

Effects of Inhibitors of Carbohydrate Absorption or Lipid Metabolism on Meal Patterns of Zucker Rats

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DREWNOWSKI, A., J. A. GRINKER, R. GRUEN AND A. C. SULLIVAN. *Effects of inhibitors of carbohydrate absorption or lipid metabolism on meal patterns of Zucker rats.* PHARMACOL BIOCHEM BEHAV 23(5) 811-821, 1985.—Peripherally active anorectic agents represent a new approach to the pharmacological management of obesity. Two inhibitors of carbohydrate absorption: an alpha-glucosidase inhibitor, acarbose (Bay g 5421) and an alpha-amylase inhibitor, Ro 12-2272, were compared with two novel inhibitors of lipid metabolism: an inhibitor of human pancreatic lipase (Ro 20-0083) and of hepatic fatty acid synthesis (Ro 22-0654). All drugs were presented as diet admixtures over 3 or 4 consecutive days. Total food and water intakes, the temporal pattern of feeding, and the average meal frequency and meal size were measured using computerized data collection procedures. Inhibitors of carbohydrate absorption failed to suppress food intake in either obese or lean Zucker rats and had no effect on the parameters of feeding. In contrast, inhibitors of lipid metabolism reduced food intake by 56-77% by reducing both meal frequency and meal size. Direct inhibition of lipid metabolism may be a viable mechanism for anti-obesity agents.

Meal patterns Zucker rats Antiobesity drugs Carbohydrate absorption inhibitors
Lipid metabolism inhibitors

CURRENTLY employed anorectic agents such as mazindol or fenfluramine suppress appetite through direct action on the central nervous system [26]. Although these compounds may be useful as short-term adjuncts to weight reduction programs, their chronic use has been restricted due to undesirable CNS side effects, potential for addiction, and in particular, the development of tolerance [26,28].

Anorectic agents acting through peripheral mechanisms represent a new approach to the pharmacological management of obesity. The primary effects of these compounds may include a delay in gastric emptying, changes in intestinal absorption, or interference with hepatic carbohydrate or lipid metabolism [7, 26, 28]. However, these compounds may also affect food intake [26-28, 30], and so alter the energy balance of the organism. The peripheral mechanisms underlying the regulation of food intake are still unclear, but are thought to include hormonal and metabolic feedback signals operating between gastrointestinal and hepatic sites and the central nervous system [28].

Peripheral anorectic agents currently under investigation include compounds that inhibit the absorption of carbohydrates in the gastrointestinal tract. Among these are acarbose (Bay g 5421, Bayer) and Ro 12-2272 (Hoffmann-La Roche). Both are complex oligosaccharides of microbial origin. Acarbose is a potent alpha-glucosidase inhibitor and has shown activity against several carbohydrate digestive enzymes including alpha-amylase, sucrase and maltase [18,19]. It is reported to inhibit the breakdown of starches and sugar polymers containing alpha 1,4-glucose linkages by

glucosidase enzymes located in the mucosa of the small intestine, and to block the rise of serum glucose, insulin and triglycerides following a glucose-rich meal [18,19]. At sufficiently high doses it inhibits the absorption of carbohydrate completely [4]. Ro 12-2272 is an inhibitor of alpha-amylase similar in its structure and effects to the alpha-amylase inhibitor Bay e 4609 (Bayer) [18]. The latter drug is also a complex oligosaccharide that is a specific and potent inhibitor of alpha-amylase *in vitro* as well as *in vivo*. It reduces the increase in blood glucose and insulin levels in humans and rats following a starch load [18].

A second class of novel pharmacological agents directly inhibits lipid absorption or synthesis. These include Ro 22-0654 (4-amino-5-ethyl-3-thiophene carboxylic acid methyl ester HCl, Hoffmann-LaRoche), which is a novel and potent inhibitor of hepatic fatty acid synthesis [29] and Ro 20-0083 (dinitrodecarboxylalocid, Hoffmann-LaRoche), an *in vitro* inhibitor of human pancreatic lipase which also inhibits *in vivo* lipid absorption and synthesis in rats [6]. Body composition studies indicated that the weight loss in obese and lean Zucker rats following chronic treatment with Ro 22-0654 was attributable primarily to a significant decrease in carcass lipid content [29]. Oral administration of Ro 20-0083 in doses of 56, 120, and 240 mg/kg body weight produced significant and dose-dependent inhibition of lipid absorption and *de novo* fatty acid synthesis [6]. Chronic administration of Ro 20-0083 to obese female Zucker rats at doses of 250 mg/kg body weight resulted in reduced body weight gain and lower carcass fat and triglyceride levels [6].

TABLE 1
EFFECTS OF ACARBOSE ON DAILY FOOD AND WATER INTAKES AND MEAL
PARAMETERS (15 MIN IMI) OF OBESE AND LEAN ZUCKER RATS (MEAN \pm S.E.M.)

	Condition					
	Baseline		Drug		Recovery	
	Dark	Light	Dark	Light	Dark	Light
Food intake (g)						
lean	16.10 ± 0.71	3.26 ± 0.21	18.33 ± 1.48	3.93 ± 1.18	17.20 ± 1.09	2.82 ± 0.54
obese	22.77 ± 1.39	6.36 ± 0.86	22.35 ± 0.41	6.82 ± 0.64	23.71 ± 1.08	6.57 ± 1.02
Meal size (g)						
lean	1.72 ± 0.17	1.20 ± 0.23	2.22 ± 0.18	1.51 ± 0.23	2.15 ± 0.18	1.18 ± 0.17
obese	2.53 ± 0.26	2.83 ± 0.40	4.14 ± 1.36	2.29 ± 0.27	2.80 ± 0.12	2.97 ± 0.38
Meal frequency						
lean	9.87 ± 0.67	3.03 ± 0.52	8.44 ± 0.32	2.72 ± 0.77	8.16 ± 0.42	2.25 ± 0.65
obese	9.37 ± 0.69	2.37 ± 0.25	7.44 ± 1.22	3.22 ± 0.20	8.58 ± 0.39	2.33 ± 0.28
Water intake (ml)						
lean	30.9 ± 1.1		36.2 ± 2.5		31.1 ± 2.4	
obese	43.5 ± 5.0		46.7 ± 3.0		50.3 ± 2.9	

Most of these peripheral anorectic agents have been reported to suppress body weight gain or to reduce body weight in rats following chronic treatment ranging from 4 to 38 weeks. However, it is uncertain to what extent these effects are mediated by a reduction in food intake. For example, some investigators have reported concordant decreases in food intake and body weight gain following chronic treatment with acarbose [17,18], while others reported an increase in food intake but a reduction in body weight [11]. The two inhibitors of lipid metabolism under investigation, Ro 22-0654 and Ro 20-0083, are also reported to reduce body weight following chronic treatment [6,29]. However, their effects on food intake have not been investigated in detail.

To understand the mode of action of peripheral anorectic agents, it is important to know how pharmacological activity is related to the diurnal shifts in energy metabolism or to the regulatory systems of appetite and satiety [8,13]. The technique of meal pattern analysis allows the measurement of food intake in freely feeding rats over several days. Study of the parameters of feeding behavior can show how putative anorectic agents influence the circadian distribution of food intake, mean daily meal frequency and average meal size. This methodology has a clear advantage over procedures whereby drugs are administered after a period of food deprivation and food intake is monitored for periods up to 24 hours. The deprivation paradigm is known to heighten the anorectic effect of certain pharmacological agents such as d-amphetamine [2,13], and may obscure the drugs' effects on

the temporal patterns of food intake. Reduction in intake combined with rebound hyperphagia occurring within the 24 hour period may lead to unchanged total intakes. The present methodology permits the determination of the size of individual meals both at night and during the day, and the assessment of the effects of drugs on the initiation and termination of meals.

In the present study, we examined the effects of two classes of compounds on food and water intakes of freely feeding rats and on the microstructure of feeding. In Experiment 1 we examined the effects of two inhibitors of carbohydrate absorption (acarbose and Ro 12-2272), and in Experiment 2 we examined the effects of two inhibitors of lipid metabolism (Ro 20-0083 and Ro 22-0654). Measures of water intake were included to control against possible contributing effects of taste aversion or drug-induced general malaise. Since the effectiveness of these agents in the management of human obesity is of principal interest, all compounds were tested in the genetically obese Zucker rat, considered a useful animal model of juvenile-onset human obesity [13, 14, 16, 31].

EXPERIMENT 1

METHOD

Animals

Six genetically obese (fa/fa) and six lean (Fa/-) experi-

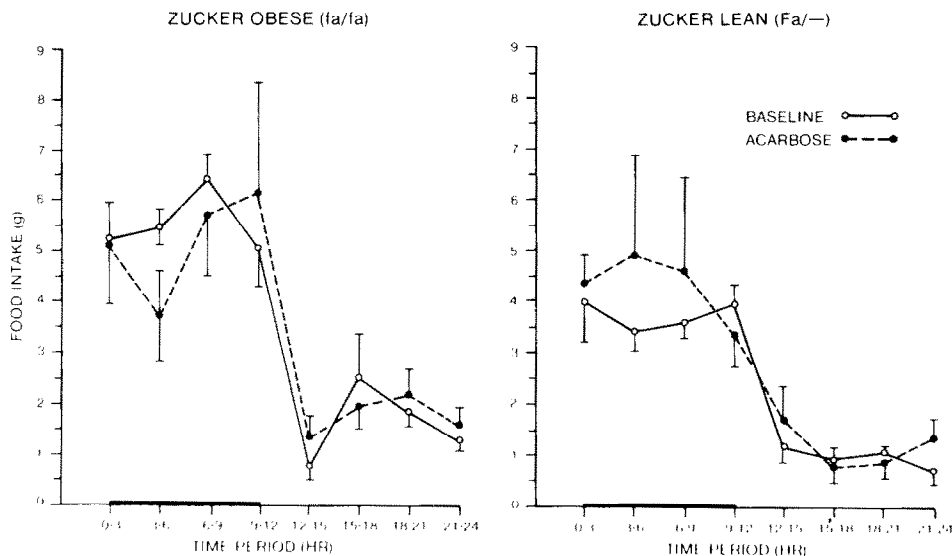


FIG. 1. Temporal effects of administration of acarbose (BAY g 5421) on food intakes of obese and lean Zucker rats relative to the baseline condition in Experiment 1. Eight consecutive 3-hr time periods are shown, with the 12 hour dark phase (beginning at 17:00) indicated by a solid bar. The data represent mean food intakes (in grams) during each 3 hr period. SEM bars are also indicated.

mentally naive male Zucker rats were obtained from the Hoffmann-La Roche breeding colony. At the start of the experiment the rats were 13 weeks old. Obese rats weighed a mean of 462 g (range 402 to 512 g) and lean rats weighed a mean of 317 g (range 307 to 326 g).

Diet

The rats were accustomed to a diet of powdered Purina rat chow for 10 days prior to the start of the experiment. Caloric density of the diet was 3.5 kcal/g, and its composition according to the manufacturer's specifications was 54% carbohydrate, 23% protein, 4.5% fat, 6.0% fiber, and 2.5% minerals, as well as moisture and ash. The rats had free access to water at all times.

Apparatus

The rats were individually housed in Plexiglas cages with stainless steel rod floors. The cages were placed in noise-attenuating chambers under a computer-controlled light-dark cycle (lights on 5 a.m. to 5 p.m.) at a constant temperature of 22°C. The rats had been accustomed to the apparatus for one week prior to the experiment. Details of the food-dispensing apparatus have been provided previously [25].

Drugs

All drugs were provided as diet admixtures. Acarbose and Ro 12-2272 were provided in effective concentrations of 80 mg/100 g of diet. This dose corresponds to the highest dose of acarbose (80 mg/100 g), also used as diet admixture in previous studies with female obese Zucker rats [18]. Since the average food intake during the baseline period was 29.1 g for obese and 19.4 g for lean rats, the effective doses of acarbose were 5.0 mg/100 g body weight for the obese and 4.9 mg/100 g body weight for lean rats. The corresponding doses of Ro 12-2272 were 5.3 mg/100 g body weight for the obese and 4.9 mg/100 g body weight for lean rats.

Procedures

Experiment 1 began with a 4 day baseline period during which the rats ate the standard chow diet, followed by 4 consecutive days of acarbose administration. At the end of acarbose treatment, the rats were again provided with the standard chow diet during 4 days of recovery. The rats were then given a rest period of one week to recover fully from any residual effects of acarbose. During the second phase of the experiment, the rats were monitored during a 4-day baseline period and during 4 days of Ro 12-2272 administration. Meal patterns were not monitored during the recovery period following Ro 12-2272 administration.

Data Collection

The apparatus used to monitor food intakes consisted of solid-food eatometers connected to an on-line PDP-8 computer [25]. Total food intake (minus spillage) was measured daily, and water intake was read from 100 ml graduated cylinders.

All animals were serviced daily between 3:30 p.m. and 5 p.m., immediately preceding the onset of the dark cycle. Food intakes, water intakes and body weights were recorded at that time, and food cups were cleaned, filled with fresh food or with the drug admixture and re-weighed. The rats' feeding responses were continuously monitored by means of programs written to record all licks (or bites) of food between 5 p.m. (the start of the dark period) and service time on the following day. Daily records for up to 12 rats (6 obese and 6 lean) were obtained at one time. The servicing and data collection procedures have been described previously [25].

The computer-based collection criteria were set as low as possible to avoid eliminating potentially valuable data prior to inspection. For data collection purposes only, a minimum of one bite of food occurring within two seconds constituted the threshold criterion for the initiation of a recorded bout of feeding. A feeding bout was considered as terminated when no bites had been recorded for at least one minute. Analyses

of the temporal course of intake and of the principal parameters of meal-taking behavior were carried out subsequently. During data analyses, we excluded from consideration as noise all feeding responses below 0.1 g, and we used 15 minutes as the intermeal interval (IMI) criterion. Thus, periods longer than 15 minutes constituted true intermeal intervals, while periods below 15 minutes were regarded as within-meal pauses. For a fuller discussion of the suitability of 15 minutes IMI see previous literature [8, 9, 13].

Data Analysis

The data were analyzed using analyses of variance (BMDP) for repeated measures. Rat genotype (obese vs. lean) was the between-subjects variable, while condition (baseline, drug or recovery), light phase (light vs. dark) or time periods [8] were the within-subject variables. Analyses of variance for each drug were conducted separately for the measures of food intake, meal frequency and meal size. Additional ANOVAS of condition by genotype were carried out for measures of 24 hour water intake. Significant ANOVA F values are presented for both main effects and for interactions. Differences between treatment means and baseline control were tested for significance using Dunnett's *t*-statistic. When the main effects were not significant and in the absence of significant interactions, *post hoc* tests were not performed.

RESULTS

The effects of acarbose on food and water intakes are shown in Table 1. Analysis of data collected during the baseline period preceding drug administration showed that obese Zucker rats ate more food, ANOVA $F(1,10)=62.67$, $p<0.01$, than did lean rats. All rats ate more food at night than during the day. Obese Zucker rats also ate larger meals than did lean rats, $F(1,10)=15.90$, $p<0.01$, but both sets of rats ate the same number of meals per day [8,13].

Acarbose had no significant effects on the daily food intakes of either lean or obese rats, $F(2,20)=1.75$. Meal size, meal frequency, and the circadian distribution of food intake were unchanged by drug treatment and the temporal profile of intake (see Fig. 1) remained unaffected. Analysis of water intakes showed only that obese rats drank more water than the lean rats, $F(1,30)=42.5$, $p<0.01$, and no effects of the drug were observed.

The effects of Ro 12-2272 on food and water intakes and meal parameters are summarized in Table 2. During the baseline period obese rats ate more food, $F(1,10)=52.16$, $p<0.01$, and drank more water, $F(1,20)=29.2$, $p<0.01$, than did lean rats. All rats ate more food at night than during the day, $F(1,10)=209.97$, $p<0.01$. Ro 12-2272 had no significant effect on the daily food or water intakes, meal size, or meal frequency of obese or lean rats. As shown in Fig. 2, the drug had no effect on the temporal profile of intake.

EXPERIMENT 2

Among the most effective obesity promoting diets are those that are high in fat content [9, 15, 23]. Diets containing 55% Crisco oil or lard, or "supermarket" diets consisting of sweetened condensed milk, cheese and cookies promote body weight gain and the proliferation of adipose tissue. Genetically obese Zucker rats in a 9-day self-selection study [5] as well as obese ob/ob mice [21] have been reported to overeat fat at the expense of other nutrients, although the amount

TABLE 2
EFFECTS OF RO 12-2272 ON DAILY FOOD AND WATER INTAKES AND MEAL PARAMETERS (15 MIN IMI) OF OBESE AND LEAN ZUCKER RATS (MEAN \pm S.E.M.)

	Condition			
	Baseline		Drug	
	Dark	Light	Dark	Light
Food intake (g)				
lean	16.24 ± 0.76	3.26 ± 0.40	17.89 ± 0.43	3.71 ± 0.52
obese	26.11 ± 1.58	4.65 ± 1.35	27.27 ± 1.58	4.43 ± 1.21
Meal size (g)				
lean	2.21 ± 0.30	1.74 ± 0.65	2.16 ± 0.20	1.38 ± 0.23
obese	2.80 ± 0.23	2.04 ± 0.70	3.84 ± 0.62	2.36 ± 0.54
Meal frequency				
lean	8.08 ± 0.76	2.53 ± 0.61	8.65 ± 0.65	2.13 ± 0.22
obese	9.69 ± 0.65	1.64 ± 0.37	7.65 ± 0.60	1.21 ± 0.26
Water intake (ml)				
lean	33.3 ± 3.3		31.5 ± 1.8	
obese	47.3 ± 3.4		47.9 ± 3.4	

of fat consumed may depend on the palatability of the other dietary selections available [10].

Inhibition of lipid synthesis and/or lipid absorption might therefore be expected to provide a direct pharmacological approach to the management of obesity. In previous studies [28] several inhibitors of lipid synthesis have demonstrated antiobesity activity in rodents. These agents include (-)-hydroxycitrate, an inhibitor of citrate cleavage enzyme; dehydroepiandrosterone, an inhibitor of glucose-6-phosphate dehydrogenase, and nafenopin, an inhibitor of acetyl CoA carboxylase [28]. In the next portion of this study we examined the effects of a novel inhibitor of hepatic fatty acid synthesis, Ro 22-0654, and an inhibitor of human pancreatic lipase, Ro 20-0083.

METHOD

Animals

Five mature obese and five lean male Zucker rats from Experiment 1 were used. At the start of Experiment 2 the rats were 8 months old. Obese rats weighed a mean of 756 g (range 720 to 821 g) and lean rats weighed a mean of 490 g (range 428 to 524 g).

Diet

For three months prior to the start of and during Experiment 2 the rats were fed a semisynthetic diet (Bio-Serv).

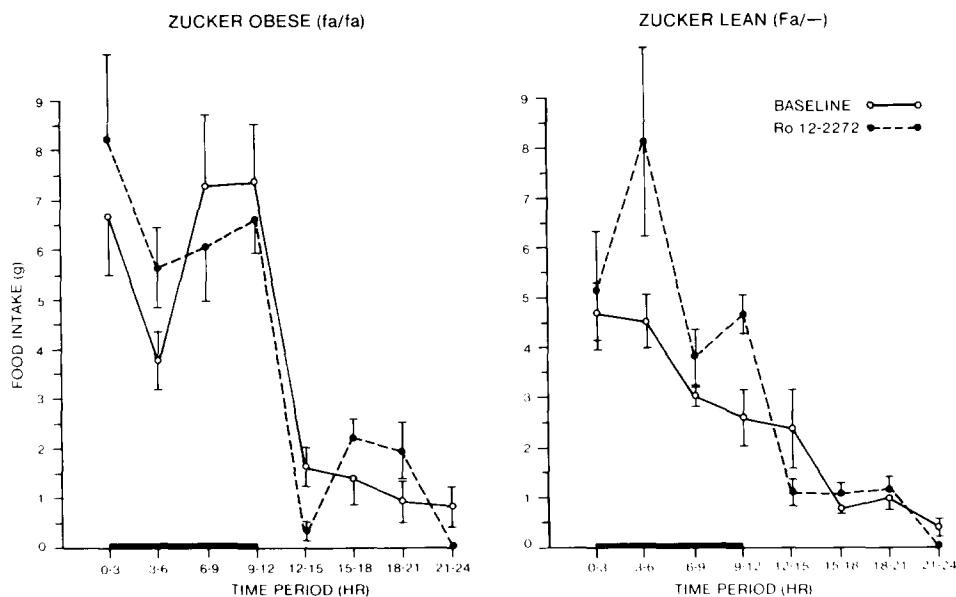


FIG. 2. Temporal effects of administration of Ro 12-2272 (alpha-amylase inhibitor) on food intakes of obese and lean Zucker rats relative to the baseline condition in Experiment 1. Eight consecutive 3-hr time periods are shown, with the 12 hour dark phase (beginning at 17:00) indicated by a solid bar. The data represent mean food intakes (in grams) during each 3 hour period. SEM bars are also indicated.

TABLE 3

EFFECTS OF RO 22-0654 ON DAILY FOOD AND WATER INTAKES AND MEAL PARAMETERS (15 MIN IMI) OF OBESE AND LEAN ZUCKER RATS (MEAN \pm S.E.M.)

	Condition					
	Baseline		Drug		Recovery	
	Dark	Light	Dark	Light	Dark	Light
Food intake (g)						
lean	13.03 ± 0.56	4.57 ± 0.66	2.08* ± 0.37	1.96* ± 0.71	13.31 ± 1.06	10.41* ± 1.34
obese	13.12 ± 1.13	11.05 ± 1.12	5.43* ± 0.65	4.67* ± 0.79	12.05 ± 0.96	13.56* ± 1.14
Meal size (g)						
lean	2.12 ± 0.29	1.27 ± 0.17	0.77* ± 0.13	0.93 ± 0.24	3.20* ± 0.39	3.79* ± 0.75
obese	2.32 ± 0.19	2.13 ± 0.25	1.67† ± 0.39	1.60 ± 0.23	3.44* ± 0.55	3.49* ± 0.48
Meal frequency						
lean	6.70 ± 0.86	3.55 ± 0.49	2.67* ± 0.33	1.62* ± 0.40	4.30* ± 0.54	3.20 ± 0.58
obese	5.72 ± 0.49	5.38 ± 0.58	3.63* ± 0.53	2.67* ± 0.53	4.30* ± 0.85	4.30 ± 0.62
Water intake (ml)						
lean	24.9 ± 1.5		26.9 ± 1.9		27.5 ± 3.0	
obese	27.7 ± 5.3		23.3 ± 9.2		26.3 ± 5.0	

*Dunnett's *t*-statistic: $p < 0.05$ (two-tailed).

†Dunnett's *t*-statistic: $p < 0.05$ (one-tailed).

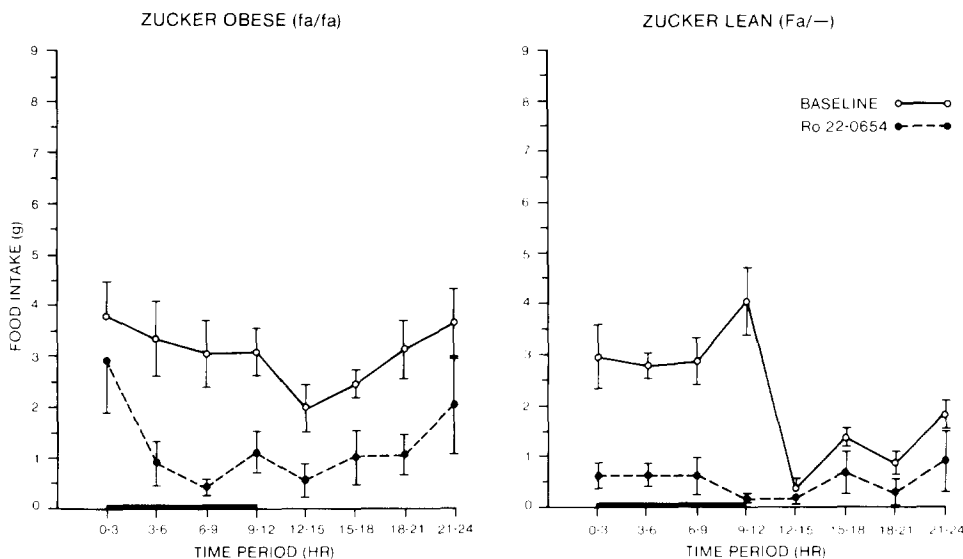


FIG. 3. Temporal effects of administration of Ro 22-0654 (inhibitor of hepatic fatty acid synthesis) on food intakes of obese and lean Zucker rats in Experiment 2. Eight consecutive 3-hr time periods are shown, with the 12 hour dark phase (beginning at 17:00) indicated by a solid bar. The data represent mean food intakes (in grams) during each 3 hour period. SEM bars are also indicated.

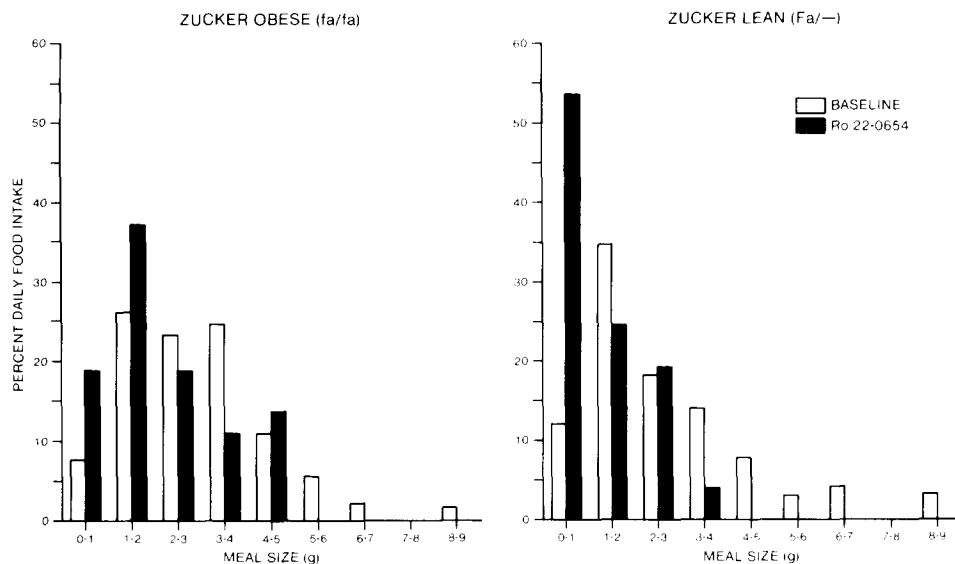


FIG. 4. Effects of Ro 22-0654 on the 24 hour distribution of meal sizes of obese and lean Zucker rats in Experiment 2. The data represent the percentage contribution of different size meals to the total daily food intake. Meal sizes are calculated on the basis of 15 minutes IMI criterion.

Caloric density of this diet was 4.1 kcal/g and its nutrient composition was 60% glucose, 10% corn oil, 23% vitamin-free casein, 5% Phillips and Hart modified salt mix IV, and 1% vitamin mix.

Drugs

Both compounds were administered as diet admixtures. Ro 22-0654 was given at a dose of 322 mg/100 g diet and Ro 20-0083 at a dose of 774 mg/100 g diet. For an obese Zucker rat weighing 750 g and consuming 25 g of diet a day, these concentrations are equivalent to daily doses of 100 mg/kg

body weight for Ro 22-0654 and 240 mg/kg body weight for Ro 20-0083. The doses selected were comparable to the dose of 80 mg/kg of Ro 22-0654, reported to suppress fatty acid synthesis *in vivo* [29], and to the chronic orally administered dose of 250 mg/kg of Ro 20-0083, reported to suppress body weight gain in obese female Zucker rats [6].

Procedures

A four-day baseline was followed by three days of Ro 22-0654 treatment, and four days of recovery. Because of apparatus malfunction, meal pattern data for two of the

TABLE 4
EFFECTS OF RO 20-0083 ON FOOD AND WATER INTAKES AND MEAL PARAMETERS
(15 MIN IMI) OF OBESE AND LEAN ZUCKER RATS (MEAN \pm S.E.M.)

	Condition					
	Baseline		Drug		Recovery	
	Dark	Light	Dark	Light	Dark	Light
Food intake (g)						
lean	15.38 ± 2.79	4.74 ± 1.36	3.97* ± 0.52	1.67 ± 0.37	18.57 ± 2.05	6.41 ± 1.63
obese	13.45 ± 1.54	10.14 ± 1.09	6.95* ± 1.93	3.46* ± 0.82	17.19 ± 2.49	8.47 ± 2.09
Meal size (g)						
lean	2.75 ± 0.84	1.60 ± 0.40	0.98* ± 0.21	0.92 ± 0.16	4.11 [†] ± 0.62	2.28 ± 0.61
obese	2.59 ± 0.32	2.70 ± 0.52	1.41 ± 0.38	1.47 ± 0.35	3.61 ± 0.64	2.62 ± 0.53
Meal frequency						
lean	6.50 ± 0.77	3.53 ± 1.20	4.40* ± 0.66	1.87* ± 0.39	5.00* ± 0.35	3.10 ± 0.73
obese	5.33 ± 0.49	4.20 ± 0.64	4.73 ± 0.62	2.20* ± 0.34	4.90 ± 0.46	3.20 [†] ± 0.64
Water intake (ml)						
lean	28.5 ± 3.4		30.7 ± 2.0		26.5 ± 1.7	
obese	31.7 ± 5.2		24.1 ± 11.5		33.3 ± 5.4	

*Dunnett's *t*-statistic: $p < 0.05$ (two-tailed).

[†]Dunnett's *t*-statistic: $p < 0.05$ (one-tailed).

animals were collected for only one day of recovery. All rats were then given a rest period of one week before the administration of Ro 20-0083. In this phase of the experiment, the rats were monitored for three baseline days, that were followed by three days of drug administration and two days of recovery.

RESULTS

The effects of Ro 22-0654 on food and water intakes are summarized in Table 3. Analysis of food intake data collected during the baseline period showed that obese rats ate more food than did the lean rats, ANOVA $F(1,8)=17.84$, $p < 0.01$, and that both sets of rats ate more food at night than during the day, $F(1,8)=10.08$, $p < 0.05$. The circadian distribution of intake for obese rats was much less pronounced than had been observed for the same rats in Experiment 1. This result raises the possibility that the circadian pattern of intake distribution of obese (though not lean) Zucker rats changes with age [8].

Ro 22-0654 reduced the daily food intake of both obese and lean rats. The main effect of drug treatment was significant, $F(2,16)=121.48$, $p < 0.01$. The 24-hr food intakes of lean rats were reduced by 77% and those of obese rats by 58%. Food intake was reduced more at night than during the day as shown by the condition by time interaction,

$F(2,16)=11.14$, $p < 0.01$. The genotype by condition by time interaction was also significant, $F(2,16)=4.99$, $p < 0.01$, confirming that the food intake of lean rats during the night was affected the most (84% reduction). During the recovery period, lean rats showed compensatory hyperphagia; food intakes during the light phase were significantly higher than light intakes during the baseline period, $t(8)=3.91$, $p < 0.05$.

The temporal profile of food intake during Ro 22-0654 treatment is summarized in Fig. 3. Food intake of both obese and lean rats declined over the 24-hour period, and no rebound feeding was observed at any time. The condition by time interaction was significant, $F(14,112)=1.93$, $p < 0.05$. As shown in Table 3, the reduction in food intake was associated with significant reductions in meal size, $F(2,16)=41.30$, $p < 0.01$, and in meal frequency, $F(2,16)=14.64$, $p < 0.01$. This was true for both genotypes and for both dark and light periods: both ANOVA main effects were significant and no significant interactions with genotype or time were observed. During the recovery period, meal size of lean and obese rats increased to above baseline values both at night and during the day. In contrast, no rebound was observed for meal frequency. The distribution of meal sizes during drug treatment days compared to baseline days is summarized in Fig. 4. Ro 22-0654 reduced the average meal sizes of both obese and lean rats by eliminating the largest meals.

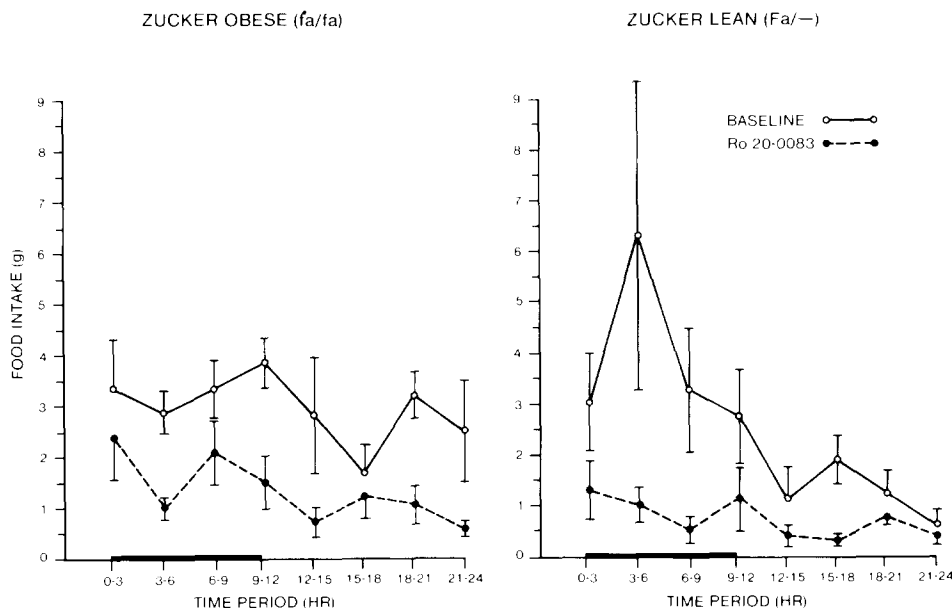


FIG. 5. Temporal effects of administration of Ro 20-0083 (inhibitor of human pancreatic lipase) on food intakes of obese and lean Zucker rats in Experiment 2. Eight consecutive 3-hr time periods are shown, with the 12 hour dark phase (beginning at 17:00) indicated by a solid bar. The data represent mean food intakes (in grams) during each 3 hour period. SEM bars are also indicated.

The effects of Ro 20-0083 on food and water intakes are summarized in Table 4. During the baseline period, the rats ate more food at night than during the day, $F(1,8)=33.20$, $p<0.01$, and obese rats ate more food than lean rats. Ro 20-0083 reduced the food intake of lean rats by 72% and that of obese rats by 56%. The main effect of condition was significant, $F(2,16)=59.69$, $p<0.01$. Food intake was reduced more at night than during the day, leading to a condition by time interaction, $F(2,16)=3.68$, $p<0.05$. Recovery following drug administration was complete within 3 days and no significant differences from baseline intake were observed.

The temporal patterns of food intake are summarized in Fig. 5. Food intake of both obese and lean rats was reduced over the entire 24 hour period and no rebound feeding occurred. The condition by time interaction was again significant, $F(14,112)=4.15$, $p<0.01$.

The observed decrease in food intake was achieved through significant reductions in meal size, $F(2,16)=18.60$, $p<0.01$, and in meal frequency, $F(2,16)=6.11$, $p<0.05$. No main effects of genotype or time-related interactions were observed. The distribution of meal sizes during treatment days as compared to baseline days is summarized in Fig. 6. Ro 20-0083 reduced meal sizes of both obese and lean rats: meals consumed during the treatment period were primarily below 2 g for the obese and below 1 g for the lean rats.

DISCUSSION

Peripherally-acting antiobesity agents may influence energy metabolism through modulation of nutrient absorption, endocrine function, or carbohydrate or lipid metabolism [26,28]. Some of the compounds have also been shown to affect overall food intake, presumably via an indirect modulation of appetite and satiety mechanisms. Therefore, one important aspect of any pharmacological evaluation of these new agents is a detailed description of their action not only

on the 24 hr food and water intakes but also on the diurnal patterns and parameters of feeding.

Of the anorectic agents tested here, acarbose (Bay g 5421) is the most extensively studied. Previous research has primarily focused on the long term effects of acarbose on body weights of growing Sprague-Dawley or Zucker rats. In one study [17], the body weight gain of male and female obese Zucker rats (mean initial weights 324 and 293 g respectively) fed a semisynthetic diet containing 45% carbohydrate (starch and sucrose in equal amounts) was dose-dependently reduced over an 8 week period. The reduced body weight gain was reported to be due to a highly significant reduction of the total lipids in the carcass of obese rats [18]. In another experiment reported by these investigators [18], food intakes of mature obese male Zucker rats were reduced by 27% following administration of 40 mg/100 g diet for 28 days and by 34% following 80 mg/100 g diet. The rats' initial bodyweights were in the range 558–585 g and were reduced by as much as 100 g by the highest dose of acarbose at the end of the test period. The same investigators have reported comparable results with female obese Zucker rats [18] after 34 weeks feeding of a sucrose containing diet to which 20, 40 or 80 mg acarbose per 100 g diet had been added.

These data showing the effects of acarbose on obese Zucker rats are at variance with the results of a more recent study [30,31], in which growing 7 week old obese and lean Zucker rats were fed either 0, 20 or 40 mg of acarbose per 100 g of diet (45% complex carbohydrate; 35% fat and 20% protein calories) for a period of 13 weeks. Obese Zucker rats showed no significant reductions in food intake. The percentage of total body fat in the obese Zucker rats given acarbose was not significantly reduced in spite of lowered final body weights. However, lean Zucker rats did show significant reductions in body weight and in cumulative food in-

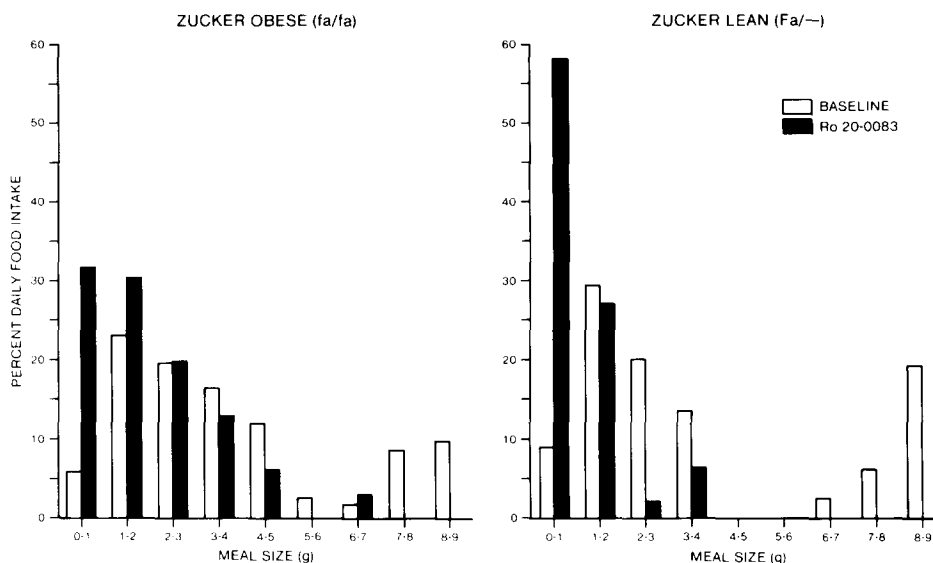


FIG. 6. Effects of Ro 20-0083 on the 24 hour distribution of meal sizes of obese and lean Zucker rats in Experiment 2. The data represent the percentage contribution of different-size meals to the total daily food intake. Meal sizes are calculated on the basis of 15 minute IMI criterion.

takes. At the highest dose of acarbose, the food intake of lean rats was reduced by 13% at the end of 13 weeks [30,31].

Further studies on the effects of acarbose were conducted on Sprague-Dawley rats also fed "obesogenic" diets containing sucrose. In one study [30,32], groups of six 12-week-old female Sprague-Dawley rats were fed a diet of powdered chow with access to a 40% sucrose solution. Acarbose was added per 100 g of chow in doses 0, 20 or 40 mg for a period of 38 weeks. Analyses of the data showed no differences in daily caloric intakes, and thus no dose-dependent effect of acarbose on food intake between the three sucrose consuming groups. These authors failed to find any differences in cumulative caloric intakes of chow and sucrose or in cumulative total calories either between weeks 1-20 or between weeks 21-38. However, in contrast to the stable food intake data, body weights of the 20 and 40 mg groups were significantly below those of the 0 mg acarbose group by the conclusion of the experiment. Moreover, while the 0 mg group had become significantly obese as determined by percentage of carcass fat (30.3%), the 20 and 40 mg groups maintained a nearly normal body composition [30,32]. Contrasting with the above data, is a recent report [24] that administration of up to 45 mg acarbose/100 g in a high sucrose diet to female Sprague-Dawley rats for 20 days resulted in a significant reduction in food intake and body weight. Meal sizes were reported to be decreased but meal frequency was increased at the highest dose of acarbose.

It should also be noted that in one study [11], acarbose added in a range of doses (including 80 mg/100 g diet) to a starch diet comparable to the one used here actually resulted in significant dose-dependent increases in daily food intake in female Sprague-Dawley rats over a period of 14 days. However, body weights were markedly reduced under this drug regimen. Mixed in a high-fat diet, acarbose had no significant effects overall, but resulted in a general trend for

suppression of food intake, which was significant only at the large dose of 80 mg/100 g diet. No significant effects on the rats' body weight were obtained following 14-days of a high-fat diet [11].

The effectiveness of acarbose as an anti-obesity agent thus appears to vary with the nature of the diet, duration of drug administration, and the type of obesity studied [11, 17, 24, 30-32]. Acarbose appears most effective in preventing body weight gain in young, growing rats fed a sucrose-supplemented diet, although as discussed above, the reported food intake data are not always in complete agreement. Acarbose proved ineffective when mixed with a high-fat diet, and actually resulted in increased food intake when mixed into a high starch diet [11]. Using powdered laboratory chow (high starch) and a high dose of acarbose, we failed to modify the rats' food intakes or influence the parameters of meal-taking behavior.

It is also possible that the effects of acarbose on food intake may be influenced by the rats' age or the duration of treatment. Zucker rats used in the present study were 13 weeks old with mean body weights of 462 g for the obese and 317 g for the lean group. In the study by Vasselli *et al.* [31] male Zucker rats weighed less than 200 g at 7 weeks of age and presumably showed a more rapid rate of growth. In that study [31] food intake of obese rats (age 7 weeks) was significantly reduced following the high dose of acarbose (40 mg/100 g diet) during the first week of acarbose treatment but at no time thereafter. Furthermore, obese rats fed the medium dose of acarbose (20 mg/100 g diet) showed a gradual tendency towards an increased daily intake [31]. As noted previously, acarbose-fed obese rats maintained their obese body composition on a percentage basis at levels not significantly different from that of the 0 mg obese control group. The only other study [18] to report the effects of acarbose on food intakes of obese Zucker rats used mature

male Zucker rats (weight range 558–585 g) over a 28-day period. These rats were reported to lose between 30–100 g of body weight when fed both 40 and 80 mg/100 g acarbose and to reduce their food consumption by up to 34%, although it is not clear how the percentage reduction in food intake was calculated. These two reports are clearly at odds, and our data on the short-term effects of two inhibitors of carbohydrate absorption appear to be more consistent with those of Vasselli *et al.* [31] in demonstrating that the food intakes of obese rats were unaffected by acarbose.

While acarbose may prevent the development of dietary obesity in young rapidly growing rats, there is little information on the effects of acarbose on body composition of mature and already obese Zucker rats. Given the demonstrated ability of the Zucker rat to divert ingested energy towards fat deposition even in the face of caloric restriction [16], it is possible that acarbose would not be effective in altering body composition of mature, obese Zucker rats. The clinical usefulness of commercial inhibitors of carbohydrate absorption in the management of human obesity remains doubtful [3], especially given the undesirable side effects produced by carbohydrate malabsorption [1]. More recently, it has been suggested that inhibitors of carbohydrate absorption might be more useful in the treatment of diabetes than as antiobesity agents [12,22]. Several non-oligosaccharide inhibitors of carbohydrate absorption which appear to avoid the malabsorption problems of acarbose and similar compounds have recently been described and are in early clinical trials [20].

Previous research on antiobesity drugs has primarily focused on modulation of carbohydrate rather than lipid metabolism. However, animal studies on hyperphagia and dietary obesity indicate that some of the most effective obesity promoting diets are high in fat as well as sugar. These include "supermarket" diets (chocolate chip cookies, salami) or powdered chow supplemented with Crisco or with sweetened condensed milk [9,23]. The more recently developed inhibitors of lipid absorption or synthesis may be better suited to deal with excessive ingestion of dietary fats. Previous studies on Ro 22-0654 and Ro-0083 have addressed primarily their effects on metabolism and body composition. Chronic administration of Ro 22-0654 produced a marked

weight loss in both obese and lean Zucker rats [29]. Preliminary reports suggest that inhibition of fatty acid synthesis produced by this compound was not the result of changes in the activities of acetyl CoA carboxylase, fatty acid synthetase, or citrate cleavage enzyme (Triscari and Sullivan, unpublished observations). Body composition studies indicate that the weight loss in obese and lean Zucker rats following chronic treatment with Ro 22-0654 was attributable primarily to a significant decrease in carcass lipid content, although an initial transitory decrease in food intake was observed as well [29]. The present data indicate that inhibitors of peripheral lipid metabolism have significant acute effects on the daily food intakes of laboratory rats, which is consistent with their reported effects on body composition.

The present findings that inhibitors of lipid metabolism reduced food intake during three days of treatment suggest a link between lipid metabolism and the regulation of food intake that may be exploited further in the pharmacological management of obesity. The inhibitors of lipid synthesis (Ro 22-0654) and of pancreatic lipase (Ro 20-0083) reduced food intakes of both obese and lean rats primarily through their action on meal size. The observed reduction in meal frequency suggests further that these agents may delay meal initiation and may promote earlier satiation during a meal. While compensatory hyperphagia during recovery was observed in lean rats, no rebound feeding was observed in the obese. It should be noted here that the reported effects are for acute drug treatment only, and that preliminary long term studies of food intake suggest that some of the anorectic effects of peripherally acting compounds may be abolished following several consecutive days of drug administration [29]. Nevertheless the potential short-term anorexia in combination with the long term effects of these agents on lipid metabolism may provide a basis for new treatment modalities in obesity management.

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