on circulating Acrp30. Equilibrated micro-osmotic pumps containing either prolactin or the dopamine agonist bromocriptine were placed in the dorsal subcutaneous area of mature female C57BL/6J mice (n = 4 per group). The rate of release of prolactin and bromocriptine were 50–100 pg/day and 100–150 pg/day, respectively. Blood samples were obtained before (light bars) and 72-h after (■) continuous hormone exposure. Two-way ANOVA identified a significant (P < 0.05) effect of both treatment and time after treatment. *Significant (P < 0.05) effect of both treatment and time after treatment. B: Circulating Acrp30 in Pit-1 (−/−) mice. Blood was sampled from young (4-month-old) female Snell dwarf mice (■, −/−, body weight = 10.9 + 1.1 g, n = 5) and their litter mate controls (light bar; +/+ or +/−, body weight = 25.7 + 2.3 g, n = 5) and analyzed for Acrp30 as previously described. Nonparametric two-sample t test indicated a significant difference between groups (P < 0.05). *Significant difference (P < 0.05) between groups.

CR in rodents has also been used to understand the process of aging since the life-extending effects of food restriction were first described in rats (41). One theory is that CR delays the onset of changes associated with senescence, and we tested this by measuring Acrp30 levels in young and old ad libitum–fed and CR mice. The progression of biological senescence in common strains of laboratory mice is well advanced by 23–30 months, yet ad libitum–fed males maintained the same levels of Acrp30 as seen at 3–5 months. Moreover, CR led to a similar induction of Acrp30 in young and old males. On the other hand, old female mice had higher Acrp30 levels than young mice, and CR failed to stimulate Acrp30. The induction of Acrp30 we observed with the combination of CX and prednisolone treatment in Swiss Webster mice may be related to the mild stress response and secondary hypogonadism triggered by CR (42,43).

Because of a mutation in Pit-1, the Snell dwarf mouse (−/−) is a genetic model of anterior pituitary hormone deficiency of prolactin, thyroid-stimulating hormone, and growth hormone (42,44,45). We observed a significant elevation of plasma Acrp30 in the Snell dwarf mouse that is consistent with its marked elevation in insulin sensitivity (46,47). Elevated insulin sensitivity is considered to play a key role in life extension across phyla (48).
and Acrp30 (−/−) mice have decreased insulin sensitivity (49,50). Future studies may compare the insulin sensitivity between male and female Acrp30 (−/−) mice to determine the significance of Acrp30 in sex-specific differences or CR.

How is it possible that a female steroid hormone like estrogen could suppress Acrp30 if basal Acrp30 values are clearly greater in females than males? This paradox suggests that the inhibitory effect of elevated estrogen exposure in females cannot eliminate the sex-specific “set point.” We showed that early postnatal CX is the only manipulation that could alter the sexual dimorphism, suggesting that neonatal testicular secretions play a major role in establishing a sex-specific set point for Acrp30. Therefore, we propose that the set point, which determines basal Acrp30 levels in males and females, is established by the neonatal testosterone surge. Estrogen in adults only modulates Acrp30 values.

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