Metabolic features of diet-induced obesity without hyperphagia in young rats

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LEVIN, BARRY E., JOSEPH TRISCARI, AND ANN C. SULLIVAN. *Metabolic features of diet-induced obesity without hyperphagia in young rats.* Am. J. Physiol. 251 (Regulatory Integrative Comp. Physiol. 20): R433–R440, 1986.—Diet-induced obesity (DIO) developed in 1-mo-old male Sprague-Dawley rats over an 8-wk period on a relatively high-fat (16%) high-calorie (4.6 kcal/g) diet (DIO diet). Percent carcass lipid (56%) and body weight gain (15%) were greater, whereas food intake was decreased over the first 3–5 wk in DIO diet- compared with chow-fed controls. Overall, 8-wk body weight gain (15%), percent carcass lipid (26%), and feed efficiency (15%) were greater, but food intake was not increased. Norepinephrine (NE) turnover rate, indicative of organ sympathetic activity, increased in interscapular brown adipose tissue (IBAT; 57–218%), heart (21–44%), and pancreas (25%) during the first 3 wk and remained elevated for the entire 8 wk. IBAT weight (51%) and in vitro lipolytic capacity (68%) increased by 1 wk and brown adipocyte size (43%) by 3 wk; IBAT thermogenic capacity (maximal NE-stimulated in vitro O_2 consumption) increased by 5 wk (39%). Plasma insulin levels were similar in both diet groups over the entire 8-wk period. Why DIO diet-fed rats had increased metabolic efficiency is unknown, but activation of IBAT metabolism and thermogenesis failed to prevent the development of DIO.

brown adipose tissue; sympathetic nervous system; norepinephrine; oxygen consumption; lipolysis; thermogenesis; insulin

WHEN RATS ARE CHRONICALLY EXPOSED to diets with high energy content, they usually overeat and become obese (5, 24, 25). Young rats fed high-energy diets characteristically decrease their metabolic efficiency with the end result that they become less obese than would be predicted from the amount of energy consumed (21). This phenomenon is usually associated with an increase in thermogenic capacity of the animal as mediated by the sympathetic nervous system [SNS (21, 22)]. We previously found that when 3-mo-old Sprague-Dawley rats were fed a high-energy semisynthetic diet for 3–5 mo, about half of the animals became obese, usually without a demonstratable increase in overall food intake (16, 19). Although increased sympathetic activity was seen in brown adipose tissue (BAT) after 1 wk of exposure to the diet, sympathetic activity had returned to control levels in BAT and was below control levels in their pancreases by 3 mo on the diet (18, 19). Those rats that did not become obese on this diet tended to decrease their caloric intake even more than chow-fed controls and obese rats (16). Fischer F-344 rats fed the same diet became only half as obese as the Sprague-Dawley rats and, like the obesity-resistant Sprague-Dawley rats, showed no change in pancreatic SNS activity (19). This suggested that either differences in organ sympathetic activity and/or the amount of diet consumed might be responsible for the degree to which animals resisted the development of diet-induced obesity (DIO). Because young rats appear to have an even greater capacity to resist the onset of obesity (21), we fed 1-mo-old male rats our high-energy diet for an 8-wk period in an attempt to identify those factors that might contribute to this obesity resistance.

METHODS

Animals and diet. One-month-old male Sprague-Dawley rats (Charles River Breeding Labs) were received at least 1 wk before use and kept on a 12-h light-dark cycle at 23–24°C. Rats were randomized by weight and fed either powdered Purina rat chow (4.0 kcal/g) or a powdered semisynthetic diet (BioServ) composed of 47% rat chow, 8% corn oil, and 47% sweetened condensed milk that contained (by weight) 4.6 kcal/g, 16% fat, 55% carbohydrate, and 16% protein [DIO diet (18)]. Caloric contents of the two diets were determined by bomb calorimetry. In total, two complete sets of experiments were carried out in each of which individual groups of 20 rats each were fed either chow or the DIO diet for periods of 1, 3, 5, or 8 wk. In the first set of experiments, food intake and body weight were monitored every 2–3 days, and the animals were used for carcass composition, organ norepinephrine (NE) turnover, plasma insulin, and BAT lipolysis experiments. In the second set of studies, body weights only were followed at weekly intervals, and rats were used for BAT composition, cell size and number, and O_2 consumption (V_{O_2}) determinations.

Organ NE turnover. Rats in each diet and time period group were divided into three groups of six to seven rats each: one group was not injected and served as a control for endogenous norepinephrine levels; a second group was injected with the NE synthesis inhibitor α-methyl-*p*-tyrosine (AMPT; 250 mg/kg ip) at 0 h and decapitated at 3.5 h, while a third group received 250 mg/kg AMPT at 0 h and 125 mg/kg at 3.5 h and were decapitated 7 h after the initial injection (1, 18). Rats were allowed ad libitum access to food and water throughout the experi-

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mental period. Whole heart, pancreas, and interscapular BAT (IBAT) pads were quickly removed, cleaned, weighed, and frozen at −7°C. NE levels were determined in aliquots of tissue homogenized in 0.1 N perchloric acid containing 10 pg/μl (free base) dihydroxybenzylamine as an internal standard. Catecholamines were adsorbed to alumina, and the acidic eluates were analyzed for NE by reversed-phase high-performance liquid chromatography (11).

BAT composition and metabolism. IBAT pads were removed and weighed, and aliquots were taken for lipid (6) and protein (20) determinations. The remainder of the tissue was subjected to collagenase digestion by modification of the method of Bukowiecki et al. (3). Briefly, minced tissue was incubated at 37°C for 20 min in Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 4 mg/ml type II collagenase (Worthington) in the presence of fatty acid-free albumin. Separated cells were washed and counted by hemacytometer, and correction was made for recovery (15–20%) using determination of the lipid content of the separated cells vs. that seen in the whole pad (15). Cell size and type (multi- vs. unilocular) were determined microscopically (12).

In vitro lipolysis was determined on collagenase-separated cells using the liberation of glycerol as an index of lipolysis (12). In vitro Vo_2 was determined polarographically using a Clarke style electrode (Yellow Springs Instruments) according to the method of Bukowiecki et al. (3). NE (10^{-4} to 10^{-9} M) was used to stimulate both lipolysis and Vo_2.

Plasma constituents and body composition. Trunk blood from decapitated rats not treated with AMPT was assayed for insulin by radioimmunoassay using authentic rat insulin (Novo) as a standard (13). Carcass composition was determined after saponification of six to eight individual rats from each diet and time period group (not treated with AMPT) selected on random weight distribution within that group. Carcass lipid was determined after saponification in 85% ethanol containing 10% KOH (26). Carcass protein was determined on an acidified aliquot of the saponified carcass material using the Kjeldahl nitrogen procedure (26).

Statistics. Body weight gain and food intake were analyzed by two-way analysis of variance using repeated measures design. Feed efficiency was calculated by dividing the average weight gain (in $g \cdot rat^{-1} \cdot day^{-1}$) by the average food intake (in $kcal \cdot rat^{-1} \cdot day^{-1}$) for each individual rat over a given 1-wk period, and data thus derived were analyzed as above. NE turnover data were analyzed by the method of Brodie et al. (1), and the fractional turnover rates (k = 1/slope of log NE vs. time) were compared by analysis of covariance. All data found to be significant by analysis of variance were further analyzed by Duncan's multiple range test where differences were considered statistically significant if $P = 0.05$ or less. All other parameters were compared between the two diet groups by t test for unpaired groups.

RESULTS

Body weight gain, food intake, feed efficiency, and plasma insulin levels. Rats fed the DIO diet gained weight at the same rate as chow-fed rats until the fifth week at which time they had gained 15% more weight (Fig. 1). Thereafter, they maintained a similarly accelerated rate of gain so that for the remaining 3 wk the DIO diet-fed rats still had gained an average of only 15% more weight than chow-fed controls [$F(1,38) = 12.35$, $P = 0.001$]. Overall, the DIO diet-fed rats had a 15% greater total body weight gain over the entire 8-wk period (Table 1). Despite the greater weight gain in the DIO diet-fed rats, their total food intake over the 8-wk period was the same as that for chow-fed rats (Table 1) and was actually significantly lower than controls from the third to sixth

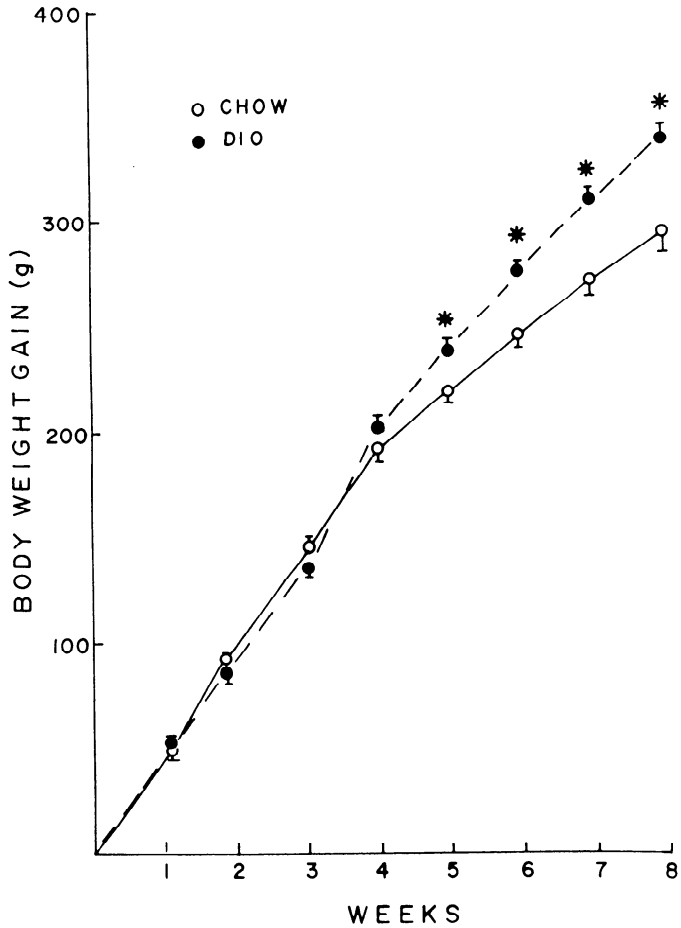

FIG. 1. Body weight gain in chow- (*open circles*) and diet-induced obesity (DIO) diet-fed (*closed circles*) rats over 8-wk period. Data represent means ± SE (*horizontal bars*) body weight gains (g) for groups of 20 rats in each diet group. * $P = 0.05$ or less when body weight gain at given time period was compared by Duncan's multiple range test between diet groups after overall significant difference was found by analysis of variance for repeated measures.

TABLE 1. *Total body weight gain, food intake, and feed efficiency for rats fed chow or DIO diet for 8 wk*

	Chow	DIO
Initial body wt, g	122±3	121±2
Final body wt, g	415±9	460±12*
Body wt gain, g	292±7	336±7*
Total food intake, $kcal \cdot rat^{-1}$	5,944±357	6,097±427
Total feed efficiency, $g \cdot kcal^{-1}$	0.052±0.002	0.060±0.003*

Values are means ± SE for data from rats fed chow ($n = 20$) or diet-induced obesity (DIO) diet ($n = 20$) for total of 8 wk beginning at 1 mo of age. * $P = 0.05$ or less when DIO diet-fed rats were compared with chow-fed rats.

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weeks (Fig. 2). When feed efficiency was estimated by dividing the weight gained in any given 1-wk period by the caloric intake (Fig. 3), the DIO rats showed a decreased efficiency during the first week when their food intake was greater than controls, but weight gain was comparable to controls. Thereafter, however, feed efficiency in the DIO rats was generally greater than controls for the remaining 7-wk period [$F(1,38) = 9.83$; $P = 0.001$]; this amounted to a 15% greater efficiency over the total 8-wk period (Table 1).

The increased metabolic efficiency of the DIO rats expressed itself in a 46% increase in carcass lipid content by the third week on the diet, 2 wk before the difference in body weight gain between the two groups became evident (Table 2). By the fifth week the chow-fed animals had also increased their carcass lipid content so that the 24% difference between the two diet groups did not reach statistical significance ($P < 0.1$). By the eighth week the DIO rats had 84% more total carcass lipid and 74% more lipid as a percent of total body weight. At no time was there a significant difference between carcass protein contents in the two groups. Neither were there significant differences between the plasma insulin levels obtained from trunk blood at the time of decapitation (Table 3).

Organ NE turnover. Turnover of NE in the individual organs was used to assess endogenous sympathetic activity during the development of DIO. Such a relationship has been shown to hold when there is a monoexponential decline in organ NE levels seen after synthesis inhibition with AMPT (1). Such a monoexponential decline of organ NE levels after AMPT was seen in all organs at all time periods studied and is exemplified by Fig. 4, which shows the data for the third week of dietary feeding. Increased fractional turnover of NE, as reflected in a shortened half time of NE disappearance (Table 4, Fig. 4), was seen in both IBAT and pancreas of the DIO rats by the third week on the diet and was still present at the fifth week in IBAT and additionally in heart. NE turnover rate, calculated as the product of endogenous

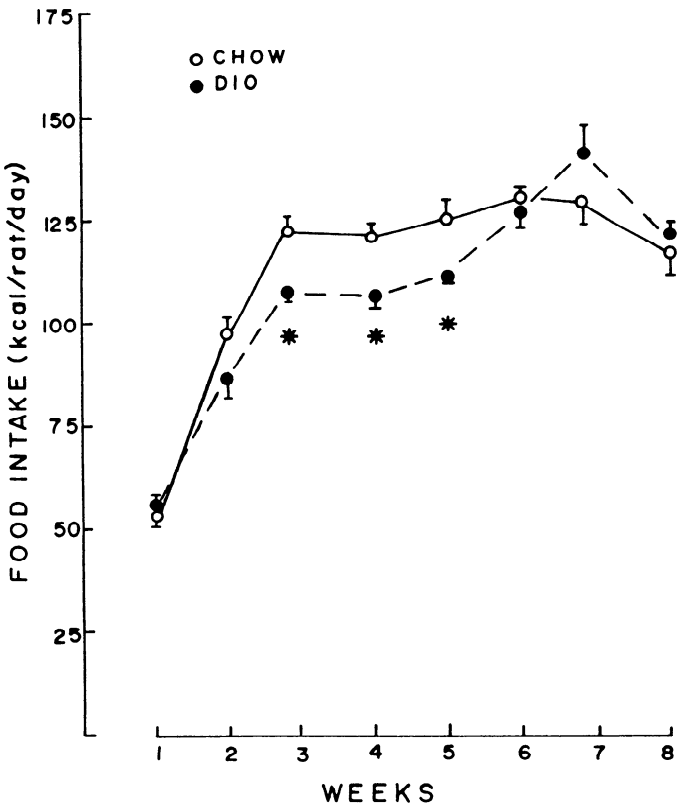

FIG. 2. Food intake (kcal·rat^{-1}·day^{-1}) for chow- (*open circles*) and diet-induced obesity (DIO) diet-fed (*closed circles*) rats over 8-wk period. Data represent means ± SE (*horizontal bars*) of food intake for 20 rats in each diet group. * $P = 0.05$ or less when food intake at given time period was compared by Duncan's multiple range test between diet groups after overall significant difference was found by analysis of variance for repeated measures.

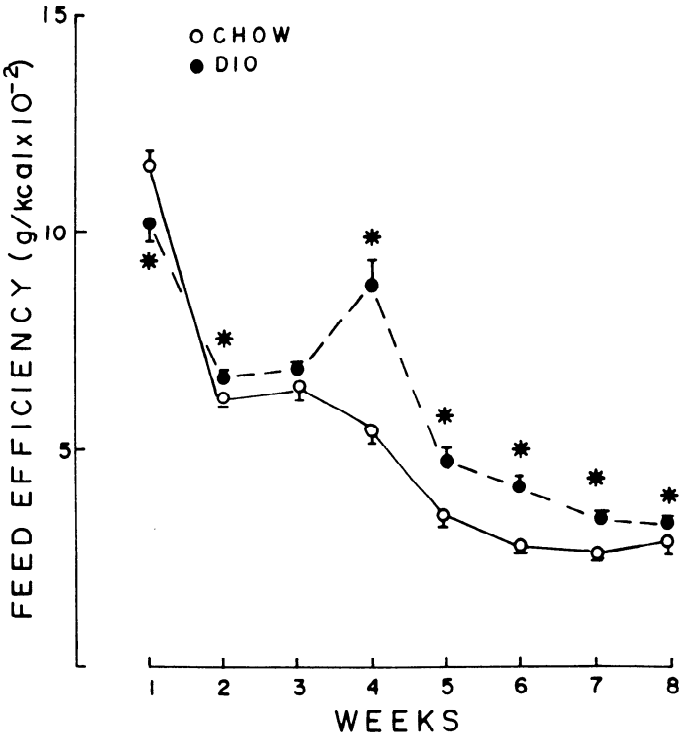

FIG. 3. Feed efficiency (g·kcal^{-1}·10^{-2}) in chow- (*open circles*) and diet-induced obesity (DIO) diet-fed (*closed circles*) rats over 8-wk period. Data represent means ± SE (*horizontal bars*) of feed efficiency as determined by dividing daily weight gain (g·rat^{-1}) by daily energy intake (kcal·rat^{-1}·day^{-1}) for each individual rat from data derived from Figs. 1 and 2. * $P = 0.05$ or less when feed efficiency at given time period was compared by Duncan's multiple range test between diet groups after overall significant difference was found by analysis of variance for repeated measures.

TABLE 2. *Carcass composition in rats fed chow or DIO diet for periods up to 8 wk*

	n	Body wt, g	Protein, g	Protein, %	Lipid, g	Lipid, %
1 wk						
Chow	7	210±4	31.5±2.3	15.1±1.0	12.9±0.9	6.15±0.55
DIO	8	207±6	33.7±2.7	16.3±1.3	14.5±1.2	7.01±0.56
3 wk						
Chow	7	234±6	45.9±1.6	16.1±1.7	16.4±0.5	7.04±0.30
DIO	6	220±8	46.0±3.4	20.9±0.9	24.0±1.0*	11.0±0.7*
5 wk						
Chow	6	325±9	73.4±4.2	22.6±1.4	28.9±2.8	8.90±0.74
DIO	6	320±9	72.3±3.7	23.5±1.2	35.7±3.5	11.2±0.96
8 wk						
Chow	8	439±46	95.3±4.0	21.7±0.9	39.8±4.2	9.07±1.02
DIO	8	464±41	104±5	22.4±1.1	73.1±6.4*	15.8±1.2*

Values are means ± SE for rats fed chow or diet-induced obesity (DIO) diet and killed at time intervals given. * $P = 0.05$ or less when DIO diet-fed rats were compared with chow-fed rats.

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TABLE 3. *Plasma insulin levels for rats fed chow or DIO diet for periods up to 8 wk*

	Chow	DIO
1 wk	8.86±0.17 (10)	8.41±0.19 (10)
3 wk	19.9±4.6 (6)	25.4±5.2 (6)
5 wk	17.0±1.5 (9)	20.7±2.7 (16)
8 wk	20.0±3.6 (5)	24.8±3.8 (10)

Values are means ± SE of resting plasma insulin levels (μU/ml) obtained from trunk blood at time of decapitation from rats fed chow or diet-induced obesity (DIO) diet. Number of animals per group is given in parentheses. Animals were allowed ad libitum access to food and water up until time of decapitation.

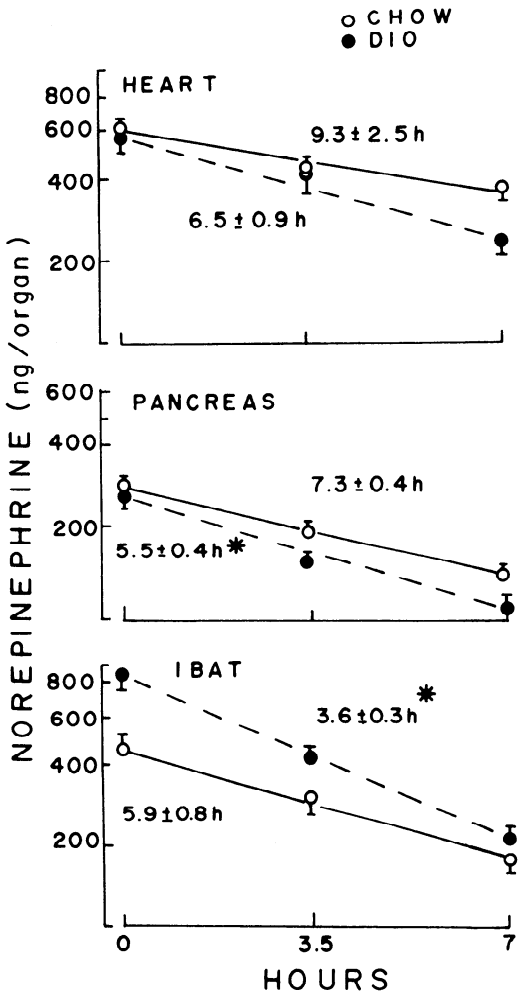

FIG. 4. Norepinephrine (NE) turnover in organs of rats fed chow (*open circles*) or diet-induced obesity (DIO) diet (*closed circles*) for 3 wk. Groups of 5–7 rats from each diet group were either killed for endogenous organ NE levels (0 h) or treated with synthesis inhibitor α-methyl-*p*-tyrosine (AMPT; 250 mg·kg^{-1}) at 0 h and 125 mg·kg^{-1} at 3.5 h. Data represent means ± SE of NE level (ng·organ^{-1}) at each time interval. Half-lives (h) for disappearance of NE after AMPT treatment are given above or below respective lines drawn through mean NE levels as derived from least-squares linear regression analysis (r = 0.89–0.98). * P = 0.05 or less when half-lives were compared between 2 diet groups by analysis of covariance.

NE content and the fractional turnover of NE (Table 4, Fig. 4), was significantly increased in DIO rats in both their hearts and pancreases by the first week and thereafter was increased in all three organs for the remainder of the 8-wk experiment. This increase reached its greatest level at 300% of chow-fed controls in IBAT after 3

TABLE 4. *Organ norepinephrine turnover from rats fed chow or DIO diet for periods up to 8 wk*

	Heart	Pancreas	IBAT
	Endogenous NE levels, ng·organ^{-1}		
1 wk			
Chow	389±26	163±21	538±66
DIO	427±62	154±14	750±55
3 wk			
Chow	608±15	253±4	419±20
DIO	558±16	249±21	855±38*
5 wk			
Chow	547±17	437±21	634±29
DIO	708±25*	370±31	775±33*
8 wk			
Chow	590±50	343±15	863±50
DIO	447±38	370±13	994±77
	Half time for NE disappearance, h		
1 wk			
Chow	6.25±0.95	4.20±0.69	3.37±0.37
DIO	5.42±0.93	4.36±0.39	3.15±0.41
3 wk			
Chow	9.32±2.52	7.34±0.35	5.94±0.77
DIO	6.46±0.91	5.48±0.44*	3.59±0.31*
5 wk			
Chow	12.2±1.6	7.22±0.74	7.66±0.74
DIO	6.52±0.62*	4.00±0.37*	6.30±0.74
8 wk			
Chow	13.9±4.3	10.5±1.9	7.91±1.55
DIO	7.09±0.96	9.95±1.85	6.44±1.24
	NE turnover, ng·organ^{-1}·h^{-1}		
1 wk			
Chow	42.3±1.3	23.5±1.3	103±9
DIO	51.2±1.4*	23.4±1.1	162±12*
3 wk			
Chow	43.4±1.2	24.0±1.4	49.2±1.3
DIO	62.4±1.2*	30.0±1.1*	156±10*
5 wk			
Chow	30.7±5.8	39.9±1.2	56.0±2.2
DIO	75.7±1.2*	64.2±2.3*	84.8±4.3*
8 wk			
Chow	32.8±1.2	20.5±0.9	72.4±4.2
DIO	49.2±2.6*	27.5±0.9*	107±9*

Values are means ± SE for groups of 15–20 rats per diet group at each time interval. DIO, diet-induced obesity; NE, norepinephrine; IBAT, interscapular brown adipose tissue. Endogenous NE levels were determined in organs from untreated rats. Half time for NE disappearance and NE turnover were determined according to Brodie et al. (1) from disappearance of NE after synthesis inhibition with α-methyl-*p*-tyrosine. * P = 0.05 or less when values from DIO diet-fed rats were compared with those from chow-fed controls. Correlation coefficients for decline of NE levels after synthesis inhibition were r = 0.88–0.98.

wk on the DIO diet. In many cases the increased turnover rate in the absence of increased fractional turnover reflected the increase in endogenous organ NE levels (Table 4, Fig. 5). Again, this increase was greatest in the IBAT pads of DIO rats after 3 wk on the DIO diet when NE levels were 200% of chow-fed controls.

Brown adipose composition and metabolism. IBAT pads in the DIO rats were significantly heavier than those from chow-fed controls after only 1 wk on the DIO diet (Fig. 6). This increase in weight was not reflected as an increase in cell size until the third week when multilocular cells were larger in the DIO IBAT pads; thereafter, the DIO IBAT unilocular cells were larger than controls (Table 5). At no time was there a significant difference between the number of cells or percentage of multilocular

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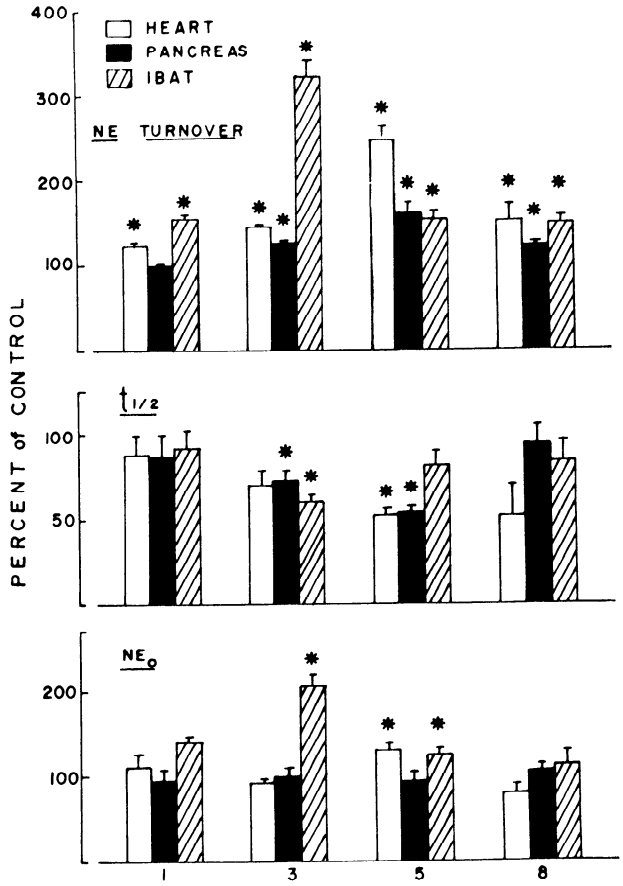

FIG. 5. Summary of organ norepinephrine (NE) turnover data for groups of 15–20 rats per time period that were fed either chow or diet-induced obesity (DIO) diet for up to 8 wk. Data are given for DIO diet-fed rats as means ± SE percent of chow-fed controls. NE_0, endogenous NE levels; $t_{1/2}$, half-life; NE turnover, product of NE_0 levels and fractional disappearance constant (k) for NE after synthesis inhibition with α-methyl-p-tyrosine; IBAT, interscapular brown adipose tissue. Data for each diet group are given in Table 4. * $P = 0.05$ or less when data were compared between 2 diet groups.

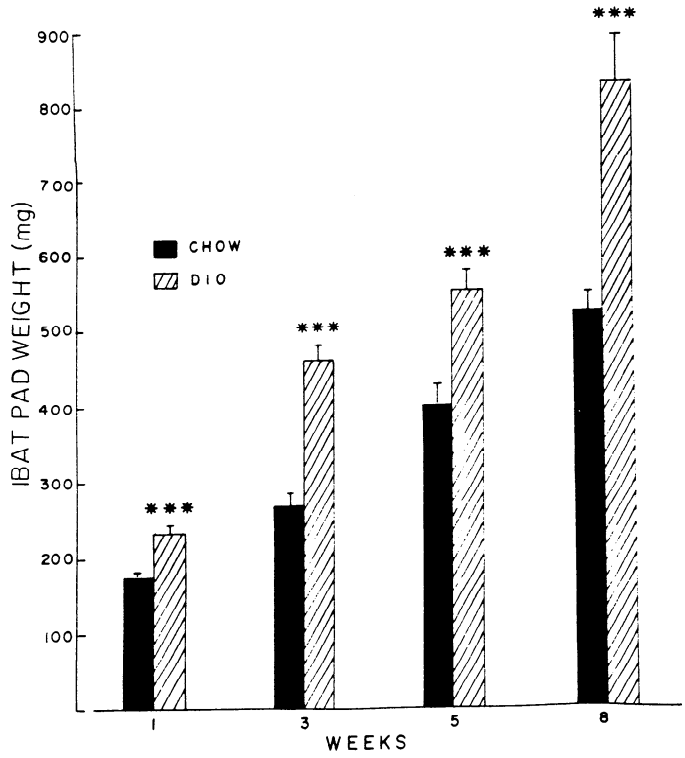

FIG. 6. Interscapular brown adipose tissue (IBAT) pad weights in groups of 15–20 rats per time period fed either chow or diet-induced obesity (DIO) diet for up to 8 wk. Data are means ± SE for pad weights (mg). *** $P < 0.001$ when pad weights in rats from 2 diet groups were compared by t test for unpaired groups.

cells per pad in IBAT pads from the two diet groups (Table 5). By the eighth week on the diet, when DIO IBAT pads weighed 60% more than those from controls, the fractional protein content of DIO pads (23.6 ± 1.5%) was 61% greater ($P < 0.05$) than chow-fed controls (14.7 ± 2.4%), while the fractional lipid content of DIO pads (57.1 ± 6.4%) was only 29% greater ($P < 0.05$) than controls (44.2 ± 2.2%). Therefore, most of the hypertrophy of the IBAT pads in DIO diet-fed rats was due to an increase in protein rather than lipid content.

Assessment of IBAT metabolic activity was performed in vitro by measuring basal and NE-stimulated increases in both lipolysis (Table 6) and V_{O_2} (Fig. 7). Although maximal in vitro lipolytic activity was increased to 168% of controls in DIO IBAT pads after only 1 wk on the DIO diet, it was not until the fifth week that this increase in precursor availability (fatty acids released by lipolysis) was reflected in an actual increase in thermogenic capacity of the multilocular cells in the pad. At this time the sensitivity for NE stimulation of V_{O_2} was only 36% of control (Fig. 7), probably reflecting downregulation of the receptors due to continued sympathetic stimulation. It was not until the eighth week on the DIO diet that there was an overall increase in whole IBAT (multi- plus unilocular cells) thermogenic capacity. Therefore, continued exposure to a high-energy diet without the development of hyperphagia led to sympathetic and metabolic activation in BAT, but animals on the diet still became obese.

DISCUSSION

Previous studies have shown that when young rats are fed high-calorie diets of various compositions they generally decrease their metabolic efficiency so as to resist the development of DIO (21, 22, 27). The ability to resist DIO appears to be dependent on a number of varied factors including hyperphagia and dietary composition as well as the age, strain, and sex of the rat tested (19, 24, 25). In general, presentation of high-energy diets leads to increased energy intake followed by stimulation of SNS activity in various organs (10, 18) and an eventual increase in the thermogenic capacity of the whole animal (9, 21, 22, 27). Although our 1-mo-old Sprague-Dawley rats fed the DIO diet in the current studies never developed hyperphagia, there was a sustained increase in basal SNS activity in their hearts, pancreases, and IBAT pads. Based on previous studies in older (3 mo) Sprague-Dawley rats that had normal or decreased organ sympathetic activity after chronic exposure to this diet (16, 18, 19), it was expected that younger rats might resist the development of DIO by continued activation of their SNS. Yet the 1-mo-old rats, in the absence of any significant increase in energy intake and the presence of elevated sympathetic activity, still became as obese, rel-

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TABLE 5. *Brown adipocyte cell size and number in IBAT pads from rats fed chow or DIO diet for periods up to 8 wk*

	Chow	DIO
	Cell size, μm	
1 wk		
Multi	18.4±1.6	18.4±1.5
Uni	26.2±2.2	24.2±2.0
3 wk		
Multi	20.1±1.9	28.8±2.8*
Uni	28.5±2.0	36.2±4.0
5 wk		
Multi	24.1±2.2	22.9±2.1
Uni	26.4±2.4	34.1±2.1*
8 wk		
Multi	31.4±2.0	35.7±0.9
Uni	33.7±0.9	39.8±1.2*
	Cell number, $10^6 \cdot pad^{-1}$	
1 wk		
Total	5.53±0.43	6.13±0.60
% Multi	36±4	35±7
3 wk		
Total	6.60±0.59	7.32±0.66
% Multi	29±3	28±3
5 wk		
Total	8.12±0.79	6.52±0.71
% Multi	28±6	27±4
8 wk		
Total	8.77±0.97	6.00±0.60
% Multi	15.2±3.6	11.5±2.7

Values are mean ± SE for IBAT pads from groups of 6–10 rats per diet group at each time interval. IBAT, interscapular brown adipose tissue; DIO, diet-induced obesity; multi, multilocular cells; uni, unilocular cells. * $P = 0.05$ or less when data from DIO diet-fed rats were compared with those from chow-fed controls.

TABLE 6. *In vitro lipolysis in brown adipocytes from IBAT pads from rats fed chow or DIO diet for periods up to 5 wk*

	n	Basal	V_{max}
1 wk			
Chow	5	25.8±1.5	190±3
DIO	5	30.1±4.5	320±10*
3 wk			
Chow	4	68.9±1.7	94.9±6.6
DIO	4	141±4*	210±11*
5 wk			
Chow	4	156±2	286±25
DIO	4	310±18*	546±36*

Values are means ± SE of glycerol release ($nmol \cdot 10^6 cells^{-1} \cdot h$) under basal and maximally stimulated (V_{max}) conditions (1 μM norepinephrine). DIO, diet-induced obesity. * $P = 0.05$ or less when DIO diet-fed rats were compared with chow-fed controls.

ative to chow-fed controls, as 3-mo-old rats fed the DIO for 3 (18) or 5 mo (16).

In studies similar to ours, Hogan et al. (9) fed the identical DIO diet to 6-wk-old Sprague-Dawley rats for 8 wk. After 4 and 8 wk on the DIO diet their rats showed an increase in whole animal thermogenic capacity; as measured by in vivo Vo_2, maximal NE-stimulated but not basal Vo_2 was increased. Like our rats, their animals also became obese in the absence of hyperphagia. Therefore, despite tonically elevated sympathetic activity in selected organs and a likely increase in total body thermic capacity to sympathetic stimulation (NE), our rats

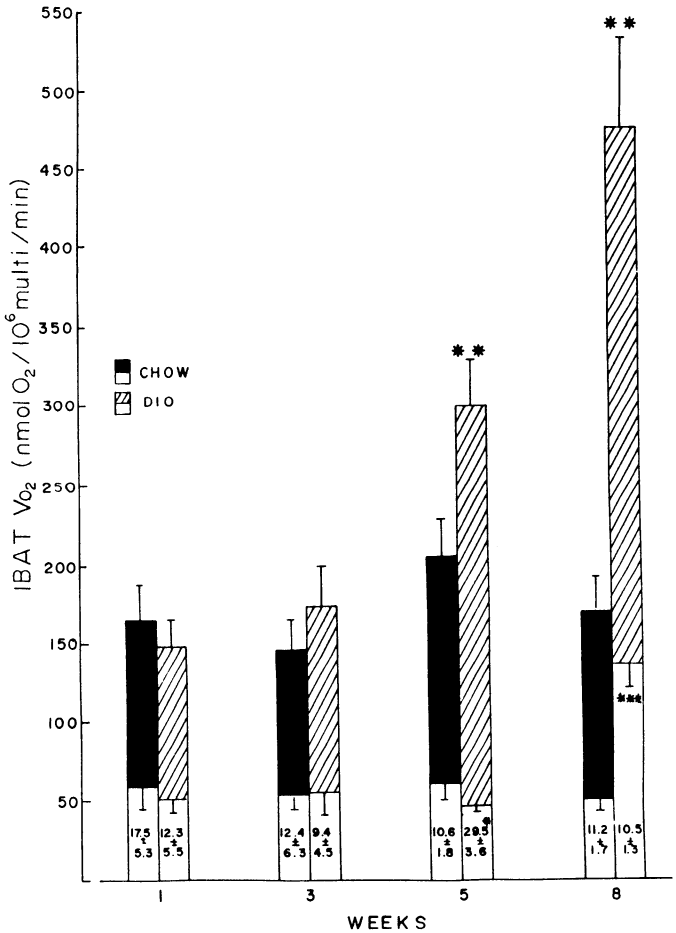

FIG. 7. In vitro O_2 consumption (Vo_2) in 3–4 sets of interscapular brown adipose tissue (IBAT) pads per time period pooled from 2 rats fed chow or diet-induced obesity (DIO) diet for up to 8 wk. Data are means ± SE of Vo_2 ($nmol\ O_2 \cdot 10^{-6}$ multilocular cells $\cdot min^{-1}$). *Open bars*, basal levels of Vo_2; *solid* (chow-fed) or *cross-hatched* (DIO diet-fed) *bars*, maximally stimulated rates of Vo_2 in presence of norepinephrine (NE); *Numbers* in *open bars*, means ± SE of EC_{50} dose (nM) of NE (dose producing half-maximal stimulation) as derived from dose-response curves. * $P < 0.05$; ** $P < 0.01$ when data were compared between 2 diet groups by t test for unpaired samples.

showed an increased rather than decreased metabolic efficiency. This suggests that the diet-induced increase in basal sympathetic activity in the organs studied was insufficient to increase basal or diet-induced thermic output and played little role in combating the development of DIO. Alternatively, the thermogenic output of other organ systems that were not evaluated (e.g., liver and muscle) may have been decreased so as to negate any increased basal or diet-induced thermic output in BAT, heart, and pancreas.

Since the studies of Foster and Frydman (7), it has been widely accepted that BAT is the predominant site of nonshivering thermogenesis in the adult rat. A case has also been made for a primary role for BAT in diet-induced thermogenesis (2, 21, 22). However, the current studies, as well as previous ones from our laboratory, cast some doubt on the functional importance of BAT in defending against DIO. Despite the early increase in IBAT sympathetic activity, NE-stimulated lipolysis, and Vo_2, with peaks at 3, 5, and 8 wk, respectively, rats on the DIO diet continued to deposit increasing amounts of carcass lipid compared with chow-fed controls. Previous

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studies have used each of the three parameters of BAT metabolism as indexes of in vivo thermogenic output; our studies suggest that only in vitro Vo_2 may reflect the functional state of BAT in the intact animal. There was a clear-cut dissociation between the early increase in both IBAT SNS activity and lipolytic capacity, on the one hand, and the much delayed onset of enhanced oxidative capacity on the other. Our current and previous data (16) clearly show that increased lipolytic capacity in BAT is not necessarily accompanied by an increase in oxidative capacity and therefore should not be used as an index of heightened thermic output as has been previously proposed (3, 21). This dissociation can best be explained by the fact that multilocular brown adipocytes account for the majority of Vo_2 in this tissue, while both uni- and multilocular cells are capable of NE-stimulated lipolysis (14). Therefore, although sustained sympathetic stimulation appeared to induce a rapid increase in lipolytic capacity of all of the IBAT cells, this was not translated into increased oxidative capacity in the mitochondria of multilocular cells for a full 5 wk.

Binding of GDP to the 32,000 mol wt protein in BAT mitochondria has also been used as an index of thermogenic capacity of BAT (2, 8). However, Himms-Hagen et al. (8) showed that the increase in BAT GDP binding seen in hyperphagic cafeteria-fed rats was due to an unmasking of existing binding sites as is seen with continued NE exposure and not to an actual increase in the amount of binding protein as is seen in cold-acclimated animals. Although Rothwell and Stock (23) have also reported an increase in NE-stimulated blood flow to IBAT of cafeteria-fed rats in vivo, the results of Himms-Hagen et al. (8) strongly suggest that there is a basic difference between the change in BAT induced by increased energy intake vs. chronic cold exposure. In fact, our results, when combined with the similar studies of Hogan et al. (9), also suggest that GDP binding in BAT may not represent a true measure of the in vivo functional status of this tissue. After 8 wk on the DIO diet, basal and NE-stimulated Vo_2 were increased in both the individual multilocular cells and the whole IBAT pads of our rats. However, there was no increase in GDP binding to IBAT mitochondrial membranes from 6-wk-old rats fed the same diet for 8 wk (8). Clearly, the individual cells had increased their thermic capacity at that time, but GDP binding gave no hint of this.

If we then accept increased in vitro Vo_2 as a reasonable index of increased in vivo thermic capacity of IBAT, then the combination of increased capacity and tonically elevated sympathetic activity in this tissue was clearly insufficient to prevent the increase in metabolic efficiency and consequent obesity of rats fed the DIO diet. This failure of IBAT to effectively combat the development of DIO, even in the face of a sustained increase in IBAT sympathetic activity, is in keeping with our previous studies (17) in which near total destruction of the sympathetic innervation of BAT in the neonatal period had little effect on body weight gain, carcass composition, or metabolic efficiency. Such results raise serious doubts about the importance of BAT in the regulation of body weight and diet-induced thermogenesis.

If BAT did not play a major role, how else can we explain the inability of our rats to resist the development of DIO despite the absence of hyperphagia? Glucose intolerance and insulin insensitivity have been proposed as factors that predispose rats to develop DIO; rats that become obese on cafeteria diets tend to have such abnormalities of glucose metabolism (4). In addition, when older (3 mo) male Sprague-Dawley rats were fed the DIO diet for 3–5 mo, those that became obese were hyperinsulinemic and/or had decreased pancreatic NE turnover (16, 18, 19). Fischer F-344 rats, fed the same diet for 3 mo, developed no abnormality in pancreatic sympathetic activity and became only half as obese as comparable Sprague-Dawley rats fed this same diet (19). Although this suggests that insulin resistance and/or decreased pancreatic sympathetic activity might predispose older animals to develop DIO, the 1-mo-old rats that became obese in the current studies had normal plasma insulin levels and increased pancreatic NE turnover. Therefore, no major abnormalities in insulin metabolism appeared to accompany or predispose these young rats to the development of DIO.

The degree to which rats overeat on a given diet may be one important factor in determining how well a given rat will resist the development of DIO. Others have shown that obesity can develop in the absence of hyperphagia on certain diets (see, e.g., Ref. 24). Hyperphagia has been a common feature seen in rats fed cafeteria diets where decreased metabolic efficiency and obesity resistance develop (21, 22). Also, when both 3-mo-old male Sprague-Dawley and Fischer F-344 rats were fed our DIO diet for a 3-mo-period the Fischer rats were hyperphagic for the first 3 wk and subsequently became only half as obese as Sprague-Dawley rats, which generally develop little or no hyperphagia on this diet (16, 18, 19). Alteration in dietary content alone does not appear to be adequate to product this change in efficiency. When dietary carbohydrate or fat content have been manipulated in the absence of increased energy intake, little stimulation of sympathetic activity has been shown to occur (28). Only low-protein diets (9.9%) appear to provide such a stimulus for sympathetic activation. The DIO diet is relatively high in fat, sucrose, and energy content compared with chow, and our rats still showed increased sympathetic activity in selected organs despite their lack of hyperphagia. This increase could not be attributed to low protein content of the diet (16%) because carcass protein was similar in chow- and DIO diet-fed rats. Even so, the increased sympathetic activity was not associated with decreased metabolic efficiency. Therefore, hyperphagia may stimulate some nonsympathetically mediated process that enables the animal to resist the development of DIO.

Another obvious way to prevent the onset of obesity is to decrease caloric intake enough to balance any positive deposition of calories in the form of body fat. In 3-mo-old rats exposed to the DIO diet for a 3- to 5-mo period, about half gain no more weight than chow-fed controls. This apparent resistance to obesity appears to be due to a significantly reduced caloric intake (of the DIO diet) compared with both obese and chow-fed control rats (16). Clearly the rats in the current study neither decreased their caloric intake sufficiently to prevent the

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development of obesity nor appropriately stimulated those metabolic processes that allow hyperphagic cafeteria-fed rats to resist the onset of obesity. Because these rats did increase their sympathetic and BAT activity similarly to cafeteria-fed rats, it seems likely that some other explanation for obesity resistance in such animals must be sought. Some of the possible explanations for this finding include *1*) insulin release in response to food intake may have been enhanced in the DIO rats, thus contributing to their increased metabolic efficiency; *2*) meal patterns may have differed on the DIO diet, thus influencing feed efficiency; *3*) the DIO diet probably had more absorbable energy than chow in light of its lower fiber content; however, we have not documented this by measuring fecal or urinary energy losses; and *4*) the higher fat content of the DIO diet may have led to lower energy costs of incorporating dietary energy into the body. Although any of these possibilities may have been operant here, the striking finding of this study was that obesity still developed in the presence of marked sympathetic stimulation and eventual activation of thermogenically active BAT.

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