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OBESITY AS A RISK FACTOR FOR DRUG-INDUCED ORGAN INJURY. VI. INCREASED HEPATIC P450 CONCENTRATION AND MICROSOMAL ETHANOL OXIDIZING ACTIVITY IN THE OBESE OVERFED RAT [1]

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The obese overfed rat effectively models many of the pharmacological changes in human obesity. Recent data show that the obese rat is unusually susceptible to liver damage by several metabolically activated drugs that may be more toxic in obese humans. Results of the present study suggest a specific molecular locus for this interaction. In obese rats, P450 content of liver and the microsomal concentration of P450 were elevated 88% and 31%, respectively, over nonobese controls. Increases in microsomal ethanol oxidation were of identical magnitude. The ethanol-inducible form of P450 that is responsible for microsomal ethanol oxidation, P450IIE1, bioactivates several drugs that are shown to cause increased injury in obese rats. Collectively, these findings indicate that specific forms of P450 may become up-regulated in obesity, increasing the risk of a biochemically defined spectrum of drug-induced organ injuries.

Obesity ranks as the most prevalent disease in the United States and carries with it substantial increases in morbidity and mortality. Although many negative consequences of obesity remain unexplained, several may arise from changes in the P450 system. Pharmacokinetic studies have established that the oxidative clearance of many drugs is increased in obese humans (1). This raises the concern that obese individuals may be prone to adverse drug reactions resulting from metabolic activation. Increased metabolic activation may also contribute to several adverse sequelae of obesity which are well recognized, including high rates of occult liver disease, cirrhosis, and certain malignancies. An illustration of how the risk of drug toxicity may be magnified in obesity involves general anesthetics. Patients who are

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Abbreviations: ADH (alcohol dehydrogenase), ALDH (aldehyde dehydrogenase), MEOS (microsomal ethanol-oxidizing system), GC (gas chromatography), and NADH (reduced nicotinamide adenine dinucleotide).

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substantially overweight appear to be at greater than normal risk of organ damage from halogenated anesthetics because anesthetic bioactivation to toxic metabolites is increased (see 2).

Recent studies from our laboratory show that key physiological and biochemical alterations and, importantly, many of the pharmacokinetic changes that appear in human obesity are well reproduced by the overfed rat (3-5). It is therefore of some concern that acetaminophen and furosemide, drugs that undergo metabolic activation, produce substantially greater liver and kidney injury in this model (6-7). These and other observations support the hypothesis that obesity is a risk factor for drug toxicity and suggest that the obese overfed rat may be a preferred model for identifying metabolic changes that contribute to this interaction.

A P450 form that metabolizes ethanol has been implicated in the bioactivation of many medically important drugs and chemicals. Thus, elevated activity of the microsomal ethanol oxidizing system (MEOS) may serve as a specific index of broader increases in metabolic activation and associated toxicities in obesity. Furthermore, ethanol-induced liver injury is a global health problem which we believe is potentiated by obesity (see 11-12). The present studies evaluate the obese overfed rat for changes in the hepatic P450 system and for abnormalities in ethanol metabolism by subcellular fractions.

MATERIALS AND METHODS

Materials and Animals.

Chemicals were analytical grade or higher and used without purification. Weanling male Sprague-Dawley rats (Blue Spruce Farms, Altamont NY) received a pellet control diet (RMH-1000; Agway, Syracuse NY) or an obesity producing energy-dense diet described by Wong et al. (5). Energy-dense diet consumption is such that g protein and kCal energy intake relative to metabolic body size ($kg^{0.75}$), and exposure to additives known to induce metabolism, such as phenolic antioxidants, are equivalent to animals on control diet (5). Obesity was defined as body mass exceeding 125% of the mean for pellet control rats.

Procedures and Assays.

Animals maintained on the diets for 57 wks were euthanized by decapitation and livers were removed for microsome preparation (13). Total P450 concentration was measured by dithionite difference spectroscopy (14) and protein by Lowry (15). MEOS was determined spectrophotometrically by semicarbazide trapping (16) and confirmed by headspace GC analysis. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities in cytosol were calculated from the rate of NADH reduction by 100,000xg supernatant, with 10 mM glyceraldehyde serving as substrate in the latter assay (17,18). Hepatic mitochondria of animals on the diets for 40 wks were evaluated for ADH activity using 5 mM acetaldehyde and 10 mM glyceraldehyde as substrates (19).

Data Analysis and Statistics.

Results are mean ± SD. Group comparisons were made by Student's t-test and data analyzed by linear regression and correlation (20). Differences were attributed to treatment rather than chance variation when $p < 0.05$.

RESULTS

Obesity developed readily in the Sprague-Dawley rat, as reflected in the higher body mass of overfed animals (Table 1,2). Ethanol metabolism also changed in obese rats. MEOS activity per mg microsomal protein increased by 28% and MEOS activity per liver increased 87% (Table 1). Conversely, cytosolic and mitochondrial dehydrogenase activities remained unchanged or declined slightly per mg protein whereas dehydrogenase activities per liver increased 44%-69% (Table 1,2).

Other parameters governing overall drug metabolism also changed in obese animals. The amount of spectrally detectable P450 per mg microsomal protein increased by 31% and liver content of P450 rose 88% (Table 1). Although the yield of microsomal protein per gram of liver remained constant, mitochondrial protein concentration increased greatly and cytosolic protein was marginally lower (Table 1,2). Liver mass rose in direct proportion to increasing body mass in obese rats.

Obese and control rat groups were pooled for regression analysis to determine whether the liver content of P450 (Figure 1A), MEOS (Figure 1B), cytosolic ADH and ALDH, or mitochondrial acetaldehyde dehydrogenase varied as

Table 1. Changes in Hepatic Microsomal Ethanol Oxidation, P450, and Cytosolic Dehydrogenase Activities in Obesity

PARAMETER [a]	CONTROL	OBESE		% DIFFERENCE
ANIMAL CHARACTERISTICS				
Body Mass, g	583 ± 83.7	978 ± 233	[b]	+ 68
Liver Mass, g	14.3 ± 2.38	22.8 ± 5.00	[b]	+ 60
Microsomal Protein, mg/g	41.2 ± 4.57	38.3 ± 3.52		- 7
Cytosolic Protein, mg/g	113 ± 11.1	98.5 ± 8.29	[b]	- 13
P450 (nmol)				
Per mg Microsomal Protein	0.789 ± 0.093	1.04 ± 0.064	[b]	+ 31
Per g Liver	32.7 ± 6.49	39.7 ± 5.41		+ 21
Per Liver	471 ± 121	885 ± 117	[b]	+ 88
MICROSOMAL ETHANOL OXIDIZING ACTIVITY (nmol/min)				
Per mg Microsomal Protein	1.86 ± 0.271	2.38 ± 0.193	[b]	+ 28
Per g Liver	75.9 ± 8.56	90.6 ± 6.53	[b]	+ 19
Per Liver	1098 ± 276	2049 ± 391	[b]	+ 87
Per nmol P450	2.41 ± 0.58	2.31 ± 0.30		- 4
ALCOHOL DEHYDROGENASE ACTIVITY (nmol/min)				
Per mg Cytosolic Protein	9.96 ± 2.98	11.0 ± 2.00		+ 10
Per g Liver	1140 ± 385	1090 ± 245		- 4
Per Liver	17000 ± 8320	24500 ± 6340		+ 44
ALDEHYDE DEHYDROGENASE ACTIVITY (nmol/min)				
Per mg Cytosolic Protein	16.5 ± 0.661	17.4 ± 2.04		+ 5
Per g Liver	1860 ± 153	1700 ± 106		- 8
Per Liver	26800 ± 5670	38500 ± 7770	[b]	+ 44

[a] Mean ± SD (n = 6) for animals on diets for 57 weeks.
[b] $p < 0.05$ vs. control (Student's t-test).

Table 2. Changes in Hepatic Mitochondrial Acetaldehyde and Glyceraldehyde Dehydrogenase Activities in Obesity

PARAMETER a	CONTROL	OBESE		% DIFFERENCE
ANIMAL CHARACTERISTICS				
Body Mass, g	509 ± 59.4	787 ± 82.0	b	+ 55
Liver Mass, g	15.5 ± 2.51	21.4 ± 3.84	b	+ 38
Mitochondrial Protein, mg/g	29.7 ± 6.75	42.2 ± 1.67	b	+ 42
ACETALDEHYDE DEHYDROGENASE ACTIVITY (nmol/min)				
Per mg Mitochondrial Protein	79.7 ± 6.03	67.2 ± 7.33	b	- 16
Per g Liver	2370 ± 596	2840 ± 415		+ 20
Per Liver	35900 ± 8030	60800 ± 14100	b	+ 69
Per 100 g Body Mass	7160 ± 1910	7710 ± 1630		+ 8
GLYCERALDEHYDE DEHYDROGENASE ACTIVITY (nmol/min)				
Per mg Mitochondrial Protein	120 ± 11.7	100 ± 13.7		- 16
Per g Liver	3600 ± 1060	4240 ± 742		+ 18
Per Liver	54300 ± 14400	89900 ± 19200	b	+ 66
Per 100 g Body Mass	10900 ± 3330	11500 ± 2480		+ 5

[a] Mean ± SD (Control n = 4, Obese n = 3) for animals on diets for 40 weeks.
[b] $p < 0.05$ vs. control (Student's t-test).

a direct function of body mass. Each parameter correlated strongly with body mass (cytosolic ADH [Y = 0.020 X + 5.02, r = 0.66, $p < 0.02$], cytosolic ALDH [Y = 0.029 X + 10.2, r = 0.86, $p < 0.001$], and mitochondrial ADH [Y = 0.079 X

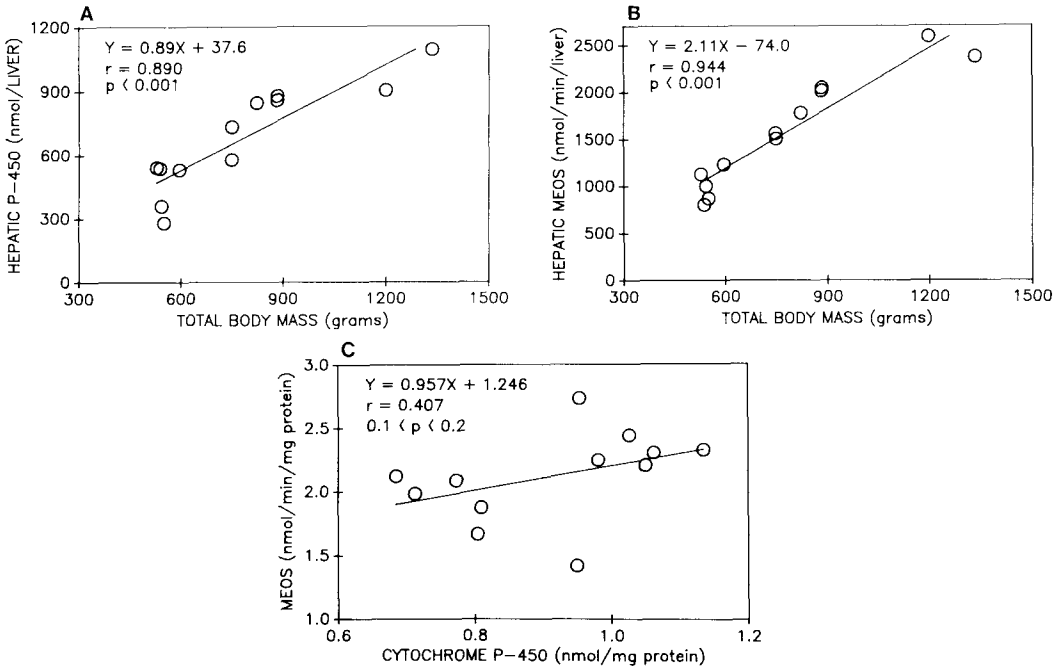

Figure 1. Several hepatic enzymes that are involved in ethanol metabolism were increased in the obese rat. The liver content of P450 (Panel A) and the activity of MEOS per liver (Panel B) rose with the body mass of control and obese animals ($p < 0.02$). Linear regression of MEOS activity versus the amount of P450 per mg microsomal protein showed these variables to be only weakly correlated (Panel C).

- 29.6; $r = 0.77$, $p < 0.05$]). MEOS activity correlated only weakly with the amount P450 per mg microsomal protein (Figure 1C), as affirmed by the failure of obesity to change MEOS activity per nmol P450 (Table 1).

DISCUSSION

The principal findings of this study, increases in the microsomal concentration, liver content, and catalytic activity of P450 (Table 1), corroborate results from obese humans showing that absolute clearance rate is increased for many drugs that are oxidized by hepatic P450 (1). Because the P450s catalyze a spectrum of cellular processes, two important questions arise. Are detrimental processes increased in obesity, and if they are, which pose genuine health risks. Increases in acetaminophen and furosemide hepatotoxicity in the obese rat (6,7) show that metabolically activated drugs have the potential for being more toxic in obese humans. The rise in MEOS activity (Table 1) carries this one step further by identifying a form of P450 that may increase selectively in obesity and by singling out known substrates of this enzyme as candidates for drug-obesity interactions.

Ethanol metabolism in microsomes is mediated by P450IIE1 in human and rat (21,22). The P450IIE1 enzyme also metabolizes halothane and acetaminophen to hepatotoxic metabolites and N-dimethylnitrosamines to ultimate carcinogens (8-10). Elevation of P450IIE1 in obese humans could explain why an unusually high fraction of patients with unexplained halothane hepatitis are obese (23,24) and why these individuals form more reactive metabolites from anesthetics (2). It would appear likely that this enzyme form is elevated in the obese rat based on increases in acetaminophen and anesthetic toxicities (6,25). Raucy and colleagues now show with an antibody to human P450IIE1 that this enzyme accounts for most acetaminophen bioactivation by human microsomes (26) and that rat P450IIE1 increases parallel MEOS activity in obese rat microsomes (Personal Communication). Each of these findings adds support to the hypothesis that P450IIE1 is increased in obese individuals and that a host of defined toxicities is thus exacerbated. The absence of a MEOS/P450 correlation (Figure 1C) points out that other forms of P450 increase in obesity and that other toxicities may also be magnified.

A role for obesity in alcoholic liver injury should also be considered. More than 90% of obese individuals have abnormal liver histopathology, with the most severe fibrotic damage in liver correlating closely with ethanol consumption (11). Biochemical signs of liver injury are prevalent even in those who are only moderately overweight, and again are greatest in consumers of ethanol (12). Microsomal and total hepatic MEOS activities were increased in the obese rat (Table 1) but presently, there is no evidence that microsomal induction of an ethanol-inducible form of P450 contributes

materially to the hepatotoxic effects of ethanol. The small changes in cellular ADH and ALDH activities and parallel increases in activities per liver (Table 1,2) suggest that ethanol elimination may increase in obesity. Such a finding would discount speculation that ethanol clearance is impaired in overweight individuals and greater plasma exposure leads to increased toxicity.

In conclusion, obesity appears to be accompanied by meaningful changes in the P450 system. Knowledge of the specific P450 forms that undergo change may offer a molecular explanation for several adverse consequences of this disease. Such information could also provide successful rationales for therapeutic interventions to combat the major adverse effects of obesity.

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