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IN VITRO HEPATIC DRUG METABOLISM AND MICROSOMAL ENZYME INDUCTION IN GENETICALLY OBESE RATS

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Abstract—Genetically obese Zucker-strain rats were used to determine if obesity produced alterations in *in vitro* drug metabolism in hepatic microsomes or cytosol. Adult homozygous obese rats of both sexes had significantly less active drug-metabolizing enzymes *in vitro* than homozygous lean Zucker rats or Sprague–Dawley rats. Specific activities of aniline hydroxylase, aminopyrine demethylase and aryl-hydrocarbon hydroxylase (AHH) from male obese rats were only 25–30 percent of control activities, while glutathione *S*-aryltransferase, biphenyl 4-hydroxylase, and cytochrome P-450 activities were 50–70 percent of control activities. K_m and V_{max} values for aminopyrine demethylation were significantly less in obese rats than in lean rats. Enzyme activities in weanling obese rats were nearly equivalent to activities in weanling lean rats. Pretreatment of obese and lean rats with 3-methylcholanthrene produced expected increases in activities of biphenyl 4-hydroxylase, AHH and cytochrome P-450. Phenobarbital (PB), however killed half of the PB-treated obese rats at 60 mg/kg, but killed no lean rats. At lower doses, PB pretreatment produced only marginal increases in specific activities of aniline hydroxylase, aminopyrine demethylase and cytochrome P-450 in obese rats, while producing larger increases in activities in lean controls. When genetically obese rats were pair-fed to body weights that were equal to lean rats, the deficiency in *in vitro* drug metabolism between lean and fat rats was qualitatively as great as in *ad lib.* fed rats. Testosterone treatment of obese rats produced no significant increase in *in vitro* drug metabolism. Heterozygous lean Zucker rats responded to all treatments in a manner similar to that of homozygous lean Zucker rats. The explanation for these results is complex and they cannot be accounted for by a simple increase in body weight.

Although it is recognized that grossly overweight patients must receive different drug dosages than lean individuals [1–3], the relation between clinical obesity and drug metabolism, disposition and excretion is poorly characterized. Thus, Gal *et al.* [1] related increased half-life of theophylline in obese patients to increased volume of distribution due to partitioning of the lipid soluble drug into the large fat stores, while Samuelson *et al.* [2] related apparent increased half-life of methoxyflurane to a different manner of handling of the drug by liver drug-metabolizing enxymes. Young *et al.* [3] speculated that differences in serum F^- concentration in obese patients after the administration of fluorinated anesthetics were due to increased biotransformation, due possibly to increased mixed function oxidase activity in obese patients. In non-humans, meaningful studies of obesity and drug metabolism are rare, possibly because many studies have been compromised by drug, dietary or surgical intervention, leaving open to question the true cause of any observed change in drug metabolism. An uncompromised non-human model for studying the effect of obesity on drug metabolism has not been used to date. In an attempt to determine whether obesity might influence hepatic drug-metabolizing enzyme activity, genetically obese Zucker rats were utilized in the following studies to examine the endogenous activity of the hepatic drug-metabolizing enzyme system and the response of this system to enzyme-inducing chemicals.

METHODS AND MATERIALS

Animals. Male or female Zucker rats were purchased as weanlings (Bird Memorial Laboratories, Stow, MA) and housed two per cage in clear plastic cages until use. Rats were used either immediately (following 3 days of acclimatization to laboratory conditions) in studies utilizing weanlings, or at 19 ± 2 weeks of age for all other studies. Three genotypes were studied: homozygous dominant lean rats (FaFa), homozygous recessive obese rats (fafa) and heterozygous lean littermates of the obese rats (Fa?). Where appropriate, sex- and age-matched Sprague–Dawley (SD) rats (Taconic Farms, Germantown, NY) also were used. *Ad libitum (ad lib.)* access was provided to tap water and to Purina laboratory chow except where noted, and rats were fed *ad lib.* prior to enzyme studies.

Animals were weighed, and then decapitated. Livers were removed, rinsed in cold 0.15 M KCl containing 50 mM Tris–HCl, pH 7.4 (KCl–Tris), blotted dry and weighed. All subsequent manipulations were performed at 0–4°. Livers were minced with scissors, and homogenized in 3 vol. of cold KCl–Tris in a Potter-type glass homogenizer with a motor-driven Teflon pestle. The homogenates were centrifuged for 20 min at 9000 *g*, after which the resulting supernatant fractions were further centrifuged for an additional 60 min at 105,000 *g*. The post-microsomal supernatant fractions (cytosol) were carefully collected, and the microsomal pellets were suspended in KCl–Tris.

Analytical procedures. Protein was estimated as described by Lowry *et al.* [4]. All enzyme assays have been described in detail elsewhere [5]. Briefly, microsomes (1 mg protein/ml) were incubated aerobically at 37° with substrates (aniline HCl, 10 mM; aminopyrine, 25 mM; *p*-nitrophenol, 0.5 mM; biphenyl, 16.7 mM; benzypyrene, 0.066 mM), Tris–HCl buffer (150 mM, pH 7.4), and appropriate cofactors in a total volume of 1.5 ml. The final concentrations of the components of the NADPH generating system were: NADP, 1.1 mM; glucose-6-phosphate, 11.5 mM; $MgCl_2$, 5 mM; and glucose-6-phosphate dehydrogenase, 1.0 unit/ml. *N*-Acetyltransferase activity was determined by incubating *p*-aminobenzoic acid (PABA, 0.05 mM) with cytosol (2.0 mg protein/ml) and acetyl Coenzyme A (0.2 mM). Glutathione (GSH) *S*-aryltransferase activity was estimated using recrystallized 1, 2-dichloro-4-nitrobenzene (1.0 mM), cytosol (0.1 mg protein/ml) and glutathione (10 mM). NADPH cytochrome *c* reductase activity was determined by the method of Williams and Kamin [6], as described by Gigon *et al.* [7]. Cytochrome P-450 was determined by its dithionite difference spectrum [8], and total heme was determined as described by Falk [9]. Incubations for the determination of the kinetic constants of aminopyrine *N*-demethylation were performed at a microsomal protein concentration of 1.0 mg/ml, an incubation time of 10 min, and substrate concentrations ranging from 0.25 to 25 mM. Enzyme specific activity was plotted vs substrate concentration, and a line of best fit was estimated using a least squares method. The fit of the line to the experimental data points and the subsequent estimation of V_{max} and K_m were accomplished by the MLAB computer package established at the NIH by Knott and Reece [10].

Drug treatments. In studies to evaluate the possible effects of enzyme-inducing agents, male Zucker obese and lean rats and age-matched Sprague–Dawley controls were treated intraperitoneally (i.p.) with either Na-phenobarbital (PB; 45 mg/kg daily for 3 days prior to use) or 3-methylcholanthrene (3-MC; 25 mg/kg daily for 2 days prior to use). Weanling rats used in induction studies were approximately 5 weeks of age and were treated with 45 mg/kg PB daily for 4 days prior to use. Sleeping times were monitored following i.p. injection of Na-hexobarbital (125 mg/kg). Animals were considered conscious when they could fully right themselves three times in a 10-sec time period. In order to determine if a deficiency in circulating androgen was responsible for decreased drug metabolism, male lean and obese Zucker rats and age-matched female Sprague–Dawley controls were treated i.p. with testosterone propionate (2.5 mg/kg) twice weekly for 3 weeks prior to being killed. Vehicle controls (0.9% NaCl or corn oil) were run in all experiments.

Controlled feeding studies. In studies designed to determine if drug-metabolizing enzyme activity was related to body weight rather than to a genetic defect, weanling obese and lean Zucker rats were divided into two groups. One group of each genotype was allowed free access to normal chow, and the daily consumption of food was measured. The second group of each genotype was fed daily an amount of food equal to that consumed by the *ad lib.* fed lean rats on the preceding day. After 10 weeks, when the rate of food consumption appeared to plateau, consumption was adjusted every Monday based on the average daily consumption for the preceding week. Rats on the restricted diet regimens were given one-third of their daily ration at 8:00 a.m. and two-thirds of the daily ration at 5:00 p.m. each day. All pair-fed lean rats gained weight at rates similar to the *ad lib.* fed lean rats and rats of both genotypes appeared to be in good health at the time of use. On the night prior to death, *ad lib.* fed rats were allowed an amount of diet equal to that given to the pair-fed rats for that day. No attempt was made to control coprophagy by pair-fed obese rats, but none was apparent.

Histologic evaluation. Sections of liver from obese rats and appropriate controls were fixed in 10% formaldehyde and stained with either Oil Red O or hematoxylin–eosin prior to histologic study. Electron microscopic evaluation was conducted on liver sections that had been finely minced with a razor blade and fixed in ice-cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Details of the fixation, staining and electron microscopy have been published previously [11].

Lipid determination. In order to determine if differences in drug metabolism might be related to different lipid composition of the whole liver or the microsomal fraction, total lipids, phospholipids and free fatty acids were determined by standard clinical chemistry techniques.

RESULTS

Hexobarbital sleeping time in obese Zucker rats (*fafa*) was 73 ± 8 min (mean ± S.D.; N = 7), compared to 42 ± 4 min for the normal lean controls (*FaFa*). Plasma levels of hexobarbital at awakening were equal in both groups. Yields of microsomal and soluble protein were equal in obese and lean rats and comparable to those published previously for Sprague–Dawley rats [12]. As can be seen in Table 1, activities of cytochrome P-450-dependent enzymes were greatly reduced in male obese rats, with activities that were 25–75 percent of the activities in homozygous lean rats. Aniline hydroxylase, aminopyrine demethylase and arylhydrocarbon hydroxylase activities were most significantly affected. Levels of cytochrome P-450 also were significantly less in obese rats, although levels of total heme were equal to those in the lean controls. NADPH cytochrome *c* reductase and UDP glucuronyltransferase activities were not decreased in obese rats. Heterozygous lean rats (*Fa*?) had enzyme activities that were like the homozygous lean controls (*FaFa*).

When female obese rats were examined for *in vitro* drug-metabolizing ability, it was observed (Table 2) that enzyme activities were more nearly equal to lean controls than was seen with the obese males. Only aniline hydroxylase activity was as low as that seen in males. The decrease in aminopyrine demethylase activity was less dramatic in the obese females than in obese males (22 vs 74 percent

Table 1. Variables of *in vitro* drug metabolism in adult male obese (*fafa*) and lean (*Fa?*, *FaFa*) Zucker rats*.

Variable	Lean (*FaFa*)	Lean (*Fa?*)	Fat (*fafa*)
Body wt (g)	377 ± 7	424 ± 33†	621 ± 25†
Liver wt (g)	11.6 ± 0.7	13.8 ± 1.8	24.1 ± 3.6†
Total heme ‡§	1.20 ± 0.14		1.05 ± 0.03
Cytochrome P-450‡	0.74 ± 0.08	0.65 ± 0.03	0.43 ± 0.07†
NADPH cytochrome *c* reductase‖	131 ± 21	209 ± 20†	134 ± 24
Aniline hydroxylase ‖	0.99 ± 0.20	0.85 ± 0.18	0.26 ± 0.15†
Aminopyrine *N*-demethylase‖	5.47 ± 0.89	4.49 ± 1.40	1.46 ± 0.19†
UDP-glucuronyltransferase‖	5.49 ± 1.61	3.86 ± 1.32†	6.03 ± 1.30
Biphenyl 4-hydroxylase‖	0.82 ± 0.06		0.60 ± 0.05†
Arylhydrocarbon hydroxylase¶	15.2 ± 3.5		5.6 ± 2.6†
N-Acetyltransferase‖	1.07 ± 0.14	1.27 ± 0.45	0.86 ± 0.12
GSH *S*-aryltransferase§,‖	62.8 ± 2.9		46.6 ± 4.4†

* Data are the mean ± S.D.; N = 4.
† Significantly different from the homozygous lean controls (*FaFa*), $P \leq 0.05$.
‡ Expressed in nmoles/mg of protein.
§ Data from Table 3.
‖ Expressed in nmoles/mg of protein/min.
¶ Total benzpyrene metabolites are expressed in relative fluorescence units/mg of protein/min; activation 388 nm, emission 510 nm.

Table 2. Variables of *in vitro* drug metabolism in adult female obese (*fafa*) and lean (*Fa?*, *FaFa*) Zucker rats*

Variable	Lean (*FaFa*)	Lean (*Fa?*)	Obese (*fafa*)
Body wt (g)	228 ± 14	232 ± 11	424 ± 28†
Liver wt (g)	7.7 ± 1.5	7.4 ± 0.5	13.8 ± 0.9†
Microsomal protein (mg/g)	27.5 ± 1.8	27.0 ± 1.4	26.0 ± 0.8
Soluble protein (mg/g)	70.3 ± 6.4	75.3 ± 8.0	67.5 ± 2.2
Cytochrome P-450‡	0.88 ± 0.11	0.93 ± 0.11	0.91 ± 0.07
NADPH cytochrome *c*§ reductase	228 ± 26	223 ± 29	185 ± 21
Aniline hydroxylase§	0.51 ± 0.04	0.58 ± 0.13	0.27 ± 0.30
Aminopyrine *N*-demethylase§	3.40 ± 0.25	3.51 ± 0.32	2.67 ± 0.11†
UDP-glucuronyltransferase§	7.05 ± 0.77	6.65 ± 1.02	6.94 ± 0.92
N-Acetyltransferase§	3.45 ± 0.68	2.89 ± 0.50	2.89 ± 0.62

* Data are the means ± S.D.; N = 6.
† Significantly different from the homozygous lean control (*FaFa*), P = 0.05.
‡ Expressed in nmoles/mg of protein.
§ Expressed in nmoles/mg of protein/min.

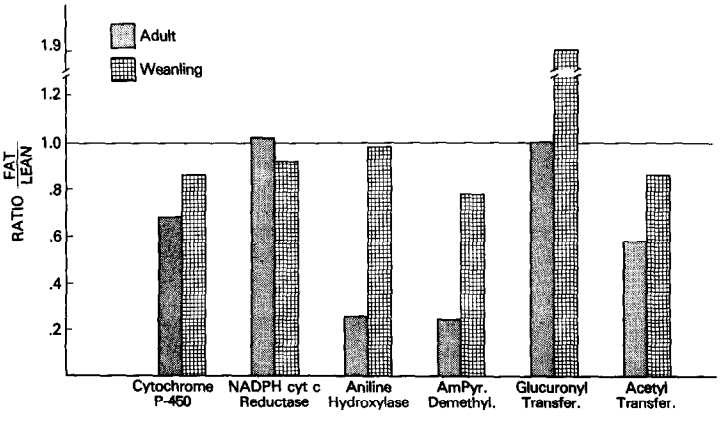

Fig. 1. Effect of age on *in vitro* hepatic drug-metabolizing enzyme activity in male obese rodents. Bars represent the mean values (N = 4) from obese (*fafa*) rats expressed as a fraction of lean (*FaFa*) rat values for adult (⊡) and weanlings (▦).

Table 3. *In vitro* hepatic drug metabolism in *ad lib.* fed and pair-fed lean (*FaFa*) and obese (*fafa*) male Zucker rats*

Variable	Lean (*FaFa*)		Obese (*fafa*)	
	ad lib.	pair-fed	*ad lib.*	pair-fed
Liver wt (g)	14.4 ± 1.4	9.1 ± 1.2†	21.5 ± 2.3	10.5 ± 0.9†
Cytochrome P-450‡	0.71 ± 0.04	0.77 ± 0.06	0.48 ± 0.03	0.50 ± 0.08
Total heme‡	1.20 ± 0.14	1.42 ± 0.17	1.05 ± 0.03	1.05 ± 0.12
NADPH cytochrome *c*§ reductase	212 ± 13	201 ± 16	161 ± 17	160 ± 12
Aminopyrine *N*-demethylase§	6.15 ± 0.31	7.49 ± 0.85†	2.86 ± 0.51	3.47 ± 0.49
Aniline hydroxylase§	0.47 ± 0.04	0.68 ± 0.07†	0.26 ± 0.05	0.60 ± 0.11†
UDP glucuronyl transferase§	2.27 ± 0.78	2.86 ± 1.16	3.86 ± 0.71	2.76 ± 1.51
N-Acetyltransferase§	0.84 ± 0.25	0.74 ± 0.02	0.77 ± 0.19	0.59 ± 0.11
GSH *S*-aryltransferase§	62.8 ± 2.9	50.0 ± 4.1†	46.6 ± 4.4	46.7 ± 7.8

* Data are the means ± S.D.; N = 4.
† Significantly different from the *ad lib.* fed rats $P \leq 0.05$.
‡ Expressed in nmoles/mg of protein.
§ Expressed in nmoles/mg of protein/min.

decrease, respectively). Cytochrome P-450 levels were equal in obese and lean female rats. It can be further noted by a comparison of Tables 1 and 2 that lean female rats of the Zucker strain demonstrate the same sexual dimorphism with respect to *in vitro* microsomal drug metabolism that has been reported for other rat strains [13].

In order to investigate more fully the impaired drug metabolism in obese rats, weanling male rats were examined. As shown in Fig. 1, enzyme activities in weanling obese rats were reduced less than were enzyme activities in the adults. UDP glucuronyltransferase activity, however, was greater in obese than in lean weanlings, although no difference in this enzyme was observed between lean and obese adults (Table 1).

From a comparison of adult and weanling obese rat data, it appeared as if the impairment in drug metabolism might be related not to a direct genetic defect in the obese mutants, but to excessive body weight. In order to examine this possibility, rats of both lean and obese genotypes were put on a pair-feeding protocol, and body weight and food consumption were carefully monitored. At 19 weeks of age, body weight in pair-fed obese rats was 355 ± 7 g, compared to 626 ± 40 g for *ad lib.* fed obese rats. Body weights in pair-fed and *ad lib.* fed lean rats were 353 ± 14 and 335 ± 41 g, respectively. Obese rodents that had been pair-fed appeared healthy. An unexpected finding, however, was large subcutaneous and intraperitoneal fat deposits in the pair-fed obese rats. These fat deposits were smaller than in the *ad lib.* fed obese rats, but substantially larger than in Sprague–Dawley or *FaFa* rats of a similar body weight. In addition, serum lipid levels were comparable in the *ad lib.* fed and the pair-fed obese rats. Microsomal and soluble protein yields were equal in *ad lib.* and in pair-fed rats. It can be seen (Table 3) that restricting the weight gain in genetically obese (*fafa*) animals by pair-feeding did not significantly affect the impairment in drug metabolism normally seen in these animals. Accordingly, enzyme activities in *ad lib.* and pair-fed animals were generally similar and, in turn, were markedly lower than in lean (*FaFa*) rats.

Table 4 presents some kinetic parameters of aminopyrine demethylase activity for male Sprague–Dawley (SD), obese (*fafa*) and lean (*FaFa*) Zucker rats. Data are presented separately and combined for high and low substrate concentrations. The V_{max}

Table 4. Kinetic parameters for adult male Sprague–Dawley (SD), lean (FF) and obese (ff) Zucker rats*

Parameter	Substrate concn	Genotype		
		SD	FF	ff
V_{max}	0.25–25	6.78 ± 0.33	2.89 ± 0.02†	1.45 ± 0.08‡,§
	1.5–25	5.08 ± 0.34	2.36 ± 0.02†	1.12 ± 0.07‡,§
	0.25–1.0	7.87 ± 0.07	2.90 ± 0.02†	2.05 ± 0.12‡
K_m	0.25–25	0.62 ± 0.08	0.65 ± 0.04	0.40 ± 0.06‡,§
	1.5–25	0.34 ± 0.02	0.27 ± 0.01†	0.22 ± 0.04‡
	0.25–1.0	1.22 ± 0.04	1.20 ± 0.16	0.67 ± 0.03‡,§

* Values are the means ± S.D. of four separate determinations.
† Difference between FF and SD is significant at $P \leq 0.05$.
‡ Difference between ff and SD is significant at $P \leq 0.05$.
§ Difference between ff and FF is significant at $P \leq 0.05$.

Table 5. Effects of testosterone treatment on hepatic drug metabolism in male lean (*FaFa*) and obese (*fafa*) Zucker rats*

Variable	Lean (*FaFa*)		Obese (*fafa*)	
	Corn oil	Testosterone	Corn oil	Testosterone
Total heme†	1.36 ± 0.08	1.33 ± 0.11	0.99 ± 0.10	1.08 ± 0.12
Cytochrome P-450†	0.89 ± 0.06	0.82 ± 0.06	0.60 ± 0.08	0.78 ± 0.04‡
NADPH cytochrome *c*§ reductase	233 ± 15	231 ± 20	194 ± 34	179 ± 6
Aniline hydroxylase§	0.43 ± 0.11	0.51 ± 0.13	0.21 ± 0.04	0.24 ± 0.03
Aminopyrine *N*-demethylase§	5.25 ± 0.76	6.49 ± 0.53	2.79 ± 0.50	3.04 ± 0.18
N-Acetyltransferase§	0.43 ± 0.03	0.37 ± 0.08	0.38 ± 0.02	0.64 ± 0.07‡
GSH *S*-aryltransferase§	5.54 ± 0.70	6.33 ± 1.14	4.14 ± 0.40	4.13 ± 1.06

* Testosterone treatment: 25 mg/kg twice weekly for 3 weeks. Data are the means ± S.D.; N = 4.
† Expressed in nmoles/mg of protein.
‡ Significantly different from the corn oil-treated control, $P \leq 0.05$.
§ Expressed in nmoles/mg of protein/min.

for obese rats was only 20 percent of the comparable value for Sprague–Dawley rats and 50 percent of the value of lean (*FaFa*) Zucker rats. In addition, the K_m in the fat (*fafa*) rat was different from either the lean (*FaFa*) or the Sprague–Dawley rat. The biphasic kinetics seen with aminopyrine demethylase is a phenomenon which has been demonstrated previously [14].

Because testosterone is responsible for the sexual dimorphism in rats [13], and obese (*fafa*) rats of both sexes are known to be sterile, the possibility was explored that the impaired drug-metabolizing enzyme activity in obese male rats might be related to deficient androgen levels. Serum testosterone in obese males was found to be 44 percent of the value in normal Sprague–Dawley males (0.8 ± 0.3 vs 1.9 ± 0.6 ng/ml, respectively), but only slightly less than the lean (*FaFa*) Zucker rat (1.1 ± 0.2 ng/ml). Seminal vesicle weights in obese rats were 30 percent lower than in lean (*FaFa*) controls, although there were no differences in testes weight between lean and obese rats. Table 5 shows *in vitro* drug metabolism in obese and lean (*FaFa*) male rats that had been supplemented with injections of testosterone. No marked increases in drug metabolism were observed in either obese or lean rats as a result of testosterone administration. No increase in testes weight was noted in either lean or obese rats after testosterone, although 30–40 percent increases in both serum testosterone and in seminal vesicle weight were seen in both lean and obese rats after testosterone treatment. When the same testosterone treatment regimen was employed in female Sprague–Dawley rats, increases in enzyme activity were seen, indicating that the treatment regimen was sufficient to increase enzyme activity when testosterone deficiency was responsible for low enzyme activity.

The question then arose as to the responsiveness of obese rats to microsomal enzyme inducers. Figure 2 shows the response of cytochrome P-450, biphenyl hydroxylase and arylhydrocarbon hydroxylase in obese male rodents to an inducing regimen of 3-MC. All three parameters were greatly increased in both lean and obese rats. By contrast, when a standard

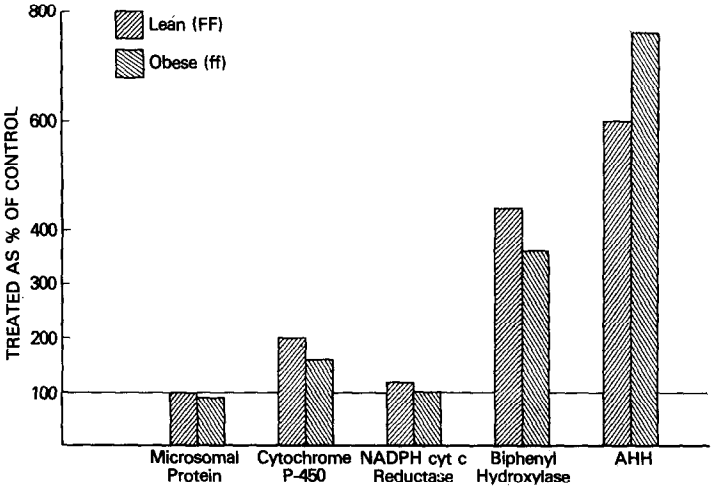

Fig. 2. Response of hepatic microsomal drug-metabolizing enzymes in lean (▨) and obese (▧) male Zucker rats to treatment with 3-methylcholanthrene. Bars represent mean values (N = 4) for 3-MC-treated rats expressed as a percentage of control (corn oil-treated) values.

Table 6. Effects of phenobarbital on male adult and weanling obese (*fafa*) and lean (Sprague–Dawley) rats*

Variable	Adult lean (SD)		Adult obese (*fafa*)		Weanling lean (SD)		Weanling obese (*fafa*)	
	Control	PB	Control	PB	Control	PB	Control	PB
Microsomal protein (mg/g)	30.8 ± 1.1	36.4 ± 2.5†	23.7 ± 0.6	25.5 ± 1.9	29.6 ± 2.1	40.6 ± 5.1†	30.2 ± 0.8	30.9 ± 5.0
Cytochrome P-450‡	1.03 ± 0.06	1.91 ± 0.21†	0.61 ± 0.04	1.02 ± 0.09†	0.86 ± 0.14	1.87 ± 0.17†	0.79 ± 0.05	1.09 ± 0.10†
NADPH cytochrome *c* reductase§	180 ± 21	264 ± 32†	153 ± 20	199 ± 25	141 ± 11	208 ± 21†	98 ± 4	163 ± 6†
Aminopyrine demethylase§	7.63 ± 0.66	15.9 ± 2.3†	2.49 ± 0.26	4.29 ± 1.18†	2.72 ± 0.19	9.35 ± 0.76†	2.60 ± 0.35	5.20 ± 1.43†
Aniline hydroxylase§	0.65 ± 0.04	0.83 ± 0.11	0.20 ± 0.02	0.23 ± 0.07	0.73 ± 0.07	0.75 ± 0.09	0.27 ± 0.04	0.38 ± 0.06
UDP glucuronyltransferase§	2.97 ± 0.44	7.20 ± 1.81†	3.71 ± 0.49	4.51 ± 1.40	6.17 ± 1.24	10.77 ± 1.48†	3.16 ± 0.84	4.52 ± 0.57
N-Acetyltransferase§	ND‖	ND	ND	ND	18.9 ± 1.5	27.4 ± 3.3†	0.55 ± 0.16	0.44 ± 0.15
GSH *S*-aryltransferase§	ND	ND	ND	ND	53.8 ± 6.0	97.8 ± 12.2†	45.3 ± 4.2	42.3 ± 2.9

* PB (45 mg/kg) was administered i.p. in 0.9% NaCl for 3 days to adults and for 4 days to weanlings. Data are means ± S.D.; N = 4.
† Values from PB-treated animals differ significantly from control values at $P \leq 0.05$.
‡ Expressed in nmoles/mg of protein.
§ Expressed in nmoles/mg of protein/min.
‖ ND = not determined.

enzyme-inducing regimen of PB (75 mg/kg daily for 4 days) was administered to obese male rats, all treated animals were dead 8 hr after the second dose. When the dose was reduced to 60 mg/kg daily for 4 days, half of the treated obese rats had died by the conclusion of the treatment regimen. No control (*FaFa*) rats died at either dose. Induction was then attempted using 30 mg/kg daily for 4 days and 45 mg/kg daily for 3 days. The lower dose regimen produced no significant increases in enzyme activity in obese rats. The response produced by the higher dose regimen is shown in Table 6. This dose of PB produced a 20–60 percent increase in most parameters studied in the obese rats. Aniline hydroxylase was nonresponsive to this dose of PB in all genotypes of rats. This dose of PB produced a 50–140 percent increase in enzyme activity in Sprague–Dawley controls, including an 18 percent increase in microsomal protein content. Although the Sprague–Dawley rats responded quantitatively more than the obese rats, the obese animals nonetheless demonstrated a mild response to PB at this dose.

Weanling obese and lean rats were then treated with PB to determine if the sensitivity to PB and the minimal induction produced by this drug was a function of the obese state or a secondary function of the genetic defect. Table 6 shows that the weanling obese rats responded to PB similarly to Sprague–Dawley weanlings, but with a less dramatic increase in cytochrome P-450, aminopyrine demethylase and UDP glucuronyltransferase.

Histology. Histologic evaluation of livers from adult obese rats demonstrated a mild fatty infiltration that was present in some, but not all obese individuals. This occasional fatty infiltration is consistent with observations of other authors [15, 16], who report only some livers from grossly fat individuals to be moderately fatty. Livers in obese rats treated with PB showed no more significant increase in endoplasmic reticulum than was demonstrable in saline-treated controls.

This variable increase in microscopically visible liver fat is consistent with the result of lipid determinations in liver, which showed no significant increases in total lipid, phospholipid or free fatty acids in livers of obese rats. The range of total lipid values in the analyzed livers was from normal to twice normal, indicating a large variation between individuals.

DISCUSSION

In addition to the well recognized adverse health effects of being overweight, empiric evidence suggests that obesity also may influence drug disposition and effects. Thus, obese rodents are more sensitive to development of both spontaneous and drug-induced tumors [17], and limited clinical experience shows a prolonged therapeutic effect of drugs in obese patients [1–3]. The underlying reason for the discrepant response of obese patients to some drugs is unknown. Among the hypotheses put forth to explain these responses were several relating to altered drug disposition and metabolism in obese patients [1–3]. In the present investigation, the use

of a genetically obese rat, uncomplicated by drug, dietary or surgical intervention, clearly shows that the cytochrome P-450-dependent microsomal enzyme system is dramatically deficient in obese rats of both sexes, and that the deficiency is less severe in weanlings than in adults, and in females than in males. This latter observation suggests that the enzyme deficiency may not be a primary effect of the genetic mutation, as has been shown for gluconeogenic enzymes in obese rodents [16], but rather may be an adaptive response to obesity as concluded for obesity-induced changes in other hepatic enzymes [16]. In this same context, Bulfield [18] reported that enzyme changes in genetically obese mice are identical to those in genetically unrelated mice made obese by injections of gold thioglucose, supporting the suggestion that enzyme changes in obesity are independent of genetic make-up or mutation. The restricted diet experiment (Table 3), however, demonstrated that, even when genotypically obese rats were not allowed to become obese, levels of drug-metabolizing enzymes were still low. Furthermore, activities of some drug-metabilizing enzymes from microsomes of lean genotype Zucker rats (*FaFa*) were different from corresponding activities from lean, age-matched Sprague–Dawley rats (Tables 1, 4 and 6), suggesting the possibility of a minor strain difference in drug metabolism. Consequently, results of different experiments in this study provide conflicting evidence as to the relationship between obesity and decreased drug-metabolizing enzyme activity, making it difficult to draw a conclusion regarding a possible mechanism for the lower enzyme activities.

Activity of the hepatic microsomal drug-metabolizing enzyme system has been related to various endocrine functions [19, 20]. The possibility exists that the lower enzyme activities observed in the obese rodents could, therefore, be related to thyroid, testes, or some other endocrine malfunction. Unpublished observations from this laboratory that indicate normal serum levels of triiodothyronine and thyroxine in obese rats can be taken as presumptive evidence of normal function of the thyroid. Other authors, however, report decreased thyroid function in obese (*fafa*) rats when other parameters of thyroid function are utilized [21]. Although significant work has been done to document the hormonal status of obese female rats [22], no similar work has been done for the obese male, although most obese males are sterile [23]. The present observation that serum testosterone levels in male obese rats were 56 percent less than in normal Sprague–Dawley male rats confirmed the suspicion that the obese males are at least partially hypogonadal. The failure of testosterone treatment to stimulate drug metabolism suggests, however, that androgen levels may be sufficient to support microsomal drug metabolism or that the testosterone-mediated mechanism for increasing enzyme activity is defective in the obese rats.

The sensitivity to PB was not entirely unexpected because hexobarbital sleep was found to be prolonged in the obese rats. This prolonged sleep time is similar to the prolonged hexobarbital sleep time reported for starved rats [24], suggesting a possible relationship between the increased hexobarbital sleep times reported in this study and starvation. A contributing factor in rodent obesity may be a partial protent starvation resulting from a defective ability to convert dietary calories into protein [25]. Lipid soluble barbiturates are known to distribute into fat stores, and plasma half-lives of barbiturates in obese animals are prolonged relative to lean controls [26]. The extremely large amounts of adipose tissue present in the peritoneal cavity of obese rats might be expected to provide a reservoir for the prolonged release of PB into the blood. Equal blood levels of hexobarbital at the time of awakening in both obese and lean rats suggest, however, that the sensitivity of the central nervous system to barbiturates is the same in obese and lean rats.

Another explanation for the PB sensitivity, however, might be related to the calculation of drug dose based on total body weight rather that on fat-free body weight [27]. In obese rodents, blood volume does not increase proportionally to increasing body weight [28], as is the case when increased body weight is made up of increased muscle mass. This would produce larger than expected blood concentrations of PB and hence larger than necessary receptor concentrations, potentially leading to overdosing. The dose–response curve for PB lethality in normal Sprague–Dawley rats, as determined in this laboratory, appears quite steep, with 4/4 rats treated i.p. at 300 mg/kg dying, but 0/4 dying at 200 mg/kg, so that the additional amount of PB administered to an obese rat because of the use of whole body weight might have been sufficient to produce lethal concentrations of PB in the brain.

The diminished inductive response to PB in obese rats is unexplained. Although some authors suggest a decreased protein-synthesizing ability in obese rats [25], other authors [29] have shown that microsomal protein synthesis occurs at a greater rate in obese than in lean rats, and data in Table 2 show equal hepatic microsomal and cytosolic protein contents in obese and lean rats. Furthermore, obese rats responded normally to 3-MC, a prerequisite of which is increased protein synthesis. The differential response of obese rats to 3-MC and PB may suggest that the primary drug-metabolizing hemoprotein present endogenously in obese rats may be of the P-448 rather than the P-450 type.

The relationship between lipids and microsomal drug metabolism is well established. A decrease in total dietary fat has been shown to cause a decrease in drug metabolism [30], while administration of linoleic acid to rats also has been shown to decrease drug metabolism [31]. Livers of obese rats have been shown to contain less linoleic acid than livers of lean rats [32], however, and results of the present study showed no significant change in free fatty acid content of livers or microsomes from obese rats. Furthermore, phospholipid is recognized as being an integral part of the microsomal drug-metabolizing system [33], and Buchar *et al.* [34] correlated decreased pentobarbital metabolism with altered microsomal membrane phospholipid composition in rats treated with lipid. Again, in the present study no change was seen in phospholipid content of livers or microsomal suspensions between fat and lean rats. These results suggest that the decrease in drug metabolism is not due to dramatic shifts in free fatty

acid or phospholipid contents in the microsomes.

In conclusion, genetically obese rats have been shown to be substantially deficient in *in vitro* hepatic drug-metabolizing enzyme activity, The deficiency is less obvious in females than in males and less obvious in weanlings than in adults. The deficiency is maintained when obese genotypes are pair-fed to body weights equal to lean genotype rats. Testosterone administration produced no significant increase in enzyme activity, and 3-MC but not PB produced expected increases in enzyme activities. The most likely explanation for the decreased activity of drug-metabolizing enzymes in obese Zucker rats is probably a complex genetic relationship that may only coincidentally associate obesity with decreased drug metabolism.

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