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PRELIMINARY REPORT

Ovariectomy Leads to Increased Insulin Resistance in Human Apolipoprotein B Transgenic Mice Lacking Brown Adipose Tissue

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We studied the role of estrogen in the gender differences in insulin resistance observed in the apoB/BATless mouse, a model of obesity, insulin resistance, and hyperlipidemia. Ovariectomized apoB/BATless mice were more obese and more insulin-resistant than sham ovariectomized apoB/BATless mice. Estrogen replacement by subcutaneous pellet reversed the obesity, lowered plasma insulin levels, and normalized both glucose tolerance and insulin sensitivity associated with ovariectomy. The apoB/BATless mouse should be a good model to delineate the molecular mechanisms whereby estrogen protects against insulin resistance.

WE RECENTLY described a mouse model of insulin resistance and obesity, the apoB/BATless mouse, in which females were protected from insulin resistance and hypertriglyceridemia relative to males.[1] To determine whether estrogen was a relevant factor in the sexual dimorphism, we subjected female apoB/BATless mice to ovariectomy (OVX), OVX and estrogen-replacement (OVX + E_2), or sham surgery (SHAM). OVX mice weighed more, and had impaired glucose tolerance compared to OVX + E_2 and SHAM mice. Although, as expected, estrogen replacement led to significant increases in plasma triglyceride concentrations, OVX + E_2 mice were protected from the insulin resistance and impaired glucose tolerance present in the OVX mice. Thus, estrogen availability is a relevant determinant of the metabolic profile of female apoB/BATless mice.

MATERIALS AND METHODS

Animals

ApoB/BATless mice were generated and maintained as previously described.[1] Ovariectomies were performed at 6 to 8 weeks of age by bilateral midline incision under ketamine:xylazine anasthesia. Estrogen was replaced by subcutaneously implanting slow-release pellets designed to achieve plasma concentrations of 100 to 125 pg/mL (Innovative Research of America, Sarasota, FL). Plasma concentrations of 17-β estradiol were measured by radioimmunoassay (Diagnostic Products Corp, Los Angeles, CA).

Determination of Metabolic Profiles

Plasma glucose, insulin, cholesterol, triglycerides (TG), and free fatty acids (FFA) were determined from bloods obtained after an 8-hour fast using commercially available kits. Glucose tolerance tests (GTTs) were conducted after an overnight, 12-hour fast (1 g/kg body weight injected intrapeitoneally). Insulin tolerance tests (ITTs) were performed after a 4-hour fast at a dose of 0.2 IU/kg body weight. Responses to glucose and insulin challenges were measured by determining areas under the curve (AUCs) from baseline. Statistically significant differences ($P < .05$; 2-tailed) were determined by analysis of variance (ANOVA) with post-hoc Scheffé's analysis.

RESULTS

Plasma estradiol concentrations were 44 ± 36 in sham-operated, 22 ± 19 in OVX, and 152 ± 65 pg/mL in OVX + E_2 animals ($P < .001$). OVX mice gained weight more rapidly than SHAM mice, who gained weight more rapidly than OVX + E_2 mice. At 19 weeks, OVX + E_2 mice weighed 33 ± 9 g compared to 51 ± 4 and 42 ± 4 g in OVX and SHAM mice, respectively. SHAM animals had weights that were similar to female apoB/BATless mice that were not subjected to any surgical intervention.

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Table 1. Effects of Estrogen Availability on Plasma Chemistries of Female apoB/BATless Mice

	TG (mg/dL)	Cholesterol (mg/dL)	Glucose (mg/dL)	Insulin (ng/mL)	FFA (mEq/L)
OVX	136 ± 16	483 ± 29	205 ± 31	2.20 ± 1.16	0.62 ± 0.17
OVX + E_2	192 ± 45*	480 ± 24	161 ± 53	0.93 ± 0.72	0.45 ± 0.15
Sham	124 ± 43	448 ± 52	190 ± 19	1.81 ± 1.95	0.54 ± 0.08

N = 4-9 in each group; 13 weeks; 8-hour fast.
*$P < .05$ by ANOVA and post-hoc Scheffé's comparison.

Cholesterol, glucose, and FFA concentrations did not differ among the 3 groups (Table 1). Plasma TG were significantly raised in the OVX + E_2 group in comparison to SHAM and OVX mice ($P < .05$). Mean plasma insulin concentrations were twice as high in OVX compared to OVX + E_2 animals (2.20 ± 1.16 and 0.93 ± 0.72 ng/mL, respectively), although this difference did not reach statistical significance.

GTTs at 16 weeks revealed impaired glucose clearance in OVX mice compared to the OVX + E_2 and SHAM animals (n = 6 or 7 in each group; $P < .05$) (Fig 1A). Fasting glucose levels for the GTTs were lower than those depicted in Table 1 because the GTTs were performed after an overnight, 12-hour rather than a daytime 8-hour fast. At 19 weeks, ITTs in these animals (n = 5 in each group) showed similar reductions in plasma glucose levels between the OVX + E_2 and SHAM mice (Fig 1B). There was a trend toward less insulin sensitivity in the OVX mice ($P = .11$).

DISCUSSION

The apoB/BATless mouse is a model of obesity, insulin resistance, and dyslipidemia.[1] In our initial studies we observed that female apoB/BATless mice were partially protected from all of these abnormalities; that observation led to the present investigations in which OVX and OVX + E_2 replacement were used to determine the role of estrogen in the gender differences we observed. In female apoB/BATless mice, OVX was associated with nonsignificant trends towards increased fasting plasma levels of glucose and insulin relative to OVX + E_2 and SHAM mice. OVX mice were significantly less able to clear exogenously administered glucose and showed a trend towards impaired sensitivity to exogenously administered insulin compared to both OVX + E_2 and SHAM mice. Studies with greater numbers of mice might have allowed us to show significant differences in plasma insulin levels and insulin sensitivity between OVX mice and the other 2 groups. In any event, OVX + E_2 mice showed the "best" metabolic profile, with lower plasma glucose and insulin concentrations.

Sexual dimorphism with regard to insulin sensitivity has been well documented in rodents. Estrogen, in particular, has been shown to be a relevant and important modulator of insulin signaling and glucose homeostasis. Relevant sites of action include stimulatory effects of the hormone on glucose utilization and glycogenesis,[2] inhibitory effects on gluconeogenesis,[3,4] and potentiating effects of estrogen on both insulin-stimulated glucose metabolism,[5], and glucose-stimulated insulin secretion.[6] More recently, the aromatase knockout mouse, characterized by low estrogen levels due to the inability to convert testosterone to estrogen, was demonstrated to have insulin resistance,[7] as was a mouse with targeted-disruption of the estrogen receptor-α.[8]

In our study, OVX, leading to estrogen depletion, was associated with increased weight gain in these animals relative to OVX + E_2 and SHAM mice. Similar effects of deficient estrogen action on weight gain has been previously documented,[7,8] and estrogen treatment has been shown to abrogate this effect, possibly by decreasing food intake[9] and/or increas-

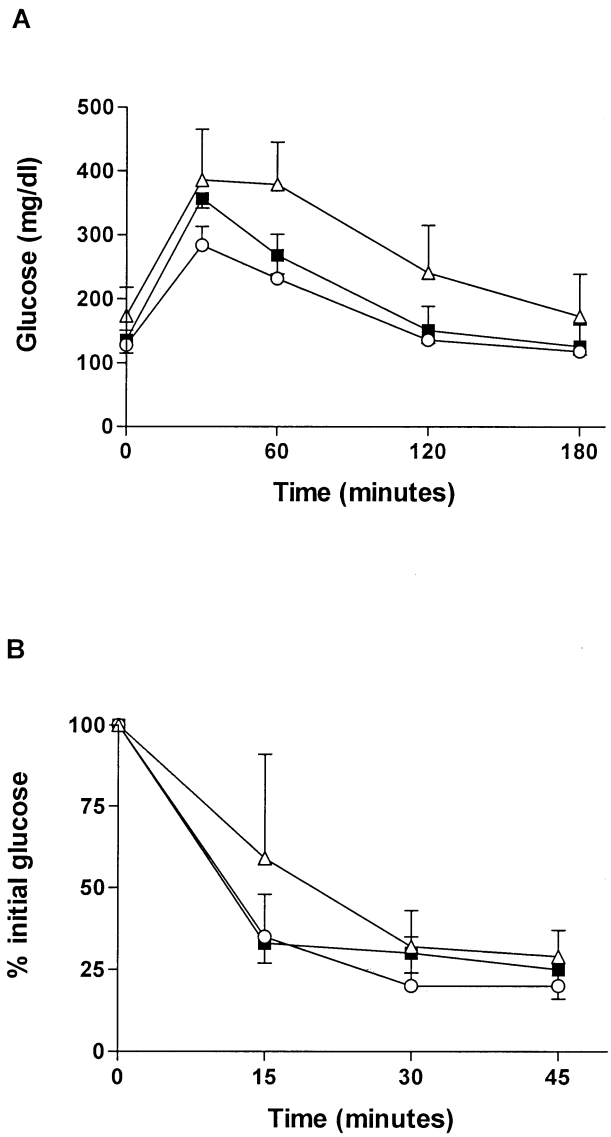

Fig 1. Glucose and insulin tolerance tests. OVX (△). OVX + E_2 (○), and SHAM (■) female apoB/BATless mice were challenged with an intraperitoneal injection of (A) glucose (1 g/kg body weight) of (B) insulin (0.2 IU/kg body weight). Values are means ± SD. OVX mice had significantly impaired glucose tolerance compared to the other 2 groups.

ing ambulatory activity.[10] The mechanisms underlying the role of estrogen in weight regulation in our OVX animals have not yet been fully defined. However, estrogen receptors are present in high numbers in rat adipocytes,[11] and in vivo treatment of rats with estrogen decreased adipocyte size.[12] Estrogen treatment of rats was also associated with lower levels of adipose tissue lipoprotein lipase,[13,14] an effect that has been demonstrated in humans as well.[15,16] These effects could lead to partitioning of energy, in the form of lipoprotein TG, away from adipose tissue.

Plasma TG levels were increased in OVX + E_2 mice relative to the 2 other groups. Treatment of an insulin resistant rat model with 17α-ethinyl estradiol was associated with increased plasma TG levels.[17] It is well known that estrogen therapy increase plasma triglycerides in women.[18] The molecular basis for the effect of estrogen on very–low-density lipoprotein (VLDL) secretion has not been completely delineated, despite intensive study. As noted above, estrogen does inhibit adipose lipoprotein lipase,[15,16] in part through an estrogen response element that has been identified on the promoter of lipoprotein lipase (LPL).[13] On the other hand, estrogen treatment increases levels and activity of hepatic lipase,[19] an effect that should reduce plasma TG levels. In transgenic mice expressing the estrogen-responsive avian apoVLDL-II gene, treatment with estrogen resulted in a 2-fold increase in plasma TG concentrations.[20] That result is in accord with studies in humans where estrogen therapy was associated with increased rates of VLDL apolipoprotein B (apoB) secretion into plasma.[21-23] Increased VLDL secretion during estrogen therapy appears to occur despite recent studies indicating that estrogen stimulates hepatic fatty acid oxidation.[24,25] Of note, while increased TG levels have usually been associated with insulin resistance, our findings indicate clearly that estrogen may affect plasma TG concentrations through mechanisms independent of its effects on insulin sensitivity.

In summary, we have shown estrogen to be a key determinant of body weight, insulin sensitivity, and lipid metabolism in the apoB/BATless mouse. This mouse model may, therefore, be a useful tool for determining the molecular basis of these effects of estrogen.

REFERENCES

1. Siri P, Candela N, Zhang YL, et al: Post-transcriptional regulation of the assembly and secretion of triglyceride-rich apolipoprotein B lipoproteins in a mouse with selective deficiency of brown adipose tissue, obesity, and insulin resistance. J Biol Chem 276:46064-46072, 2001

2. Rincon J, Holmang A, Wahlstrom EO, et al: Mechanisms behind insulin resistance in rat skeletal muscle after oophorectomy and additional testosterone treatment. Diabetes 45:615-621, 1996

3. Matute ML, Kalkhoff RK: Sex steroid influence on hepatic gluconeogenesis and glycogen formation. Endocrinology 92:762-768, 1973

4. Ahmed-Sorour H, Bailey CJ: Role of ovarian hormones in the long-term control of glucose homeostasis, glycogen formation and gluconeogenesis. Ann Nutr Metab 25:208-12, 1981

5. Puah JA, Bailey CJ: Insulinotropic effect of ovarian steroid hormones in streptozotocin diabetic female mice. Horm Metab Res 17:216-218, 1985

6. Shamoon H, Felig P: Effects of estrogen on glucose uptake by rat muscle. Yale J Biol Med 47:227-233, 1974

7. Jones ME, Thorburn AW, Britt KL, et al: Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. Proc Natl Acad Sci USA 97:12735-12740, 2000

8. Heine PA, Taylor JA, Iwamoto GA, et al: Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci USA 97:12729-12734, 2000

9. Wade GN, Gray JM: Gonadal effects on food intake and adiposity: A metabolic hypothesis. Physiol Behav 22:583-593, 1979

10. Shimomura Y, Shimizu H, Takahashi M, et al: The significance of decreased ambulatory activity during the generation by long-term observation of obesity in ovariectomized rats. Physiol Behav 47:155-159, 1990

11. Pedersen SB, Borglum JD, Eriksen EF, et al: Nuclear estradiol binding in rat adipocytes. Regional variations and regulatory influences of hormones. Biochim Biophys Acta 1093:80-86, 1991

12. Pedersen SB, Borglum JD, Moller-Pedersen T, et al: Effects of in vivo estrogen treatment on adipose tissue metabolism and nuclear estrogen receptor binding in isolated rat adipocytes. Mol Cell Endocrinol 85:13-19, 1992

13. Homma H, Kurachi H, Nishio Y, et al: Estrogen suppresses transcription of lipoprotein lipase gene. Existence of a unique estrogen response element on the lipoprotein lipase promoter. J Biol Chem 275:11404-11411, 2000

14. Peinado-Onsurbe J, Staels B, Vanderschueren D, et al: Effects of sex steroids on hepatic and lipoprotein lipase activity and mRNA in the rat. Horm Res 40:184-188, 1993

15. Price TM, O'Brien SN, Welter BH, et al: Estrogen regulation of adipose tissue lipoprotein lipase—Possible mechanism of body fat distribution. Am J Obstet Gynecol 178:101-107, 1998

16. Iverius PH, Brunzell JD: Relationship between lipoprotein lipase activity and plasma sex steroid level in obese women. J Clin Invest 82:1106-1112, 1988

17. Russell JC, McKendrick JD, Dube PJ, et al: Effects of LY117018 and the estrogen analogue, 17alpha-ethinylestradiol, on vascular reactivity, platelet aggregation, and lipid metabolism in the insulin-resistant JCR: LA-cp male rat: Role of nitric oxide. J Cardiovasc Pharmacol 37:119-128, 2001

18. The Writing Group for the PEPI Trial: Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. JAMA 273:199-208, 1995

19. Applebaum-Bowden D, McLean P, Steinmetz A, et al: Lipoprotein, apolipoprotein, and lipolytic enzyme changes following estrogen administration in postmenopausal women. J Lipid Res 30:1895-1906, 1989

20. Zsigmond E, Nakanishi MK, Ghiselli FE, et al: Transgenic mouse model for estrogen-regulated lipoprotein metabolism: Studies on apoVLDL-II expression in transgenic mice. J Lipid Res 36:1453-1462, 1995

21. Schaefer EJ, Foster DM, Zech LA, et al: The effects of estrogen administration on plasma lipoprotein metabolism in premenopausal females. J Clin Endocrinol Metab 57:262-267, 1983

22. Walsh BW, Sacks FM: Effects of low dose oral contraceptives on very low density and low density lipoprotein metabolism. J Clin Invest 91:2126-2132, 1993

23. Walsh BW, Schiff I, Rosner B, et al: Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 325:1196-1204, 1991

24. Djouadi F, Weinheimer CJ, Saffitz JE, et al: A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor alpha-deficient mice. J Clin Invest 102: 1083-1091, 1998

25. Nemoto Y, Toda K, Ono M, et al: Altered expression of fatty acid-metabolizing enzymes in aromatase-deficient mice. J Clin Invest 105:1819-1825, 2000