Rapid Publication

Synergism of Obesity Genes With Hepatic Steroid Sulfotransferases to Mediate Diabetes in Mice

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Sulfotransferases controlling the intrahepatic ratio of active androgens to estrogens represent key determinants of whether an obesity mutation will be diabetogenic in C57BL/KsJ female mice. Three unlinked genes (diabetes [*db*], obese [*ob*], and fat [*fat*]) all produced comparable obesity in C57BL/KsJ females, but only the *fat* mutation was not diabetogenic. The *fat* gene was incapable of eliciting virilizing changes in hepatic sulfotransferase activity, whereas both *db* and *ob* accelerated estrogen and suppressed androgen sulfation. Northern-blot analysis confirmed anomalous suppression of hepatic androgen sulfotransferase transcription in *db* and *ob* but not *fat* females. These findings suggest the utility of obesity genes in analyzing the interaction between hyperandrogenism, hyperinsulinemia, and diabetes. *Diabetes* 40:1360–63, 1991

Although obesity is a major risk factor for development of non-insulin-dependent diabetes mellitus (NIDDM) in humans (1), a complex interaction between obesity genes and other predisposing susceptibility modifiers is indicated because most obese individuals do not develop NIDDM. The nature of those modifiers interactive with obesity to predispose to diabetes is unknown. In inbred mice, gender-associated factors are key determinants, with male gender conferring high susceptibility to obesity gene–induced diabetes in certain strains (2). The balance of active androgens and estrogens in liver may contribute to regulation of glucose production. Sulfated androgens and estrogens do not bind to their receptors; sulfation may therefore represent a mechanism for intracellular inactivation and sequestration of sex steroids and their precursors (3). Intracellular sulfation of testosterone, its prehormone dehydroepiandrosterone (DHEA), or estrogens (estrone [E1] or 17β-estradiol [E2]) by distinct sulfotransferases (ST) represents an important catalytic mechanism for maintaining sexually dimorphic patterns of hepatic metabolism. After puberty and imprinting of the liver of male mice by testosterone, E1-ST activity is maintained, whereas DHEA-ST is suppressed (4). In contrast, both E1-ST and DHEA-ST remain active in liver of postpurbertal females. The C57BL/KsJ (BKs) inbred background is remarkable in that females are as diabetes susceptible as males when homozygous for either the diabetes (*db*; chromosome 4) or obese (*ob*; chromosome 6) mutations (5). However, not all obesity mutations are diabetogenic on the BKs background. Another recessive gene, fat (*fat*; chromosome 8), although eliciting obesity and hyperinsulinemia, is not diabetogenic (6). Note that the fat mutation in the mouse is not a homolog of the fatty (*fa*) mutation on chromosome 5 of the rat. Instead, the mouse *db* gene is the apparent homolog of the rat *fa* gene, because both mutations are linked to interferon-α and the brain glucose transporter (7). The enhanced susceptibility to *db* gene–mediated diabetes in BKs females has been correlated with aberrant shifts in the activities of hepatic ST enzymes. Accelerated (10-fold) estrogen sulfation in the late preweaning stage in BKs *db/db* mice of both sexes was followed by a male pattern of suppressed androgen sulfation in females at puberty (4). Hence, the unusual loss of ability of BKs *db/db* females to sulfate androgens coupled with an accelerated rate of estrogen sulfation would imprint a hyperandrogenized hepatic metabolism.

The purpose of this study was to extend analysis of changes in ST activities to BKs *ob/ob* (severely diabetic) and BKs *fat/fat* (diabetes-resistant) females to establish whether virilizing shifts in ST activities as observed in BKs *db/db* females were obligatory for diabetes development or represented an epiphenomenon associated with obesity/hyperinsulinemia syndromes.

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Received for publication 6 June 1991 and accepted in revised form 8 July 1991.

TABLE 1
Diabetogenic interaction between C57BL/KsJ inbred background and obesity genes on sulfotransferase (ST) specific activities

Genotype	Body weight (g)	Plasma glucose (mM)	Plasma insulin (pM)	DHEA-ST ($pmol \cdot mg^{-1} \cdot h^{-1}$)		Estrone-ST ($pmol \cdot mg^{-1} \cdot h^{-1}$)	
				0.2 μM	10 μM	0.2 μM	10 μM
+/+ (6)	20.0 ± 0.7	8.74 ± 0.45	138 ± 6	540 ± 104	7771 ± 2000	37 ± 4	906 ± 170
db/db (6)	47.3 ± 1.9*	34.38 ± 3.02*	924 ± 156*	49 ± 14*	543 ± 154*	400 ± 29*	873 ± 134
ob/ob (6)	50.1 ± 2.5*	34.61 ± 2.80*	960 ± 180*	68 ± 20*	792 ± 105*	606 ± 107*	1355 ± 264
fat/fat (4)	53.2 ± 0.4*	9.86 ± 1.06	1218 ± 36*	417 ± 72	4739 ± 482	53 ± 13	730 ± 86

Values are means ± SE for *n* (in parentheses) 10-wk-old females except *fat/fat*, which were 20 wk old. DHEA, dehydroepiandrosterone.
*$P \leq 0.01$ vs. normal (+/+) by Student's unpaired *t* test.

RESEARCH DESIGN AND METHODS

C57BL/KsJ (BKs) +/+ female mice were bred in our research colony. BKs *db/db* females and congenic BKs *ob/ob* (N5F37) and *fat/fat* (N6F9) females between 10 and 20 wk of age were kindly provided by D. Coleman and B. Paigen from their research colonies at The Jackson Laboratory. Female mice heterozygous for the X-linked testicular feminization *(Tfm)* mutation on the C57BL/6J (B6) background were obtained from the Mouse Mutant Stock Resource of The Jackson Laboratory. The mutation was transferred onto the BKs background by outcross to a BKs male followed by five cycles of backcrossing. Presence of the *Tfm* mutation in male mice with female external genitalia was confirmed by laparotomy.

DHEA-ST and E1-ST activities were assayed in 200,000 × *g* hepatic cytosols as described in detail elsewhere (4). [2,4,6,7-*N*-^{3}H]DHEA and [1,2,6,7-*N*-^{3}H]E1 were obtained from Du Pont-NEN (Boston, MA). Final specific radioactivity for each substrate was 0.8 Ci/mmol at 0.2 μM and 0.07 Ci/mmol at 10 μM. The sulfate donor molecule adenosine 3′-phosphate 5′-phosphosulfate (Sigma, St. Louis, MO) was used at a final concentration of 45 μM. Proteins were determined with the Bradford microassay procedure (Bio-Rad, Richmond, CA). Plasma insulin was determined by radioimmunoassay as described previously (4) with pork insulin as a standard. Plasma glucose was determined with a glucose analyzer (Beckman, Palo Alto, CA).

For Northern-blot analysis of ST transcription, a cDNA clone (designated *2-4*) was isolated from a rat liver λgt11 cDNA library (Strategene, La Jolla, CA) with a 34-mer oligonucleotide synthesized to the sequence of a published rat DHEA-ST gene named *Sta* (8,9). The 1015-base pair *2-4* cDNA is 90% homologous to *Sta* and contains an open reading frame for a 285-amino acid protein with 90% homology to rat DHEA-ST. The library screening and oligonucleotide labeling by a [^{32}P]kinase procedure were done with standard procedures (10). Ten micrograms of total RNA extracted from liver samples as described by Chomczynski and Sacchi (11) was analyzed by electrophoresis in a 1.25% agarose gel containing 10 mM sodium phosphate (pH 6.5) and 3% formaldehyde and transferred to a nylon membrane by capillary transfer. The *2-4* cDNA probe for DHEA-ST and a chicken β-actin probe (used to assess equivalency of intact hepatic RNA loaded per lane) were labeled with [α-^{32}P]dCTP with a random hexamer primer procedure. Simultaneous hybridization with both probes was at 42°C for 16 h in 50% formamide, 10% polyethylene glycol (8000 M_r), 5× sodium citrate–sodium chloride (SSC), 2× Denhardt's solution, 50 mM sodium phosphate buffer (pH 6.8), 0.1% sodium dodecyl sulfate, 50 mg/ml polyadenylic acid, 50 mg/ml polycytidylic acid, and 20 μg/ml denatured salmon sperm DNA. Hybridizations were followed by three 15-min washes in 2× SSC and 0.1% sodium dodecyl sulfate at room temperature and two 30-min washes in 0.1× SSC and 0.1% sodium dodecyl sulfate at 55°C.

RESULTS

Table 1 compares the strength of the diabetogenic interaction between ST activities and each of the three recessive obesity genes on the BKs genetic background. The *fat/fat* females, although comparably obese and hyperinsulinemic to the groups of *db/db* and *ob/ob* females used for comparison, are sharply contrasted by exhibiting a normal fed plasma glucose level. Correlating with the diabetes resistance was the finding that both androgen (DHEA) and estrogen (E1) ST activities in hepatic cytosols from *fat/fat* females were not different from normal (+/+) controls, indicating maintenance of a typical female estrogen-androgen ratio. In

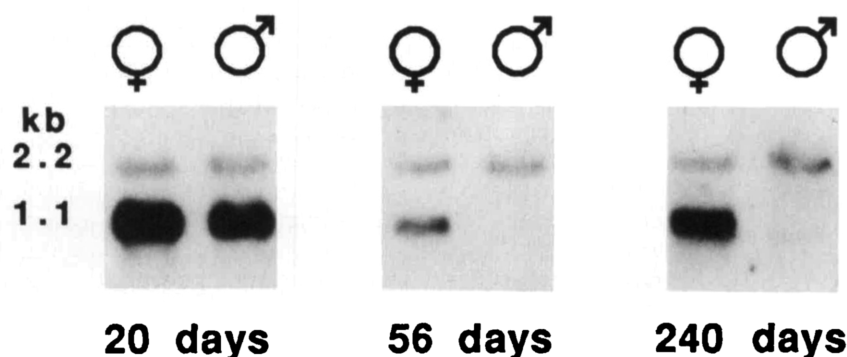

FIG. 1. Northern-blot analysis of hepatic RNA from C57BL/KsJ +/+ mice with rat *2-4* cDNA probe (detecting 1.1-kilobase [kb] sulfotransferase transcript). Transcripts are present in +/+ females throughout age range studied but are present only in 20-day-old prepubertal males, consistent with male gender-specific suppression of dehydroepiandrosterone-sulfotransferase enzyme activity after puberty. Chicken β-actin mRNA (2.2 kb) was not suppressed.

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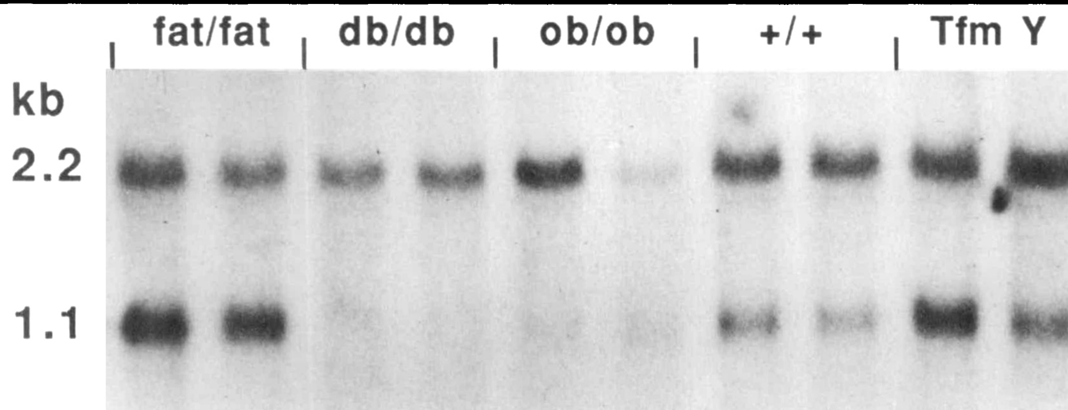

FIG. 2. Northern-blot analysis showing male-pattern suppression of 1.1-kilobase (kb) dehydroepiandrosterone-sulfotransferase (DHEA-ST) transcript in hepatic RNA from pairs of 12- to 14-wk-old C57BL/KsJ *db/db* and *ob/ob* but not *fat/fat* and +/+ females. β-Actin mRNA (2.2 kb) indicates equivalency of sample RNA per lane. Although uniformly absent in RNA from normal and mutant postpubertal males (Fig. 1), DHEA-ST transcripts were present in RNA from males carrying X-linked testicular feminization (*Tfm*) mutation, indicating that suppression of transcription in males is androgen-receptor dependent.

contrast, a male-type pattern of suppressed DHEA-ST coupled with a markedly increased E1-ST activity in both the severely hyperglycemic *db/db* and *ob/ob* females indicated a virilizing shift in the hepatic androgen-estrogen ratio. The almost 10-fold suppression of DHEA-ST activity observed at limiting (0.2-μM) and saturating (10-μM) substrate levels reflected a change in V_{max} (noncompetitive inhibition). The 10-fold increase in E1-ST activity at limiting substrate concentration reflected a reduced K_M, because it was not observed when the preparations were assayed at saturating substrate concentration. Thus, both obesity mutations *(db, ob)* capable of synergism with the BKs genome to produce diabetes were also those eliciting virilizing shifts in ST activities, whereas the nondiabetogenic *fat* mutation was unable to effect these changes. The activity changes were specific for ST; assay of microsomal sex steroid sulfohydrolase (12) catalyzing the removal of sulfate groups from estrogens and androgens showed no significant alteration in the presence of the *db* mutation (data not shown).

Northern-blot analysis of hepatic RNA of wild-type (+/+) mice demonstrated a gender-dimorphic suppression of *2–4* transcripts in postpubertal (56- and 240-day-old) males but not in prepubertal males (20 days old) or in females (Fig. 1), consistent with the known androgen-mediated suppression of hepatic DHEA-ST enzyme activity (4). Northern-blot analysis of total hepatic RNA from 12- to 14-wk-old BKs +/+, *db/db*, *ob/ob*, and *fat/fat* females showed DHEA-ST transcripts present only in the +/+ and *fat/fat* RNA (Fig. 2). Interestingly, males carrying the X-linked *Tfm* mutation encoding a defective androgen receptor (13) were distinguished from standard males by continued postpubertal expression of DHEA-ST transcripts at a female level (Fig. 2). The decreased mRNA content elicited by the *db* and *ob* mutations in females, in combination with the noncompetitive inhibition of DHEA-ST activity, indicated that the diabetes-inducing obesity genes (*db* and *ob*) were effecting an androgenized metabolic state in the liver. DHEA-ST activity is suppressible by testosterone in gonadectomized female mice (4). The presence of DHEA-ST transcripts in *Tfm* Y males (Fig. 2) but not normal postpubertal males (Fig. 1) demonstrated that this testosterone regulation was androgen-receptor dependent.

DISCUSSION

Women with polycystic ovary syndrome often exhibit excessive ovarian androgen production that, in turn, is accompanied by obesity, hyperinsulinemia, peripheral insulin insensitivity, and sometimes NIDDM (1). In humans, depressed serum levels of sex hormone–binding globulin are a humoral measure of hyperandrogenicity and represent a uniquely strong risk factor for the development of NIDDM in women (14). Because insulin can stimulate ovarian androgen production, and suppression of insulin secretion reduces serum testosterone in females with polycystic ovary syndrome, hyperandrogenism and depressed sex hormone–binding globulin concentration may be a consequence of hyperinsulinemia (15,16). However, in normal women, insulin infusion does not elicit hyperandrogenism (17), and suppression of hyperandrogenism does not reverse hyperinsulinemia (18,19).

This study comparing three different murine obesity genes on the same inbred-strain background is particularly relevant to the relationship between hyperinsulinemia and androgens. BKs *fat/fat* females were as hyperinsulinemic as both the *db/db* and *ob/ob* females, yet failed to develop the aberrant shifts in ST enzymes indicative of a virilized metabolic state (Table 1). It seems reasonable to conclude that the *fat/fat* females retained insulin sensitivity because they remained normoglycemic in contrast to the severely diabetic *db/db* and *ob/ob* females. Other salient features distinguish BKs *fat/fat* mice from either BKs *db/db* and *ob/ob* mice; fat is accumulated at a more protracted rate, and the pattern of fat deposition appears to be more uniform than in *db/db* or *ob/ob* mice (6). In obese humans, intra-abdominal (visceral) adiposity is more strongly associated with abnormalities of carbohydrate metabolism than is peripheral adiposity (1,20). Moreover, the neuroendocrine-gonadal axis appears less disturbed in *fat/fat* mice because ovarian function remains normal, and females will breed if mated before the development of obesity (6). Thus, the virilization of hepatic

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steroid metabolism effected by obesity genes expressed on the BKs inbred-strain background is not a direct function of hyperinsulinemia but more likely a function of insulin resistance associated with both the timing and the regional distribution of excess fat tissue. Further comparative analysis of the metabolic differences elicited by the *db* and *ob* versus the *fat* mutation in BKs mice should provide important insights into the complex diabetogenic interactions between obesity, hyperinsulinemia, insulin resistance, and hyperandrogenicity at the tissue level.

A severe male gender-specific hyperglycemia has recently been discovered in BKs *fat/fat* males produced at the 12th generation of inbreeding of the congenic stock N6F12 (unpublished observations).

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health Grants DK-17631 (E.H.L.) and GM-40440 (C.N.F.).

We thank Drs. Douglas L. Coleman and Beverly Paigen for generously providing mutant mice. We thank Linda Call, a participant in the 1990 Summer Student Program at The Jackson Laboratory, for assistance in this study.

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