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Effects of a Glucosidase Inhibitor (Acarbose, BAY g 5421) on the Development of Obesity and Food Motivated Behavior in Zucker (fafa) Rats[1]

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VASSELLI, J. R., E. HARACZKIEWICZ, C. A. MAGGIO AND M. R. C. GREENWOOD. *Effects of a glucosidase inhibitor (acarbose, BAY g 5421) on the development of obesity and food motivated behavior in Zucker (fafa) rats.* PHARMACOL BIOCHEM BEHAV 19(1) 85–95, 1983.—BAY g 5421 (acarbose) inhibits carbohydrate digestion in the gut, thereby reducing the rate of glucose absorption. This experiment tested whether long term administration of acarbose to developing Zucker "fatty" (fafa) rats would, by reducing several lipogenic factors, attenuate lipid deposition and reduce the hyperphagia and increased food motivated behavior of these animals. From 7 to 20 weeks of life groups of fatty and lean (FaFa) control rats were fed 0, 20 or 40 mg acarbose/100 g maintenance diet (45% carbohydrate, 35% fat, 20% protein calories), while an additional fatty and lean group were pair-fed to respective 40 mg acarbose groups. Lean groups fed acarbose exhibited dose dependent reductions of body weight, insulin, triglycerides, retroperitoneal and epididymal pad weight, adipocyte size, LPL activity/cell (retroperitoneal pad only), and lipid deposition both in total grams of fat and as a percentage of carcass weight. Fatty groups fed acarbose exhibited dose dependent reductions of insulin, blood glucose, retroperitoneal pad weight, and, at one of the two doses used, significantly lowered body weight, (40 mg), triglycerides (20 mg) and cholesterol (20 mg). However, acarbose-fed fatty groups failed to show significant reductions of adipocyte size, number or LPL activity/cell in retroperitoneal and epididymal fat pads, and maintained their obese body composition, on a percentage basis, at levels not significantly different from that of the 0 mg fatty control group. Acarbose administration led to an intitial dose dependent reduction of food intake in both genotypes, which persisted for the lean groups. Fatties fed the 20 mg dose showed a gradual tendency (ns) towards increased daily intake, lever pressed at elevated rates for food pellets, and refed at faster rates following fasting. Fatties fed the 40 mg dose maintained their daily intake at fatty control levels, did not lever press at elevated rates, and showed significantly reduced refeeding following fasting. The 40 mg fatty and both lean acarbose treated groups had decreased sucrose solution preference. Possible bases for these differing effects of the drug on feeding behavior by the groups are considered.

Acarbose Glucosidase inhibition Insulin Zucker fafa rat Lipid deposition Food intake Carbohydrate absorption Lipoprotein lipase

IN THE growing genetically obese Zucker "fatty" rat (fafa), hypercelluar-hypertrophic development of adipose tissue is accompanied by hyperphagia [6,40] and a number of metabolic alterations including hyperinsulinemia and hypertriglyceridemia [1, 46, 47]. While hyperinsulinemia is not the earliest metabolic alteration noted in the fatty rat [13,39], its presence throughout development significantly enhances the rate of lipid deposition [2], and may contribute to the hyperphagia and elevated levels of food motivated behavior seen in the developing fatty rat [11,40]. Further, it has been demonstrated in our laboratory and others that hyperinsulinemia and enhanced lipid deposition occur even when hyperphagia is prevented by caloric restriction [3,46], or when a low carbohydrate diet is fed to developing fatty rats [36]. Since persistent hyperinsulinemia in the Zucker fatty rat is highly lipogenic [2], an effective non-invasive method of lowering circulating levels of this hormone would be desirable.

Recently, the glucosidase inhibitor BAY g 5421 (acar-

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TABLE 1
EXPERIMENTAL DIET
(4.52 kcal/g)

	g/100 g	% Calories
Corn starch	26.0	22.5
Sucrose	26.0	22.5
Casein	22.2	20.0
Corn oil	8.9	17.5
Lard	8.9	17.5
Minerals*	6.0	
Vitamins†	2.0	

*Mineral Mix U.S.P. XVII.
†Bio-Serv Vitamin Fortification Mix.

bose) has been introduced as an experimental agent which inhibits the digestion of carbohydrates in the gastrointestinal tract, thereby reducing the availability of monosaccharides for immediate absorption [24, 25, 29]. When consumed daily in extremely small amounts as a dietary admixture by normal rats, this drug results in lowered levels of serum insulin, glucose and triglycerides (TG) [38,45], and, on a more long term basis, in reduced lipid deposition and body weight gain [42,43]. Reductions of body weight gain, serum insulin, glucose, TG and lipid deposition have also been reported for groups of adult Zucker fatty rats administered acarbose in the diet for various periods of time [24]. In addition, a dose dependent reduction of caloric intake was obsérved in adult fatty rats during a 28-day acarbose feeding period [19]. Thus, long term administration of acarbose to growing Zucker fatty rats may attenuate hyperinsulinemia and the development of obesity, and possibly reduce the hyperphagia and elevated food motivated behavior characteristic of this animal during development.

The present experiment was designed to examine the effects of ad lib dietary acarbose administration on insulin levels, lipid deposition, blood borne metabolites, feeding behavior, and body composition of developing Zucker fatty and lean (FaFa) control rats. Acarbose is a non-toxic [28] and slightly sweet compound. Thus, addition of extremely small amounts of acarbose as an admix to the already sweet tasting basal diet of the rats results in virtually no alteration of diet palatability, and guarantees the simultaneous arrival of the drug plus food in the gastrointestinal tract of the animals.

METHOD

Animals and Dietary Treatment

Obese and lean male Zucker rats were obtained from the colony at Vassar College. Obese rats were obtained from breedings between known heterozygous (Fafa) lean females and either (Fafa) lean males or obese (fafa) males. Homozygous (FaFa) lean rats were obtained from litters of established homozygous lean breeding pairs. After birth litter size was normalized to 8–10 pups per dam. At weaning all pups were individually housed in wire mesh hanging cages and fed pelleted laboratory chow (Charles River) ad lib. Rats were maintained in a temperature controlled room (22–24°C) on a 12 hr light-dark cycle (0800–2000 hr).

At 7 weeks of age obese and lean rats were assigned to one of four treatment groups (n=5 per group), and switched to an experimental diet containing no monosaccharides (see Table 1). Three groups of obese and lean rats were fed this diet ad lib with either 0, 20 or 40 mg of acarbose added per 100 g of diet. The fourth obese and lean group were pair-fed the diet (without acarbose) once daily in the amount consumed by the respective 40 mg groups. The groups were maintained on the experimental diet for thirteen weeks. Food intake, water intake and body weight were recorded every 48 hours throughout the study. Five deaths occurred prior to completion of the experiment. Two of these occurred accidentally under ether anesthesia (0 mg and 20 mg fatty groups), one was due to infection (pair-fed fatty group), and two occurred following weight loss (40 mg fatty and lean groups). Final group size is indicated in Table 2. At 20 weeks of age all remaining rats were killed by decapitation following a 3 hr fast. Truncal blood was collected, and plasma samples were frozen for determinations of glucose, TG, cholesterol, free fatty acids (FFA) and immunoreactive insulin.

Adipose Tissue Preparation

Left and right epididymal and retroperitoneal fat pads were removed, weighed, placed in isotonic saline, and sampled for fat cell number and size determinations using electronic counting of osmium-fixed cells as described by Hirsch and Gallian [15].

Adipose tissue homogenates (1:4; wt:vol) were prepared with ice-cold 0.25 M sucrose—1 mM EDTA buffer (pH 7.4) using ground glass-on-glass homogenizing equipment. The homogenates were centrifuged at 12,000 g for 15 min in an IEC refrigerated centrifuge at 4°C. The postmitochondrial supernatant was aspirated from under the fat cake layer and stored at −70°C for lipoprotein lipase (LPL) assay.

Organs and Body Composition

Livers, kidneys, hearts, brains, and gastrocnemius muscle were removed and weighed. The gastrointestinal tract was removed and discarded after mesenteric fat was separated and returned to the carcass. Carcasses were frozen and later homogenized for body composition determinations. Total lipids were extracted in chloroform-methanol (2:1) as described by Folch *et al.* [7] and determined gravimetrically. Total body water was determined by drying and weighing extracted tissue to a constant weight. Fat-free dry weight was calculated by subtracting total water and total fat from total weight. Values include fat depots dissected.

Assay Procedures

LPL in retroperitoneal and epididymal fat pads was assayed using minor modifications [14] of the method described by Schotz *et al.* [31]. Total lipids in adipose tissue were extracted as above. Protein was determined using the colorimetric Biuret method [22]. Insulin was determined by radioimmunoassay using the method of Rosselin *et al.* [26] and pure rat insulin standard (Novo R 171, 20–25 U/mg, Novo Laboratories, Denmark). Plasma TG [27], glucose [18], FFA [35], and cholesterol [8] were determined using established methods as adopted for the Technicon Auto-Analyzer.

Operant Training and Testing

To assess the food motivated behavior of the acarbose treated rats, the groups were trained between 9 and 11 weeks

TABLE 2

MEAN DAILY FOOD INTAKE/RAT/WEEK AND MEAN TOTAL CUMULATIVE INTAKE/RAT (g ± S.E.M.) DURING ACARBOSE TREATMENT PERIOD

		Age in Weeks													
	n	7	8	9	10	11	12	13	14	15	16	17	18	19	Mean Total
fafa															
0 mg/100 g	4	19.1† ±1.4	18.1* ±1.1	17.6 ±1.4	12.8 ±1.3	13.9 ±2.1	17.9 ±2.1	21.0 ±1.4	17.7 ±0.9	19.1 ±2.4	15.9 ±0.9	13.7 ±1.3	15.1 ±1.2	17.8 ±1.6	1379.6 ±100.1
20 mg/100 g	4	17.6† ±1.6	16.6* ±2.0	17.4 ±1.3	14.8 ±0.7	18.6 ±1.8	21.0 ±0.4	19.4 ±0.6	17.6 ±1.6	19.7 ±0.7	18.3 ±1.9	17.6 ±1.7	17.7 ±1.2	18.6 ±1.0	1454.3* ± 65.8
40 mg/100 g	4	13.8* ±1.3	15.1 ±1.2	14.6 ±1.4	12.8 ±2.1	15.6 ±2.6	16.1 ±2.1	17.6 ±2.5	15.3 ±2.1	16.0 ±2.6	17.9 ±2.2	15.6 ±2.3	15.6 ±2.0	15.2 ±1.9	1254.4 ±139.6
Pair-Fed	4	13.0 ±0.3	13.8 ±0.4	14.0 ±0.6	13.3 ±1.2	13.8 ±0.1	14.8 ±0.3	16.7 ±0.03	15.3 ±0.05	15.8 ±0.02	16.1 ±0.9	14.4 ±1.2	13.0 ±2.2	15.6 ±1.3	1108.1 ± 38.4
FaFa															
0 mg/100 g	5	13.8 ±0.5	14.8 ±0.5	16.1 ±1.4	11.0 ±1.7	18.1 ±0.9	20.5 ±1.2	19.9 ±0.9	17.9 ±0.4	18.4 ±0.7	18.3 ±0.4	16.6 ±0.9	14.9 ±0.3	16.9 ±0.4	1342.8 ± 46.9
20 mg/100 g	5	10.4§ ±0.1	12.8 ±0.4	15.0 ±0.4	13.4 ±0.8	16.2 ±0.6	17.8 ±0.8	16.9‡ ±0.8	16.9 ±0.5	18.1 ±0.6	17.6 ±0.6	15.9 ±0.5	15.9 ±0.6	16.8 ±0.5	1257.0 ± 34.5
40 mg/100 g	4	10.3§ ±0.4	12.8 ±0.9	14.1 ±0.5	12.1 ±0.4	14.0‡ ±0.8	16.2‡ ±0.4	15.6‡ ±0.9	14.7§ ±0.6	16.0 ±0.6	15.6‡ ±0.9	15.0 ±0.3	14.9 ±0.8	15.0 ±0.8	1158.1‡¶ ± 17.2
Pair-Fed	5	10.1 ±0.15	12.3 ±0.9	12.7 ±0.9	12.8 ±0.1	12.8 ±0.1	12.7 ±0.09	14.0 ±0.05	13.1 ±0.2	15.3 ±0.8	15.2 ±0.8	14.7 ±0.9	14.0 ±1.7	12.9 ±2.3	1025.6 ± 20.5

*Significantly different from respective lean group, $p<0.05$.
†Significantly different from respective lean group, $p<0.01$.
‡Significantly different from respective 0 mg group, $p<0.05$.
§Significantly different from respective 0 mg group, $p<0.01$.
¶Significantly different from pair-fed group, $p<0.01$.

of age (weeks 3–5 of acarbose treatment) to lever press for food pellets on a variable interval (VI) 80 sec schedule of reinforcement. Response rates on long interval schedules are more likely to represent an animal's motivation to initiate feeding than the amount of food consumed in a meal [32]. To minimize possible contrast effects, 45 mg food reward pellets of the exact same composition as the experimental diet were used (prepared by Bio-Serv Inc.). The groups were first trained on a continuous reinforcement schedule (CRF), i.e., one 45 mg food pellet for each lever press, then exposed to three 1 hr sessions of CRF, followed by six 1 hr sessions of VI training, with the variable intervals increasing from 20 to 80 sec as training progressed. A moderate level of food deprivation (12 hr) was sufficient for training.

At 14 weeks of age (8th week of acarbose treatment) the effect of three levels of food deprivation upon responding for food was determined. All groups were tested on VI 80 for one hour following 0, 12 and 24 hr of fasting. During the test week deprivation periods were imposed in ascending order, and the operant sessions were spaced to allow the groups' body weights to restabilize. Operant sessions were conducted during the light (1000–1700 hr), with the running order of experimental animals randomized. Operant testing was carried out in 4 Gerbrands G7321/SP rat test chambers (base 23.5×21.5 cm, height 19.5 cm), equipped with a single lever centered in one wall. The lever was 5 cm above the grid floor, extended 2 cm into the chamber, and required a force of 20 g to depress. Openings for a pellet dispenser and a liquid dipper were located on either side of the lever, but only the pellet dispenser was operative in the present study. The test chambers were housed in Grayson-Stadler 1101–9650 sound attenuating and air baffled research chests. Electromechanical programming equipment and recording devices were housed in an adjacent room.

Fasting-Refeeding Test

To assess the effect of a period of fasting on the ad lib refeeding rate of the acarbose treated rats, at 12 weeks of age (6th week of acarbose treatment) the groups were food deprived for 20 hours, and following fasting food was restored and intake measured at refeeding hours 2, 4, 6 and 24.

Sweet Preference Testing

To assess the effect of chronic acarbose treatment on the rats' preference for sweet non caloric and caloric solutions, at 16 and 17 weeks of age (10th and 11th weeks of acarbose treatment), the groups were administered 24 hr 2 bottle solution preference tests in the home cage with food continuously available. During week 16, groups were offered a choice between a 0.1% sodium saccharin (Sigma Chemical Corp.) solution or tap water for three 24 hr periods separated by 2 day periods of no testing. During week 17, the groups were offered a choice between a 10% sucrose solution or tap water according to the same test schedule. For the pair-fed groups, sucrose and saccharin preference were each tested only once.

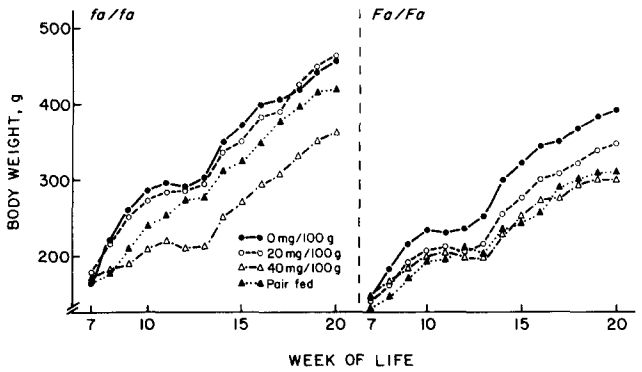

FIG. 1. Body weights of fatty and lean Zucker rats administered 0, 20 or 40 mg acarbose/100 g diet from week 7 to week 20 of life. Pair-fed fatty and lean groups were fed mean amount of diet consumed daily by respective 40 mg groups.

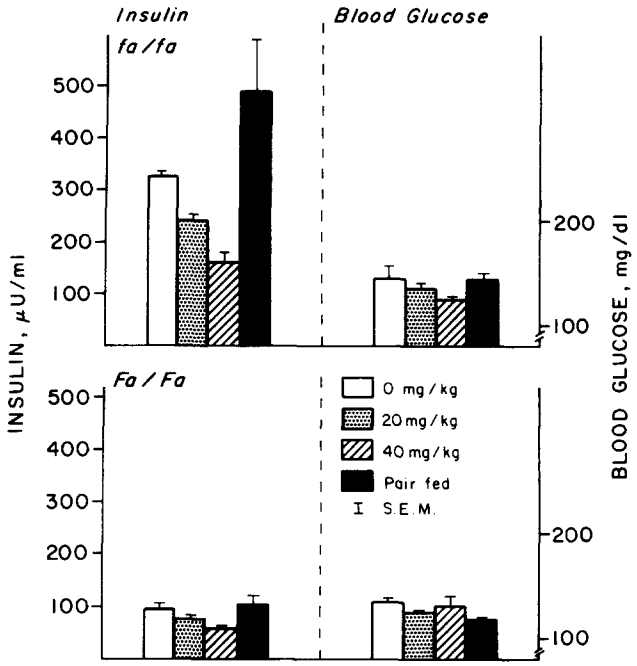

FIG. 2. Plasma insulin and glucose values of Zucker fatty and lean groups administered 0, 20 or 40 mg acarbose/100 g diet or pair-fed to 40 mg groups.

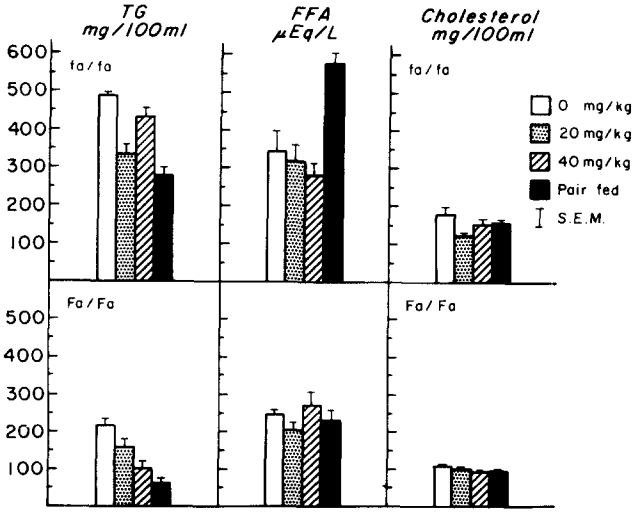

FIG. 3. Serum triglyceride, free fatty acid, and cholesterol values of Zucker fatty and lean groups administered 0, 20 or 40 mg acarbose/100 g diet or pair-fed to 40 mg groups.

Statistics

Insulin, blood-borne metabolite, adipose tissue cellularity, LPL activity, mean total food intake, and final body weight values were compared using two-factor analyses of variance ([44], p. 431). Weekly food intake, lever pressing, fasting-refeeding and sweet preference scores were compared using three-factor analyses of variance with repeated measures on the last factor ([44], p. 559). Both two- and three-factor analyses were followed by simple effect tests, when appropriate, and comparisons of individual means by the procedure of Newman-Keuls ([44], p. 191). A probability level of less than 0.05 was considered significant.

RESULTS

Food Intake and Body Weight

Table 2 presents the mean daily food intake in grams of the groups for each week of acarbose administration, and the mean total intakes of the groups for the entire experimental period. The analysis of daily intake scores revealed that fatty groups in general were significantly hyperphagic in comparison with lean groups, $F(1,32)=6.80$, $p<0.05$. Significant overall effects of dose, $F(3,32)=8.95$, $p<0.01$, and weeks, $F(12,384)=39.27$, $p<0.001$, upon daily intake were also observed. In addition, significant interactions of genotype × weeks, $F(12,384)=6.74$, $p<0.001$, dose × weeks, $F(36,384)=2.15$, $p<0.01$, and genotype × weeks × dose, $F(36,384)=2.03$, $p<0.01$, were obtained.

At the outset of treatment (week 7), each fatty group was significantly hyperphagic when compared to its lean control group (p's<0.05 or smaller). However, hyperphagia rapidly disappeared for the fatty groups prior to the start of behavioral testing (by week 9), and did not recur for any fatty group for the remainder of the experiment. Also, a trend for a dose dependent reduction of food intake was observed in both fatty and lean groups during the first week of acarbose treatment ($p<0.01$ for the lean groups). In general, this trend was maintained by the lean groups for the remainder of the study. However, the tendency for dose dependently reduced feeding by the fatty groups gradually gave way to a pattern of increased intake by the 20 mg group, in comparison with the 0 mg group. Overall feeding trends of the groups are reflected in the mean total intake scores (Table 2), which show a significant, dose dependent reduction of total intake for the lean groups ($p<0.05$), and an elevation of total intake by the 20 mg fatty group in comparison with both the 0 mg fatty group (ns) and the 20 mg lean control group ($p<0.05$). Finally, the pair-fed lean group ate significantly fewer total calories than the 40 mg lean group ($p<0.01$), but these two groups did not differ significantly in body weight at any point during the experiment.

Body weights are presented in Fig. 1. In general, fatty groups weighed significantly more than lean groups at 20

TABLE 3

FAT PAD WEIGHTS, CELL SIZE, AND CELL NUMBER (MEAN ± S.E.M.)

	Retroperitoneal						Epididymal					
	Pad Weight (g)		Cell Size (μg lipid per cell)		Cell Number $\times 10^6$		Pad Weight (g)		Cell Size (μg lipid per cell)		Cell Number $\times 10^6$	
	fafa	FaFa	fafa	FaFa	fafa	FaFa	fafa	FaFa	fafa	FaFa	fafa	FaFa
Acarbose Dose (mg/100 g diet)												
0	10.54 ±0.65	2.71 ±0.37	1.10 ±0.34	0.72 ±0.10	7.16 ±1.56	2.69 ±0.47	5.94 ±1.25	3.20 ±0.65	2.17 ±0.60	0.93 ±0.27	2.31 ±0.81	3.37 ±0.63
20	8.92 ±0.79	1.37* ±0.13	0.79 ±0.12	0.54 ±0.10	8.20 ±2.89	2.18 ±0.19	5.55 ±0.94	2.11† ±0.10	2.46 ±0.41	0.39 ±0.04	1.58 ±0.36	3.92 ±0.30
40	6.40† ±1.12	0.69† ±0.11	0.88§ ±0.03	0.27† ±0.06	4.05 ±1.18	1.86 ±0.56	5.04 ±1.25	1.27† ±0.14	2.95§ ±0.57	0.20* ±0.06	1.36‡ ±0.34	3.52 ±1.11
Pair-fed	6.72 ±0.64	0.98 ±0.11	1.69 ±0.16	0.34 ±0.05	2.94 ±0.05	2.24 ±0.13	6.41 ±1.55	1.62 ±0.10	1.29 ±0.27	0.25 ±0.03	4.15 ±1.18	5.07 ±0.81

*Significantly different from 0 mg group, $p<0.05$.
†Significantly different from 0 mg group, $p<0.01$.
‡Significantly different from pair-fed group, $p<0.05$.
§Significantly different from pair-fed group, $p<0.01$.

weeks of age, $F(1,26)=39.87$, $p<0.001$. Specifically, each fatty group weighed significantly more than its lean control group by week 20 ($p<0.02$ or smaller) with the exception of the 40 mg fatty group, which did not differ in weight from the 40 mg lean group. A significant overall effect of dose was also obtained, $F(3,26)=7.65$, $p<0.01$. Among both fatty and lean groups, acarbose administration led to reduced body weights only for the 40 mg group in comparison with the 0 mg group (p's<0.05 and 0.02, respectively). However, while no difference of body weight was seen between the 0 and 20 mg fatty groups, the same dose lean groups differed in weight by approximately 40 grams. Thus, the data suggest a dose dependent reduction of body weight gain for the lean groups only. Moreover, the final body weight of the pair-fed fatty group was approximately 90 grams greater than that of the 40 mg fatty group ($p<0.1$), while the final body weights of the 40 mg and pair-fed lean groups were not different.

Insulin and Plasma Metabolites

As Fig. 2 represents, fatty groups were in general hyperinsulinemic when compared to lean groups, $F(1,26)=150.85$, $p<0.001$. In both fatty and lean groups, a dose dependent reduction of plasma insulin was observed (p's<0.05). However, insulin levels were elevated in the pair-fed groups in comparison with respective 40 mg groups. This elevation was significant only for the pair-fed fatty group ($p<0.01$). In general, the plasma glucose levels of the fatty groups were higher than those of the lean groups, $F(1,26)=8.97$, $p<0.005$, and a mild but significant dose dependent reduction of blood glucose was observed in the fatty groups ($p<0.05$).

As Fig. 3 indicates, fatty groups in general had significantly elevated TG levels in comparison with lean groups, $F(1,26)=179.09$, $p<0.001$. Among the fatty groups, only the TG level of the 20 mg group was significantly lower than that of the 0 mg group ($p<0.01$). In the lean groups, a significant, dose dependent reduction of TG was noted ($p<0.01$). The TG levels of both pair-fed groups were reduced below those of respective 40 mg acarbose groups, significantly so in the case of the fatty groups ($p<0.01$). In general, fatty groups had significantly higher FFA levels than lean groups, $F(1,26)=31.78$, $p<0.001$. A trend for a dose dependent reduction of FFA in the fatty groups was detectable, but did not achieve significance ($p<0.1$). However, the FFA level of the pair-fed fatty group exceeded those of all other fatty groups ($p<0.01$ in all cases). In contrast, there were no significant differences of FFA level among any of the lean groups. In general, fatty groups also had significantly higher cholesterol levels than respective lean groups, $F(1,26)=76.62$, $p<0.01$. Among the fatty groups, a significant reduction of cholesterol level was observed for the 20 mg group only, in comparison with the 0 mg fatty control group ($p<0.01$). No significant differences of cholesterol level were observed among the lean groups. In summary, acarbose administration led to significant, dose dependent reductions of insulin and blood glucose in the fatty groups, and of insulin and TG in the lean groups. Also, the TG and cholesterol levels of the 20 mg fatty group were significantly reduced in comparison with those of the 0 mg fatty group.

Adipose Tissue Cellularity and LPL Activity

Data were obtained from two adipose depots, the retroperitoneal and epididymal (see Table 3). In general, fatty groups had significantly enlarged retroperitoneal pad weights, $F(1,26)=275.15$, $p<0.001$, cell sizes, $F(1,26)=53.98$, $p<0.001$, and cell numbers, $F(1,26)=17.53$, $p<0.001$, in comparison with lean control groups. A dose dependent reduction of retroperitoneal pad weight was obtained in both the fatty and lean groups (p's<0.01). However, only the lean groups displayed a dose dependent reduction of cell size ($p<0.01$), and a tendency for dose dependent reduction of cell number (ns) in the retroperitoneal pad. Fatty

groups in general also had significant elevations of epididymal pad weight, $F(1,26)=42.65$, $p<0.001$, and cell size, $F(1,26)=63.71$, $p<0.001$. However, in the epididymal pad lean groups in general displayed significantly increased cell number in comparison with fatty groups, $F(1,26)=9.37$, $p<0.01$. Only the lean groups displayed significant, dose dependent reductions of epididymal pad weight ($p<0.01$) and cell size ($p<0.05$). Finally, in comparison with the pair-fed fatty group, the 40 mg fatty group had significantly larger ($p<0.01$) and fewer ($p<0.05$) epididymal fat cells, but significantly smaller ($p<0.01$) retroperitoneal fat cells.

Figure 4 presents LPL activity/cell for both retroperitoneal and epididymal fat pads. Significant overall elevations of retroperitoneal, $F(1,26)=44.86$, $p<0.001$, and epididymal, $F(1,26)=41.44$, $p<0.001$, adipocyte LPL activity were observed for the fatty groups in comparison with lean control groups. There were no significant differences among the 0, 20 and 40 mg fatty groups in LPL activity/cell for either the retroperitoneal or epididymal fat pads. A dose dependent reduction of LPL activity ($p<0.01$) was observed for the lean acarbose-fed groups in the retroperitoneal pad, however. LPL activity/cell in the retroperitoneal fat pad of the pair-fed fatty group was significantly elevated ($p<0.01$) in comparison with that of the 40 mg group. In contrast, LPL activity/cell in the epididymal fat pad of the pair-fed fatty group was significantly reduced ($p<0.01$) in comparison with that of the pair-fed fatty group. In summary, acarbose administration resulted in dose dependent reductions of retroperitoneal and epididymal pad weight and cell size in the lean Zucker groups. Only retroperitoneal pad weight was significantly reduced in the acarbose-fed fatty groups. While the drug dose-dependently lowered retroperitoneal LPL activity/cell in the lean groups, it did not significantly alter LPL activity/cell in either pad in the fatty groups.

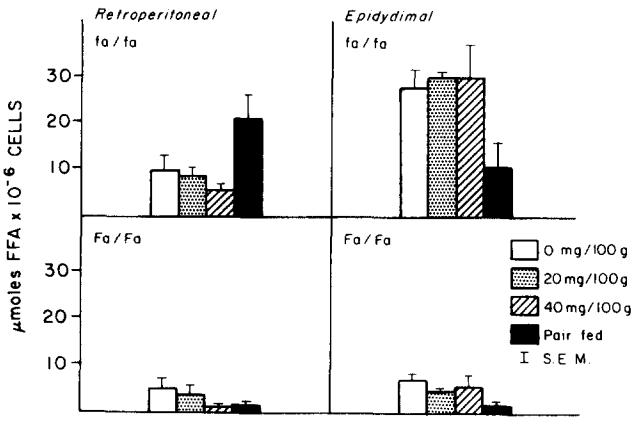

FIG. 4. Lipoprotein lipase activity per fat cell in the retroperitoneal and epididymal fat pads of Zucker fatty and lean groups administered 0, 20 or 40 mg acarbose/100 g diet or pair-fed to 40 mg groups.

Body Composition

Table 4 presents the body composition of the groups both in grams, and as a percentage of carcass weight. In general, the amount of lipid found in the fatty animals, considered either in grams, $F(1,26)=289.89$, $p<0.001$, or on a percentage basis, $F(1,26)=353.78$, $p<0.001$, was significantly greater than that found in the lean animals. In contrast, the fatty animals displayed overall significantly reduced levels of fat free dry mass on a percentage basis, $F(1,26)=34.77$, $p<0.001$, and of body water on both a gram, $F(1,26)=38.07$, $p<0.001$, and percentage of carcass basis, $F(1,26)=592.02$, $p<0.001$. In the lean acarbose treated groups, administration of the drug resulted in dose dependent reductions of lipid in both grams ($p<0.05$) and on a percentage basis ($p<0.01$), but did not significantly alter fat free dry mass. In the fatty acarbose treated groups, a dose dependent reduction of lipid deposition in grams fell just short of significance ($p<0.1$). This reduction of lipid mass was accompanied by a tendency (ns) for a dose dependent reduction of fat free dry mass in grams, and by a tendency (ns) for reduced body water in grams in the 40 mg fatty group. Thus no significant reduction of lipid as a percentage of carcass weight was observed in the acarbose treated fatty groups. Also, the pair-fed fatty group had significantly greater lipid deposition in grams ($p<0.05$) and a significantly lower percentage of fat free dry weight ($p<0.02$) than the 40 mg fatty group.

Food Motivated Behavior

The results of the fasting-refeeding test conducted at 12 weeks of age are shown in Table 5. No significant overall effects of genotype or dose upon cumulative food intake were observed, but a significant genotype × dose interaction was detected, $F(2,20)=6.27$, $p<0.01$. In addition, a significant overall effect of refeeding hour was observed, $F(3,60)=180.07$, $p<0.001$. In comparing the intakes of fatty and lean groups at each dose level, the following results were obtained. There were no significant differences of cumulative intake at any point between the 0 mg fatty and lean groups. In contrast, the 20 mg fatty group ate significantly more than the 20 mg lean group at hours 4, 6 and 24 (p's<0.05, 0.05 and 0.01, respectively). An opposite effect was observed in the 40 mg fatty group, which consumed significantly less food than the 40 mg lean group at hours 2, 4 and 6 of refeeding (p's<0.01, 0.01 and 0.05, respectively). Comparisons among the fatty groups revealed that the 40 mg fatty group ate significantly less than both the 0 and 20 mg groups at hour 2 ($p<0.05$ and 0.01, respectively), and continued to eat significantly less than the 20 mg fatty group at hours 4 and 6 (p's<0.01). Among the lean groups, no intake differences were observed until hour 24, when the 0 mg lean group ate significantly more than the 20 mg lean group ($p<0.05$). In summary, the 20 mg fatty group refed at a faster rate than its 20 mg lean control group, while the 40 mg fatty group refed at a slower rate than both its lean control group and the other fatty groups.

Figure 5 presents lever pressing for food pellets by the 0, 20 and 40 mg fatty and lean groups after 0, 12 and 24 hours of food deprivation. Significant overall effects upon lever pressing of genotype, $F(1,20)=4.83$, $p<0.05$, and dose, $F(2,20)=5.40$, $p<0.05$, as well as a significant genotype × dose interaction, $F(2,20)=8.73$, $p<0.01$, were observed. In addition, there was a significant effect of time, $F(2,40)=51.79$, $p<0.001$, indicating that, in general, lever pressing increased in the groups as a function of deprivation interval. Between genotype comparisons revealed that the fatty and lean groups fed either 0 or 40 mg of acarbose did not differ from each other in lever pressing at any deprivation level. However, for the groups fed 20 mg of acarbose, fatties lever pressed significantly more than leans following 0, 12 or 24 hours of deprivation (p's<0.05, 0.01 and 0.01, respectively). Comparisons among the fatty groups revealed that the 20 mg group also responded at an elevated rate with

TABLE 4

ABSOLUTE AND PERCENTAGE BODY COMPOSITION OF FATTY AND LEAN ZUCKER GROUPS FOLLOWING 13 WEEKS ACARBOSE ADMINISTRATION (MEAN ± S.E.M.)

		Lipid		Fat-Free Dry Weight		Water	
	n	g	%	g	%	g	%
fafa							
0 mg/100 g	4	195.6 ±18.3	50.5 ±1.6	69.0 ±8.0	17.7 ±0.1	125.4 ±21.7	31.8 ±1.7
20 mg/100 g	4	167.4 ±12.5	46.1 ±1.8	66.3 ±4.0	18.1 ±0.7	131.2 ±13.8	35.8 ±1.4
40 mg/100 g	4	123.2*‡ ±20.0	43.8 ±3.2	52.7 ±5.5	19.4§ ±0.6	99.8 ± 6.8	36.8 ±2.6
Pair-fed	4	189.3 ±15.5	51.2 ±2.4	59.4 ±4.5	16.1 ±0.8	119.4 ± 7.3	32.7 ±3.1
FaFa							
0 mg/100 g	5	52.3 ± 9.6	15.7 ±1.1	99.4 ±25.7	29.0 ±2.6	176.8 ±16.9	55.3 ±2.6
20 mg/100 g	5	32.3 ± 2.3	12.6 ±1.4	69.4 ±1.4	25.8 ±0.6	167.2 ± 4.2	62.1* ±0.9
40 mg/100 g	4	20.5* ± 1.3	9.5‡ ±0.6	57.5 ±1.3	26.7 ±0.5	137.6 ± 1.9	63.9* ±1.0
Pair-fed	5	26.2 ± 1.8	11.2 ±0.5	59.1 ±1.9	25.3 ±0.4	148.5 ± 4.8	63.5 ±0.4

*Significantly different from 0 mg group, $p<0.05$.
†Significantly different from 0 mg group, $p<0.01$.
‡Significantly different from pair-fed group, $p<0.05$.
§Significantly different from pair-fed group, $p<0.02$.

regard to both the 0 and 40 mg groups at 12 and 24 hours (p's<0.05 or smaller). Among the lean groups, the response rate of the 40 mg lean group was elevated in comparison with those of the 0 and 20 mg groups, but significantly so only at 0 hour ($p<0.05$). Thus, responding for food was significantly elevated following periods of fasting only in the 20 mg fatty group.

Figure 6 presents the results of three consecutive saccharin and sucrose preference tests conducted at weeks 16 and 17 of age, respectively. In general, fatty groups had a slightly but significantly, $F(1,20)=9.25$, $p<0.01$, lower overall percentage of preference for saccharin vs. water in comparison with lean groups. No significant effects of dose or test number were observed among either the fatty or lean groups, and no significant interactions were detected. In contrast, there was no significant overall difference between fatty and lean groups in percentage of preference for 10% sucrose vs. water. However, a significant overall effect of dose on sucrose preference was observed, $F(12,20)=8.52$, $p<0.01$. Specifically, the 40 mg fatty group had a significantly reduced preference for 10% sucrose in comparison with both the 0 and 20 mg groups (p's<0.01). Also, both the 20 and 40 mg lean groups had a significantly reduced preference for 10% sucrose in comparison with the 0 mg lean group (p's<0.01). No significant effects of test number on percentage of sucrose preference, or significant interactions, were detected. Thus, in contrast to its effects upon saccharin preference, administration of acarbose to both fatty and lean rats resulted in a significant reduction of sucrose preference at one or both dose levels. It should be noted that the 40 mg fatty group preferred water over 10% sucrose during the first two preference tests, while the opposite preference was observed in the single test conducted for the pair-fed fatty group, which, of course, was never administered acarbose.

TABLE 5

REFEEDING FOLLOWING 20 HR OF FASTING AT 12 WEEKS OF AGE; CUMULATIVE FOOD INTAKE, g (MEAN ± S.E.M.)

Acarbose Dose	Refeeding Hours	2	4	6	24
0 mg/100 g	fafa	7.5 ±1.6	9.5 ±1.9	11.7 ±2.3	18.3 ±1.9
	FaFa	7.6 ±1.6	12.3 ±1.3	15.0 ±1.1	22.2 ±1.4
20 mg/100 g	fafa	8.2 ±1.4	12.9* ±1.4	16.4* ±1.4	23.5† ±1.3
	FaFa	6.5 ±0.9	9.2 ±0.7	12.0 ±0.9	18.4‡ ±0.9
40 mg/100 g	fafa	3.3†‡§ ±0.3	5.5†§ ±0.6	3.9§ ±1.0	15.9 ±3.7
	FaFa	8.0 ±1.0	10.0 ±0.8	13.0 ±1.1	19.4 ±0.9

*Significantly different from leans at same dose, $p<0.05$.
†Significantly different from leans at same dose, $p<0.01$.
‡Significantly different from respective 0 mg group, $p<0.05$.
§Significantly different from respective 20 mg group, $p<0.01$.

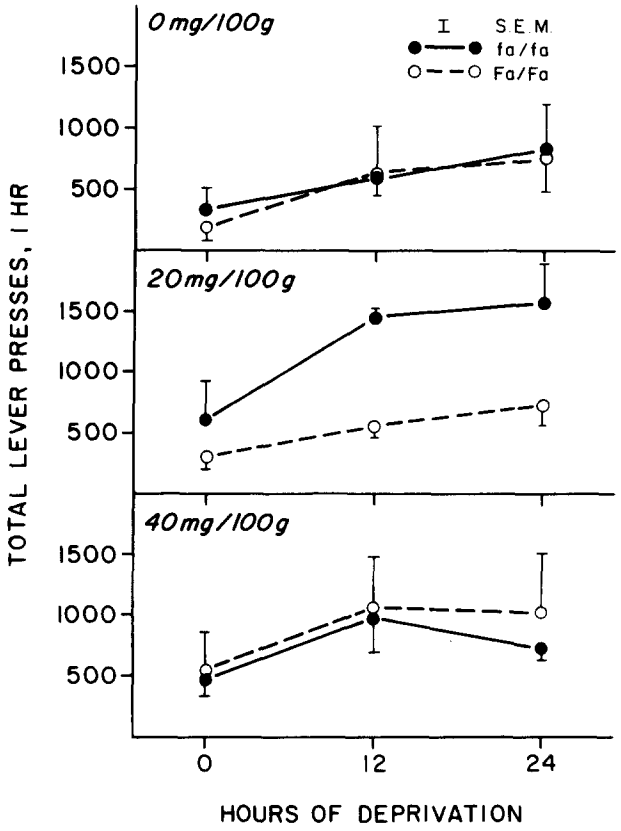

FIG. 5. Responding on VI 80 sec for 45 mg food pellets after 0, 12 or 24 hr of fasting by Zucker fatty and lean groups administered 0, 20 or 40 mg acarbose/100 g diet. Operant testing was conducted at 14 weeks of age.

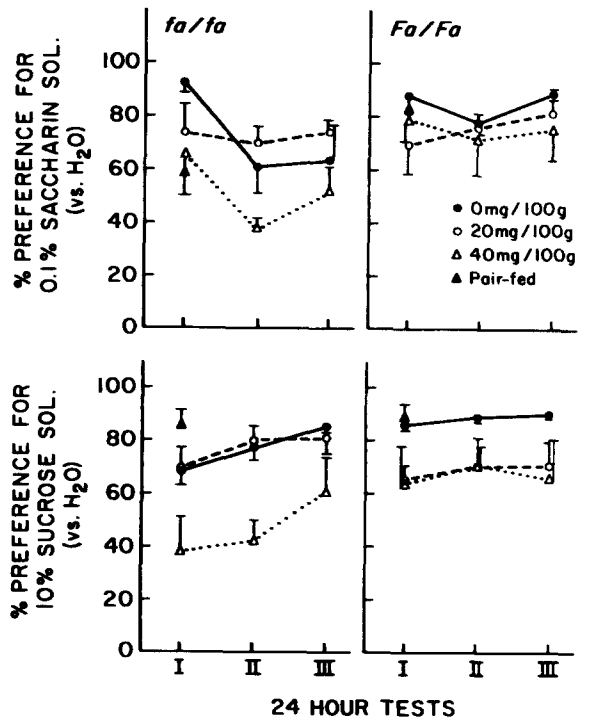

FIG. 6. Percent preference for 0.1% saccharin solution vs. water (upper panels) and 10% sucrose solution vs. water (lower panels) of Zucker fatty and lean groups administered 0, 20 or 40 mg acarbose/100 g diet or pair-fed to 40 mg groups. For all groups except pair-fed groups, three consecutive 24 hr saccharin and sucrose preference tests were conducted at weeks 16 and 17 of age, respectively. Pair-fed groups were given a single 24 hr preference test with each test solution. Values represented are means±S.E.M.

DISCUSSION

The present experiment tested whether long term administration of the glucosidase inhibitor BAY g 5421 (acarbose) to developing Zucker fatty rats would, by reducing several lipogenic factors, attenuate lipid deposition and reduce the hyperphagia and increased food motivation of the fatty rat. Results indicate that most of the predicted effects were obtained in developing lean Zucker rats, but that fatties were able to resist the action of acarbose on food intake, adipocyte LPL activity, and the development of obesity.

Lean groups fed acarbose exhibited dose dependent reductions of food intake, body weight, insulin, TG, retroperitoneal and epididymal pad weight, adipocyte size, LPL activity/cell (retroperitoneal pad only), and lipid deposition both in total grams of fat and as a percentage of carcass weight. Fatty groups fed acarbose exhibited dose dependent reductions of insulin, blood glucose, retroperitoneal pad weight, and, at one of two acarbose doses used, significantly lowered body weight (40 mg dose), TG (20 mg dose), and cholesterol (20 mg dose). However, fatty groups administered acarbose failed to show significant reductions of adipocyte size, number, or LPL activity/cell in the retroperitoneal and epididymal fat pads, and maintained their obese body composition on a percentage basis at levels not significantly different from that of the 0 mg fatty control group.

The results of this experiment are, for lean Zucker rats, in agreement with previous observations on the body weight, blood borne metabolite, and lipid lowering effects of acarbose in Sprague-Dawley rats [42, 43, 45]. Also, the present results support the data of Puls *et al.* [19, 24, 38] on some specific effects of acarbose in Zucker fatty rats, primarily those of reduced body weight gain, insulin levels, blood-borne metabolites, and lipid deposition in grams. However, the present data indicate that specific lipogenic factors remain elevated in the acarbose treated fatty rats in comparison with respective lean control groups. These factors are plasma insulin, TG, and adipocyte LPL activity. Despite an overall trend for a reduction of fat deposition in the acarbose fed fatty groups (see Table 4), the above named factors apparently favored the maintenance of an obese body composition. In particular adipocyte LPL activity, which remained at control levels or higher in the two fat depots examined in the acarbose treated fatty groups, would be expected to maintain the enlarged fat cell size characteristic of these animals.

The present results differ from previously reported effects of acarbose on adipose tissue LPL activity and body composition in Zucker fatty rats. A significant reduction of percentage carcass lipid was observed in growing male Zucker fatty rats administered 80 mg acarbose/100 g of maintenance diet (identical to that used in the present study) for 8 weeks [19]. Also, significantly reduced adipose tissue LPL activity/mg protein was observed in adult female Zucker fatty rats

fed 80 mg acarbose/100 g of fat-free diet for 17 days [38]. In both of these cases, a dose twice as high as the maximum dose used in the present study was employed, perhaps accounting for the different results obtained between the studies. It should be noted that a tendency (ns) for a reduced percentage of carcass lipid was detected in the fatties fed 20 or 40 mg of acarbose in the present study (see Table 4). Taken together, the results of the present and earlier studies on acarbose administration in Zucker fatty rats suggest that, at high enough doses, significant obesity reducing effects may be observed.

Elevations of insulin level were observed in both the pair-fed fatty and lean groups, in comparison with respective 40 mg acarbose fed groups, and, in the case of the pair-fed fatty group, in comparison with the 0 mg fatty control group (although this difference was not significant). Also, FFA levels were significantly increased in the pair-fed fatty group in comparison with both the 40 mg and 0 mg fatty groups. An elevation of insulin level in pair-fed fatty rats has been noted previously in our laboratory and another, [20,40], and may be due to the meal feeding effects associated with the method of pair-feeding used in the present experiment (entire ration presented once daily, see [20]). Likewise, increased fat cell size has been noted in pair-fed fatty rats ([20], and this study, Table 3, retroperitoneal pad), a factor which may underlie the elevated levels of FFA observed in the 3 hr fasted animals.

As noted earlier, acarbose administration resulted in a dose dependent reduction of food intake in both the fatty and lean groups at the outset of treatment (7 weeks of age). This trend persisted for the lean groups throughout the experiment, and the resulting dose dependent reduction of caloric intake in the lean acarbose fed groups (Table 2) may partially account for the reduced final body weights of these groups. However, the 40 mg acarbose fed fatty group did not differ from the 0 mg fatty control group in total caloric intake. The significantly reduced final body weight of the 40 mg acarbose fed fatty group can, therefore, be attributed to the metabolic effects of the drug. Supporting this point is the fact that the pair-fed fatty group gained a total of 90 g more body weight than the 40 mg fatty group during the experiment. No difference of body weight was observed between the 40 mg lean and respective pair-fed group, further implicating the role of reduced food intake in the decreased weight gain of the lean acarbose treated groups. Thus, two potential mechanisms for reduced body weight gain may be induced by acarbose administration: a feeding inhibitory effect of unknown origin, and a reduced rate of carbohydrate absorption with its metabolic consequences. As suggested previously [42], the metabolic consequences of acarbose most likely to underlie reduced weight gain may be the antilipogenic effect of significantly lowered insulin levels in these animals. Finally, caloric malabsorption must be considered a third possible contributor to reduced weight gain, particularly in the case of the higher acarbose dose (see [24], p. 244). Evidence of caloric malabsorption in the present study is discussed below.

The basis of the above noted feeding inhibitory effect of acarbose is worthy of further consideration. Acarbose may induce an anorectic effect based upon enhanced satiety signals of gastrointestinal origin. Presumably, these signals arise from the presence of undigested calories in the gut for longer than normal periods. Data in support of this explanation, i.e., enhanced satiety, have been obtained by Davis *et al.* [4,5], who noted that when mannitol, a poorly absorbed sugar, was added to glucose solutions, the meal size of rats consuming this solution was reduced in proportion to mannitol concentration. According to Davis, prolonged intestinal filling stimulated by mannitol activates putative tension receptors in the gut wall which signal satiety, and this process reportedly involves no illness effects [4]. Alternatively, undigested calories remaining in the gut may induce an illness-based aversion to the animal's maintenance diet, particularly if this condition leads to malabsorption. This possibility is supported by the observation that rats given jejunoileal bypass surgery, which chronically reduces their voluntary food intake, and results in some degree of malabsorption, form a long term conditioned aversion to a novel flavor introduced immediately following surgery [33]. It is difficult to determine whether one or both of these hypothesized mechanisms was acting to limit feeding in the acarbose fed groups of the present experiment, and, if so, under what circumstances. In all probability, the size of the acarbose dose being administered [16], and possibly the schedule of administration, will determine to what degree, if any, slowed absorption and/or malabsorption may be occurring and consequently whether the basis for an illness-induced feeding aversion will be present. Further investigation of the bases of the feeding inhibitory effect of acarbose clearly is required. It should be noted that while some degree of malabsorption may have been induced by acarbose administration in the present study, significantly increased intake by the acarbose treated groups was not observed, except under special test conditions (see discussion below). That lean, and to a lesser extent fatty rats, do compensate calorically on diets with non-absorbable carbohydrate or fat components has been demonstrated previously [37].

In the fatty groups, significant hyperphagia was evident relative to respective lean groups at the outset of treatment. However, food intake by the fatties rapidly declined to lean control levels when the groups had reached 9 weeks of age (Table 2). The attenuation of hyperphagia at this early age in the fatties apparently was not the result of acarbose administration, since food intake was reduced in the 0 mg fatty group as well. Previously, we have observed a reduction of what we have termed "compensatory hyperphagia" in growing Zucker fatty rats at 15–16 weeks of age [40]. The fact that the fatties in the previous study were fed a calorically less dense diet containing a lower percentage of fat suggests that the caloric density or macronutrient content of the diet used in the present study may have influenced the duration of compensatory hyperphagia in the fatty groups in this experiment.

In food motivation testing, acarbose administration led to alterations of feeding behavior in specific fatty and lean groups. Thus, the 40 mg lean group responded on VI 80 at an elevated rate in comparison with the lean control group in the non-fasted state (0 hr deprivation), while the 20 mg fatty group responded at elevated rates in comparison with both its lean acarbose fed and fatty control groups following 0, 12, and 24 hr of food deprivation. The 20 mg fatty group was generally more responsive than other acarbose fed groups in the fasting-refeeding test conducted at 12 weeks of age as well, since it ate at a faster rate than both its 20 mg lean ($p<0.05$) and 0 mg fatty control groups (ns). These observations indicate that elevated food motivation in the 20 mg fatty group was associated with periods of imposed fasting. In this case, it is possible that the reduced rate of carbohydrate absorption resulting from acarbose during refeeding following fasting was not adequate to meet the enhanced caloric needs of the fatties in the post-fasting state. In normal ad lib

feeding, however, the 20 mg fatty group may have been able to absorb an adequate amount of calories during a 24 hr period, since it consumed only slightly more calories on a daily basis, and showed no body weight gain deficit or significant alteration of body composition when compared to the 0 mg fatty control group.

In contrast, the effect of fasting upon refeeding in the 40 mg fatty group was a significant reduction in rate of feeding, in comparison with both the 40 mg lean and 0 mg fatty control groups. In this case, the possibility exists that feeding was reduced in the 40 mg fatty group as a result of malaise arising in conjunction with increased levels of carbohydrate malabsorption brought about by the higher dose. In support of this notion, it was noted that while mild to severe diarrhea occurred occasionally in the fatty and lean acarbose fed groups during the treatment period, the 40 mg fatty group was particularly susceptible to this condition. Also consistent with this possibility is the fact that the 40 mg fatty group had a significantly reduced preference for 10% sucrose (the digestion of which would also be inhibited by acarbose), in comparison with both other fatty groups. Reduced sucrose preference in the 40 mg fatty group may indicate an aversion to the sucrose solution based upon the metabolic effects of the drug. However, significantly reduced sucrose preference seen in the 20 and 40 mg lean groups was not accompanied by dramatic alterations of refeeding following fasting or responding for food pellets. Thus, acarbose treated Zucker fatty rats appear to be more sensitive to the effects of fasting upon subsequent feeding behavior than similarly treated lean controls, but the bases for this enhanced sensitivity remains uncertain. While long term acarbose administration significantly reduced the sucrose preference of both fatty and lean rats, it is unclear whether this reduced preference was related to the potential satiety inducing or aversive effects of the drug.

In general, the data reported here are consistent with the hypothesis that an effective agent for amelioration of the obesity inducing metabolic and behavioral alterations of the genetically obese rat should lower adipose tissue LPL activity [12,40]. We have proposed that elevations of adipose tissue LPL activity that occur in the preobese fatty rat in the first two weeks of life [13] lead to fat cell hypertrophy, altered body composition, and subsequent hyperphagia and excessive obesity [12]. All fatty groups in the present experiment exhibited elevated adipocyte LPL activity, in comparison with respective lean control groups, and became extremely obese despite several other metabolic alterations induced by acarbose. In previous work from this laboratory, life long pair-feeding of fatty rats to lean rats, while resulting in reduced body weights for the fatties, did not reduce the obese body composition or elevated LPL levels of these animals [4,37]. It should be noted that in another animal model of obesity with elevated adipose tissue LPL activity, the ventromedial hypothalamic lesioned or knife cut rat, reductions of LPL activity can be induced by fasting [30] or by food restriction [21]. In contrast neither moderate fasting [23], chronic food restriction [4], treatment with anti-obesity agents ([9], and present results), nor surgical intervention [10] have successfully reduced adipose tissue LPL levels in the Zucker fatty rat.

In conclusion, the effectiveness of acarbose in lowering lipid deposition and degree of adiposity in Zucker lean rats suggests that this pharmacological agent may be of use in reducing the development of obesity induced by dietary manipulations, specifically those which involve a high intake of carbohydrate [17,34]. Recent observations from our laboratory in fact indicate that acarbose can attenuate the development of sucrose-induced obesity in Sprague-Dawley rats [41,42]. It is clear that the ability of the genetically obese Zucker rat to maintain metabolic conditions which favor TG deposition renders its obesity exceedingly refractory to treatment.

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