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Palmitate Metabolism and Norepinephrine Sensitivity in Brown Adipose, Liver, and White Adipose Tissues of Zucker Rats[1] (41351)

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Abstract. Interscapular brown adipose tissue (BAT) *in vitro* utilization of palmitate (0.5 mM) and glucose (5.0 mM) were examined at 0, 0.1, 1.0, and 10.0 μg norepinephrine-bitartrate (NE)/ml. With increasing NE concentrations, *in vitro* palmitate oxidation was stimulated and glucose oxidation was inhibited in BAT of nonobese rats. This characterization of BAT metabolism provided the constraints for our analysis of fatty acid utilization and norepinephrine sensitivity at submaximal response concentrations in BAT of lean and obese Zucker rats. Liver and inguinal white adipose were used for tissue comparisons. In comparison to its lean littermates, it was demonstrated that the obese rat: (i) had heavier interscapular BAT pads, (ii) oxidized a similar amount of palmitate to CO_2 per 100 mg of BAT, (iii) oxidized more palmitate in response to NE stimulation of BAT, and (iiii) had slower rates of hepatic ketogenesis and palmitate oxidation. Altered palmitate oxidation or NE sensitivity in BAT was not contributing to the obesity of the "fatty" Zucker rat at 5 weeks of age.

Impaired thermoregulation has been established in preweanling and older obese (fa/fa) Zucker rats (1–3). Godbole *et al.* (1) detected lower body temperatures in 2-week-old fa/fa rats, when compared to lean (Fa/?) littermates. Oxygen consumption was lower in preweaned fa/fa rats, when exposed to low or moderate temperatures and similar to Fa/? at high temperatures (2). Obese Zucker rats also did not demonstrate dietary induced thermogenesis, as was the case with lean rats (4). Therefore, obese Zucker rats are unable to activate both dietary-induced and temperature-induced thermogenesis.

Brown adipose tissue is apparently the major site of both dietary-induced and temperature-induced thermogenesis (5, 6). Defective nonshivering thermogenesis has been postulated as the cause of greater energetic efficiency in obese mice (7) and some obese humans (8). Impaired brown adipose tissue metabolism has been suggested as the cause of defective thermogenesis and excessive lipid deposition in the obese mouse (7, 9). Norepinephrine infusions which produce high caloric responses and increase blood flow to brown adipose tissue also increase fatty acid turnover and reduce R.Q. (10). However, utilization of specific substrates in response to norepinephrine by brown adipose tissue has not been investigated. Our study demonstrated that with increasing concentrations of norepinephrine, *in vitro* palmitate oxidation rates increased while glucose oxidation rates decreased in brown adipose of normal rats. This characterization of *in vitro* brown adipose tissue metabolism provided the constraints for our analysis of fatty acid utilization and norepinephrine sensitivity at submaximal response concentrations in brown adipose tissue of lean and obese Zucker rats.

Materials and Methods. Experimental animals were from two strains of rats selectively bred by T. F. Zucker and L. M. Zucker. 4St line rats (11, 12) were used in the first experiment, and lean and obese Zucker rats (13) were used in the second experiment. The 4St line originated from breeding homozygous lean Zucker rats to Long Evans–Wistar crosses. All animals were housed in climate controlled rooms (22°), under controlled lighting (12 hr on, 12

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hr off) and were fed laboratory chow (Purina) and water *ad libitum*.

In the first experiment, characterization of *in vitro* glucose and palmitate utilization in response to various norepinephrine concentrations were examined in interscapular brown adipose tissue (BAT). The 4St line rats were used since an adequate number of 5-week-old lean Zucker female rats were not available. The first experiment was designed to determine linearity with time, preferred substrate for oxidation in response to norepinephrine and concentration of norepinephrine required for submaximal stimulation (sensitivity) in our system. Thirty-two 5-week-old 4St line female rats were decapitated, BAT removed, and sliced into 80- to 100-mg pieces on a Stadie–Riggs hand microtome. Slices of BAT were put in 25-ml flasks containing 2.0 ml Krebs–Ringer bicarbonate buffer (1/2 Ca^{2+}), 5.0 m*M* glucose, 0.5 m*M* palmitate, and 2% BSA. Medium also contained either 0, 0.1, 1.0, or 10.0 μg norepinephrine-bitartrate (NE)/ml and contained either [U-^{14}C]glucose (0.5 μCi/ml) or [1-^{14}C]-palmitate (0.5 μCi/ml). Flasks were gassed with O_2–CO_2 (95:5), sealed by rubber stoppers equipped with hanging center wells, and incubated for either 15, 30, 60, or 120 min at 37° in metabolic shakers (90 oscillations/min). Hyamine hydroxide (0.2 ml) was added to hanging center wells and reactions were stopped with addition of 1.0 *N* H_2SO_4 (0.5 ml) to medium. Flasks were returned to the incubator for 30 min to permit total CO_2 precipitation in the base. Center wells were placed in liquid scintillation cocktail and counted for radioactivity.

The second experiment involved 10 lean and 10 obese female littermate pairs at 5 weeks of age. Animals were decapitated (between 8:00 and 9:30) over a 2-day period (five pairs/day). Liver, BAT, and inguinal white adipose tissue (WAT) were removed and weighed. All tissues were sliced into 80- to 120-mg pieces on a Stadie–Riggs hand microtome and were incubated in the presence of [1-^{14}C]palmitate (0.5 μCi/ml) and NE (1.0 μg/ml). Production of CO_2 from palmitate was determined as in experiment 1. Tissue lipids were extracted by the procedure described by de Cingolani (14). Ketogenesis was determined by measuring radioactivity in the aqueous phase of an acidified chlorofrom–methanol (1:2, v/v) extraction medium. This procedure was similar to that described by Sauer *et al.* (15) and was found superior to a perchloric acid precipitation-centrifugation procedure. Nonradioactive chemicals were obtained from Sigma Chemical Company and Fisher Scientific Company. Radioactive substrates were purchased from New England Nuclear.

Effects due to tissue, genotype, and norepinephrine were determined by analysis of variance. Statistical significance between means was determined by Student's *t* test. Differences in absolute changes between genotypes due to norepinephrine were analyzed by paired *t*.

Results. Characterization of 4St line rat BAT in the *in vitro* system are presented in Figs. 1 and 2. Steady rates of glucose and fatty acid oxidation were demonstrated throughout 120-min incubations (Fig. 1). Addition of NE caused dose-dependent depression of glucose oxidation and stimulation of fatty acid oxidation (Fig. 2). Response to NE was linear through 2 hr; therefore, data points were a composite of 15-, 30-, 60-, and 120-min incubations and expressed as picomoles per minute.

Five-week-old obese Zucker females had body, liver, BAT, and inguinal pad weights which were 1.3-, 1.4-, 2.8-, and 4.1-fold greater than controls, respectively (Table I). Expressing organ weights relative to metabolic body size, liver, BAT, and inguinal pads were 1.2, 2.3, and 3.5-fold larger, respectively, in obese compared to lean.

Metabolic partitioning of palmitate was affected by tissue, genotype, and NE (Table II). Expression of data on a per 100 mg of tissue or total organ per metabolic body size basis affected results in most cases.

Tissue effects. On a 100 mg of tissue basis, BAT oxidized palmitate to CO_2 faster than liver or WAT. Rates of BAT ketogenesis (per 100 mg) from palmitate were slower than liver and faster than WAT for lean animals, while ketogenic rates were similar in obese BAT and liver. Esterification rates (per 100 mg) in BAT were similar

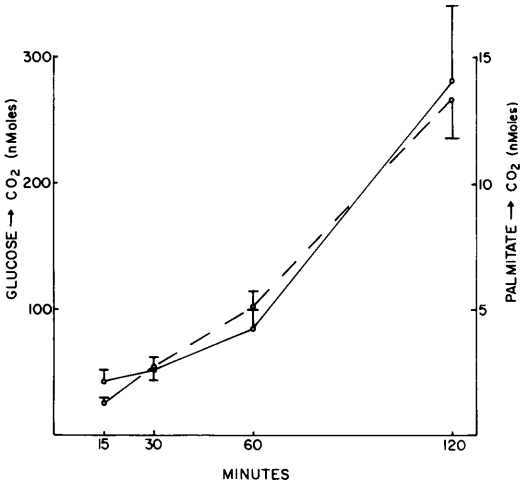

FIG. 1. Brown adipose tissue production of CO_2 from glucose (O- - -O) and palmitate (O——O) in 4ST line rats during 15-, 30-, 60-, and 120-min incubations. Values expressed as nmole converted to CO_2/100 mg tissue ± SEM.

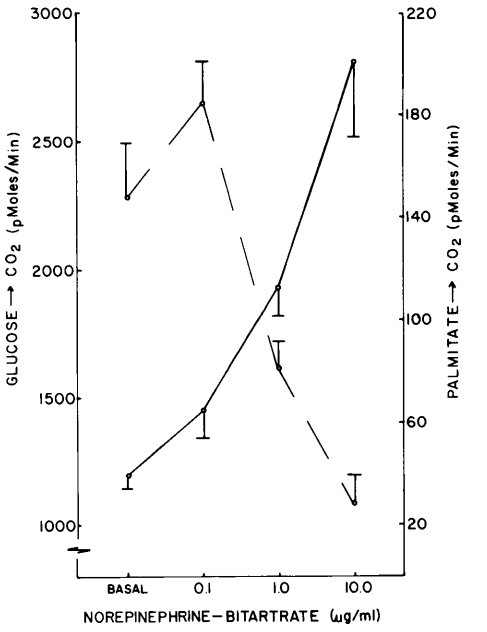

FIG. 2. Brown adipose tissue production of CO_2 from glucose (O- - -O) and palmitate (O——O) in 4St line rats at various concentrations of norepinephrine-bitartrate. Values expressed as pmole converted to CO_2/100 mg/min ± SEM.

to WAT and greater than liver. On a total organ per metabolic body size basis, BAT oxidized palmitate to CO_2 slower than liver and faster than WAT in lean animals. BAT from obese animals oxidized palmitate to CO_2 (per g/kg$^{0.75}$) at a rate similar to liver and faster than WAT. In addition, NE caused a shift to greater oxidative rates in obese BAT compared to obese liver. Ketogenesis (per g/kg$^{0.75}$) in BAT was less than liver and similar to WAT. Esterification (per g/kg$^{0.75}$) in BAT of lean rats was less than liver and WAT. Under basal conditions, obese BAT had rates of esterification (per g/kg$^{0.75}$) similar to liver and less than WAT; however, with NE, obese BAT esterification rates were less than liver and WAT.

Genotype effects. BAT from lean and obese rats oxidized palmitate to CO_2 (per 100 mg) at similar rates, while obese animals oxidized less palmitate to CO_2 in liver and WAT. Similarly, ketogenic rates (per 100 mg) were not different in lean and obese BAT but were lower in obese liver and WAT. Lean and obese rats had similar rates of esterification (per 100 mg) for all

TABLE I. INTERSCAPULAR BROWN ADIPOSE TISSUE, LIVER, AND INGUINAL WHITE ADIPOSE TISSUE WEIGHTS IN LEAN AND OBESE RATS AT 5 WEEKS OF AGE

	Weight (g)		Relative Metabolic Weight (g/kg$^{0.75}$)	
	Lean	Obese	Lean	Obese
Body	105 ± 4	135 ± 4[a]		
Interscapular BAT	0.44 ± 0.03	1.24 ± 0.12[a]	2.37 ± 0.09	5.55 ± 0.48[a]
Liver	4.75 ± 0.19	6.66 ± 0.30[a]	25.7 ± 0.5	29.9 ± 0.9[a]
Inguinal WAT	0.72 ± 0.06	2.98 ± 0.20[a]	3.83 ± 0.24	13.38 ± 0.68[a]

[a] Indicates a significant difference between lean and obese ($P < 0.05$). Values are expressed as means ± SEM ($n = 10$).

TABLE II. INTERSCAPULAR BROWN ADIPOSE TISSUE, LIVER, AND INGUINAL WHITE ADIPOSE TISSUE METABOLISM OF PALMITATE IN LEAN AND OBESE RATS AT 5 WEEKS OF AGE

	(nmole/100 mg)*				(nmole/g/kg$^{0.75}$)**			
	Basal		Norepinephrine		Basal		Norepinephrine	
	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese
Interscapular BAT								
CO_2	5.42 ± 0.52	6.29 ± 1.10	16.18 ± 2.18^{c}	22.96 ± 3.62^{c,d}	128 ± 14	385 ± 53^{b}	317 ± 39^{c}	1198 ± 169^{b,c}
Ketone	1.38 ± 0.12	1.16 ± 0.16	1.36 ± 0.09	1.19 ± 0.13	33 ± 4	60 ± 4^{b}	32 ± 2	66 ± 9^{b}
Triglyceride	114 ± 8	120 ± 9	66 ± 5^{c}	77 ± 6^{c}	2689 ± 200	6709 ± 617^{b}	1560 ± 120^{c}	4089 ± 353^{b,c}
Liver								
CO_2	2.01 ± 0.14^{a}	1.18 ± 0.08^{a,b}	1.76 ± 0.10^{a}	1.13 ± 0.08^{a,b}	516 ± 36^{a}	352 ± 26^{b}	450 ± 23^{a}	338 ± 25^{a,b}
Ketone	3.20 ± 0.32^{a}	1.32 ± 0.14^{b}	2.95 ± 0.24^{a}	1.25 ± 0.10^{b}	816 ± 79^{a}	391 ± 41^{a,b}	753 ± 56^{a}	370 ± 28^{a,b}
Triglyceride	22.8 ± 1.3^{a}	22.9 ± 1.1^{a}	24.5 ± 1.1^{a}	27.1 ± 4.4^{a}	5902 ± 388^{a}	6886 ± 434	6308 ± 617^{a}	3295 ± 997^{a}
Inguinal WAT								
CO_2	1.18 ± 0.08^{a}	0.85 ± 0.04^{a,b}	1.11 ± 0.12^{a}	0.76 ± 0.06^{a}	45 ± 4^{a}	95 ± 9^{a,b}	41 ± 4^{a}	82 ± 9^{a,b}
Ketone	0.76 ± 0.07^{a}	0.46 ± 0.07^{a,b}	0.78 ± 0.03^{a}	0.32 ± 0.04^{a}	30 ± 4	62 ± 13^{b}	26 ± 2^{a}	43 ± 5^{a,b}
Triglyceride	132 ± 9	154 ± 17	81 ± 8^{c}	117 ± 22	5188 ± 623^{a}	20,888 ± 2593^{a,b}	3111 ± 377^{a,c}	15,563 ± 2572^{a,b}

* Values are expressed as nanomoles of palmitate converted to CO_2; ketones or triglyceride per 100 mg of tissue ± SEM (n = 10) during a 2-hr incubation.

** Values are expressed as nanomoles of palmitate converted to CO_2; ketones or triglyceride per total tissue weight (g) per body weight (kg$^{0.75}$) ± SEM (n = 10) during a 2-hr incubation.

[a] Indicates a significant difference between liver and brown adipose tissue or between white adipose and brown adipose within genotype and within basal or norepinephrine treatments ($P < 0.05$).

[b] Indicates a significant difference between lean and obese within tissue and within basal or norepinephrine treatments ($P < 0.05$).

[c] Indicates a significant difference between basal and norepinephrine treatments within genotype and within tissue ($P < 0.05$).

[d] Indicates a significant difference between lean and obese littermate pairs in absolute response to norepinephrine as determined by paired t ($P < 0.05$).

tissues. In comparison to lean rats, obese rats oxidized palmitate to CO_2 (per g/kg$^{0.75}$) more rapidly in BAT and WAT, and slower in liver. Obese BAT and WAT ketogenic rates (per g/kg$^{0.75}$) were greater compared to lean, while rates were slower in obese liver. Rates of esterification (per g/kg$^{0.75}$) were greater in BAT and WAT of obese animals compared to lean, while hepatic esterification rates were similar.

Norepinephrine effects. NE increased CO_2 production from palmitate in BAT, while liver and WAT were not affected. Rates of ketogenesis were not altered with the addition of NE. Esterification rates were decreased by NE in lean and obese BAT, and lean WAT; and it was not affected by NE in lean and obese liver and obese WAT.

Paired *t* analysis of lean and obese littermates indicated that the oxidative response to NE (absolute change in palmitate oxidized in CO_2) was greater in BAT of obese rats compared to lean. The average oxidative response was 5.92 nmole greater in obese BAT, with a *t* value of 2.57 ($P < 0.025$). Paired *t* analysis indicated that esterification response difference in lean BAT was 0.6 nmole greater, with a *t* value of 0.050 (NS); while the difference was 22 nmole in lean WAT with a *t* value of 0.93 (NS).

Discussion. Previous research has demonstrated high rates of fatty acid oxidation in BAT mitochondria (16, 17). In addition, BAT slices when depleted of endogenous lipid no longer greatly increase O_2 consumption upon addition of norepinephrine (18). Lafrance *et al.* (10) demonstrated that *in vivo* norepinephrine infusion in cold-adapted rats caused greater O_2 consumption and a lower respiratory quotient, while previous work demonstrated that BAT of cold-adapted rats contributed about 60% to the *in vivo* caloric response to norepinephrine (5). These and other data have indicated that fatty acids were the predominant substrate during BAT thermogenesis. However, no data are available to demonstrate preferential BAT oxidation of fatty acids over glucose with alterations in norepinephrine status. In the present study it was demonstrated that with increasing concentrations of NE, *in vitro* palmitate oxidation rates increased while glucose oxidation rates decreased in 4St line BAT. Assuming 23 mole of O_2 consumed per mole of palmitate and 6 mole of O_2 consumed per mole of glucose, the *in vitro* data indicate no overall change in BAT O_2 consumption at the various norepinephrine concentrations (Fig. 2). This inability to demonstrate norepinephrine stimulation of overall oxidation probably was due to dilution of palmitate specific activity by intracellular fatty acids. Such a conclusion was supported by data in Fig. 1. Glucose oxidation proceeded in a constant linear fashion through 120-min incubations. Palmitate oxidation was curvilinear, suggesting either an increasing rate of palmitate oxidation with time or an increasing specific activity of palmitate with time. The latter alternative seems likely and supports the concept that the predominant substrate during BAT thermogenesis is endogenously derived fatty acid.

As a result of the preceding work, only palmitate metabolism was analyzed in BAT of lean and obese Zucker rats, using liver and WAT for tissue comparisons. Norepinephrine-bitartrate at 1.0 μg/ml was used since it significantly altered BAT palmitate oxidation but permitted analysis between genotypes at submaximal levels of norepinephrine stimulation. Palmitate oxidation rates were similar between 4St line and lean Zucker rats under both basal states ($5.00 \pm .49$ vs 5.42 ± 0.52, respectively) and NE (1.0 μg) stimulated states (14.48 ± 2.16 vs 16.18 ± 2.18, respectively).

The following observations demonstrated that substrate flux in BAT was not contributing to obesity in the "fatty" Zucker rat: (i) total interscapular BAT weight was greater in obese rats, (ii) basal palmitate oxidation in BAT was similar in lean and obese rats when expressed per 100 mg of tissue and was much greater in obese rats when expressed per total tissue weight per metabolic body size, (iii) norepinephrine stimulated oxidation of palmitate to a greater extent in BAT of obese compared to lean rats, and (iiii) hepatic oxidation and ketogenesis was reduced in obese compared to lean rats. Prior to 5 weeks of age, rats with the obese genotype have greater hepatic lipogenesis (19), larger WAT cells

(20), defective thermogenesis (1), more body fat (21), and lower O_2 consumption (6). The metabolic activity of obese BAT, as a separate entity, cannot be responsible for the alterations presented above. However, within the animal, BAT may not be functioning normally, as impaired autonomic neural activity has been related to other metabolic abnormalities in the obese Zucker rat (3, 22).

Decreased oxygen consumption of obese rats (2) was better accounted for by alterations in hepatic oxidation of palmitate, as opposed to BAT. The increased efficiency of energy deposition (23) and decreased body temperature of obese Zucker rats (1) cannot be attributed to altered BAT palmitate oxidation or impaired BAT sensitivity to norepinephrine.

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