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Normal Pancreatic and Intestinal Enzymes in Hypophagic Growth-Retarded Rats That Received Dorsomedial Hypothalamic Lesions Shortly After Weaning[1]

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BERNARDIS, L. L., P. C. LEE, S. BROOKS AND E. LEBENTHAL. *Normal pancreatic and intestinal enzymes in hypophagic growth-retarded rats that received dorsomedial hypothalamic lesions shortly after weaning.* PHARMACOL BIOCHEM BEHAV 21(2)245–253, 1984.—Male weanling Sprague-Dawley rats received bilateral electrolytic lesions in the dorsomedial hypothalamic nuclei (DMNL rats). Sham-operated rats served as controls. After being fed lab chow for two postoperative weeks, the animals were divided into four groups. One group of DMNL rats and controls received a high-caloric diet (high-fat diet, chocolate chip cookies, 32% sucrose solution, potato chips and marshmallows), whereas another group of DMNL rats and controls continued to receive lab chow. The experiment was terminated on the 185th postoperative day. In accordance with previous findings, DMNL rats, irrespective of diet, were lighter and shorter than controls. In addition, DMNL rats fed junk food were lighter than DMNL rats fed lab chow, and junk-fed controls weighed as much as chow-fed controls. Both DMNL rats and controls fed junk food were also shorter and showed higher carcass fat than their chow-fed counterparts. Also, DMNL rats fed junk food had less carcass fat than junk-fed sham-operated controls, whereas in accordance with previous findings, there was no difference between chow-fed DMNL rats and chow-fed sham-operated controls. Irrespective of diet, DMNL rats ate less calories than their respective sham-operated controls. Both absolute and percent pancreas weight and protein/pancreas were unaffected in DMNL rats but were reduced in both junk-fed groups in comparison with their chow-fed counterparts. Both concentrations and contents of pancreatic trypsinogen, amylase and lipase were unaffected in DMNL rats but total activities of all three enzymes were dramatically reduced in the junk-fed compared with the chow-fed DMNL rats. Total activities of trypsinogen and amylase but not lipase were also reduced. Duodenal mucosal weight, total protein and specific activities of enterokinase, leucine amino peptidase and maltase were uninfluenced by lesions and diet alike. In the jejunum, specific activity of enterokinase was dramatically reduced in junk-fed versus chow-fed DMNL rats; the same obtained for junk-fed vs. chow-fed sham-operated control groups. Jejunal lactase, sucrose and maltase were not affected by DMN lesions but both DMNL rats and sham-operated controls fed junk foods showed lower specific activities than their chow-fed counterparts. The data indicate that production of DMN lesions in weanling rats, although followed by dramatic alterations in ponderal and linear growth and food intake, causes little, if any, changes in a number of pancreatic and small intestinal parameters, including enzymes. The only significant changes are those related to diet effects. These findings suggest that the DMNL rat responds to dietary factors like its sham-operated control and suggest that changes in digestive function cannot be invoked as possible causes of growth retardation and food intake in the weanling DMNL rat.

Pancreatic enzymes DMN lesions Growth retardation

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WEANLING rats with bilateral electrolytic lesions, primarily destroying the dorsomedial hypothalamic nucleus (DMNL rats) show retardation of both ponderal and linear growth, hypophagia and hypodipsia but exhibit normal body composition, plasma growth hormone (GH) and insulin [5,8], T_3 and somatomedin levels [3].

Weanling DMNL rats, although grossly hypophagic and of lower body weight, show normal caloric metering [8,9], efficiency of food utilization [13] and body weight regulation [1]. We have also reported that weanling DMNL rats, despite being hypodipsic, exhibit normal water/food intake ratios and elimination of a water load [5], show normal plasma osmolality [12] and respond normally to extra and intracellular thirst challenges. Furthermore, DMNL rats respond normally to angiotensin II administration [2]. That these changes are in all likelihood not due to destruction of fibers *en passage* but rather due to the ablation of cell bodies within the DMN was recently demonstrated by Bellinger *et al.* [3] by using Kainic Acid (KA) lesions. This neurotoxin destroys neurons but leaves fibers of passage intact [31].

Most notably, KA lesions, although much smaller than electrolytic lesions, reduced food and water intake quite severely. Also, rats so operated rejected lab chow and a high-fat diet but ate a 30% sucrose-chow mix.

In view of these findings and the failure to find metabolic alterations in the weanling DMNL rat [8], we have postulated some time ago that DMN lesions bring about a "resetting" of some central nervous mechanism that allows these animals to exist "normally" with lower energy intake. More recently, we have widened this resetting hypothesis to an "organismic" hypothesis [17]. The latter is an attempt at reconciling reduced growth, food and water intake with normal body composition, anabolic hormone levels and metabolism. It states that the total animal has been harmoniously "scaled down" in all homeostatic compartments rather than showing specific disruptions such as the weanling rat with ventromedial hypothalamic lesions (VMNL rat) [15,16].

The present study was designed to explore gastrointestinal-digestive aspects of the DMNL rat in terms of several pancreatic, duodenal and jejunal parameters including the major digestive enzymes, in response to a variety of high-calorie and junk food diets.

METHOD

Animals and Operation

Weanling male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) were accommodated in individual cages and given Charles River Rat Mouse Hamster Formula and tap water ad lib. The room was temperature (23°C) and light cycle-controlled (L:D 12:12, lights on at 0600 hr). Several days later (age 27 days), they received bilateral electrolytic lesions in the dorsomedial hypothalamic nuclei, using stainless steel wires (0.37 mm diameter) that were spar varnish-coated and bared at the tip (0.2 mm). A direct anodal current of 1.0 m Amp was allowed to flow for 10 seconds. Sham-operated animals served as controls. The details of the operational procedures have been previously described [7]. Prior to the operation, body weight and nose-tail length were recorded under anesthesia to obtain precise baseline measurements.

The animals were returned to their cages and given lab chow and tap water for the next two weeks. At that point they were anesthetized with ether and body weight and length were again determined. Food intake was also recorded to obtain a mean value for the first two weeks following the operation. Using body weight and length obtained at the two-week measuring point, the animals were divided into four groups. Group 1 (DMNL rats) and Group 2 (controls) were subsequently fed a high-fat diet and 32% sucrose solution [28] as drinking fluid for 101 days. For the following 34 days they received, in addition to the above, chocolate chip cookies and for the following 25 days, two common human junk food items: potato chips and marshmallows. The reason for changing diets at that time was our failure to obtain more pronounced body weight gains in the high-fat diet-fed groups. Stephens [43] had produced increases in body weight and fat in rats fed cafeteria diets for 100 days from weaning.

Food intake was measured every third day throughout the experiment; intake from the sugar solution was measured and the bottles refilled every second day. Energy intake was calculated from food tables and information supplied by the manufacturers. According to Rothwell and Stock [35], these values come within 3% of those derived from bomb-calorimetry. The composition and caloric density of these foodstuffs are shown in Table 1. Group 3 (DMNL rats) and Group 4 (controls) were fed lab chow throughout the experiment.

On the 185th postoperative day (age 212 days) all rats were again anesthetized with ether to determine body weight and nose-tail length and were killed by decapitation on the following day.

Pancreata were removed, trimmed of fat and mesenteries and processed for the determination of protein, DNA, trypsinogen, lipase and amylase.

Pancreatic tissues were minced with sharp scissors to facilitate further homogenization in distilled water (1 mg/100 μl) at 4°C with a Potter Elvehjem homogenizer and a Teflon pestle. Enzyme activities were determined from fresh tissue homogenate.

The whole intestine from the pyloric end to the ileal-cecal region was removed, and trimmed of fat and mesentery. The length of the intestine was measured by freely suspending it and attaching a 5 g weight to one end. A length of 15 cm was removed from the proximal end representing the duodenum. Another 15 cm length was removed from the remaining 30–45 cm section and taken as the jejunal segment. Each segment was split and the intestinal content removed by gently wiping with tissue paper. The mucosa was scraped and weighed separately and the mucosal preparation from each segment was separately homogenized with a Potter-Elvehjem homogenizer using a Teflon pestle with the vessel immersed in crushed ice. Homogenates were used for the determination of DNA, protein, lactase, maltase, sucrase, enterokinase and leucine aminopeptidase.

Trysinogen was first activated with partially purified rat mucosal enterokinase at a constant ratio of enterokinase to homogenate protein for 45 min at 25°C, conditions that yielded optimal and reproducible activation of this zymogen in our laboratory. Trypsin activity was then measured from the hydrolysis of p-nitroaniline from the substrate benzoyl-DL-agrinine-p-nitroanilide (BAPNA) at pH 8.2 and 25°C. Units are expressed as nanomoles of BAPNA hydrolyzed per min per mg protein. Lipase activity was determined by potentiometric titration (at a constant pH of 8.0) of ionized fatty acids liberated from an olive oil emulsion [39]. Units are expressed as micromoles of NaOH required to neutralize the free fatty acid liberated per min per mg protein. Amylase was determined by a saccharogenic method [38], using soluble

TABLE 1

COMPOSITION AND CALORIC DENSITY OF DIETARY ITEMS USED IN THIS EXPERIMENT

Food Stuff	Carbohydrate*	Fat*	Protein*	Calories (kcal/g)
Charles River Rat Mouse Hamster Formula†	42.0	7.0	26.5	3.90
High-Fat Diet Charles River Rat Mouse Hamster Formula Powder 60% Corn Oil 35%, Sucrose 5%	35.3	45.8	18.4	5.42
Chocolate Chip Cookies‡	66.0	24.5	4.8	5.40
32% Sucrose Solution in Tap Water	32.0	0	0	1.30
Potato Chips§	51.7	36.9	7.4	5.30
Marshmallows#	83.3	6.0	6.06	3.33

*Percent by weight.
†Country Foods, Div. of Agway, Hauppauge, NY.
‡Chip-a-Roos, Sunshine Biscuits, Inc., NY.
§Dan-Dee Pretzel and Potato Chip Co., Cleveland, OH.
#Gold Crest Candy Co., Springdale, OH.

starch as the substrate. Units are expressed as micromoles of maltose liberated per min per mg protein.

Protein was determined by the Lowry technique [30] using bovine serum albumin fraction V as the standard.

Deoxyribonucleic acid was first precipitated with 0.5 N perchloric acid, hydrolyzed and the deoxyribose nucleotide residue measured by the colorimetric reaction with diphenylamine reagent according to Burton [19], using highly polymerized calf thymus DNA as the standard. A modified procedure was adapted to minimize the interference by sialic acids according to Croft and Lubran [22].

Maltase, lactase and sucrase were assayed by the method of Dahlquist [21], using maltose, lactose, and sucrose as the corresponding substrates. Disaccharidase activity was expressed as micromoles of disaccharides hydrolyzed per min per mg protein.

Enterokinase was assayed in two steps. The mucosal homogenate was preincubated with trypsinogen and aliquots of the incubation mixture were determined for trypsin formed. Trypsin activity was assayed according to the method of Erlanger [25], using benzoyl-DL-arginine-p-nitroanilide as the substrate. Activity was expressed as micromoles of substrate hydrolyzed per mg protein. Leucine aminopeptidase was assayed by the method of Szasz [44], using L-leucine-p-nitroanilide as the substrate. The activity was expressed as micromoles of substrate hydrolyzed per min per g protein.

Carcasses were skinned and eviscerated and total lipids were determined using the method of Folch [26].

The brains were dissected out and prepared for histological examination of lesion localization as previously described [6]. After elimination of animals with improperly placed lesions, the following population remained: Group 1 (DMNL rats—junk food), n=12; Group 2 (controls—junk food), n=8; Group 3 (DMNL rats—lab chow), n=10; Group 4 (controls—lab chow), n=6.

The data from these animals were analyzed using Analysis of Variance (ANOVA) and Tukey's test.

RESULTS

Lesion Localization

Figure 1 indicates that the lesions in a rat representative of Groups 1 and 3 are located in the dorsomedial hypothalamic nuclei (DMN) and that neither the ventromedial nuclei (VMN) nor the lateral hypothalamic area (LHA) were injured.

Somatic Parameters

Table 2 shows that DMN lesions resulted in significant body weight reductions in both Groups 1 and 3. They also resulted in a greater growth retardation in the DMNL rats fed junk food than in chow-fed DMNL rats. There was no difference in body weight or body weight gain (Δ body weight) between junk-fed and chow-fed sham-operated controls.

Table 2 shows that linear growth was significantly reduced in both groups with DMN lesions. In addition, it shows that both DMNL rats and sham-operated controls fed junk food were significantly shorter than their counterparts fed lab chow. Finally, Table 2 indicates that both DMNL rats and sham-operated controls fed the high-calorie junk food diets had significantly greater percentages of carcass fat than their chow-fed counterparts and that the DMNL rats fed junk food had less carcass fat than junk-fed sham-operated controls. However, chow-fed DMNL rats and chow-fed sham-operated controls had identical percentages of carcass fat.

Mean Caloric Intake

Both groups with DMN lesions ingested less calories than

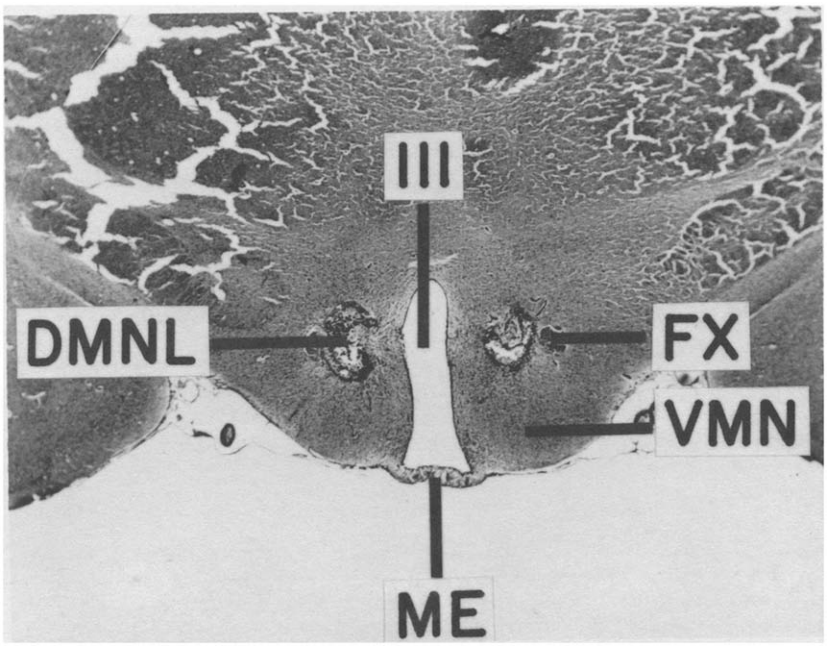

FIG. 1. Localization and size of DMN lesions in a representative rat. Note that neither the ventromedial hypothalamic nuclei nor the lateral hypothalamic area have been injured. Abbreviations: DMNL=dorsomedial nucleus lesions; VMN=ventromedial hypothalamic nucleus; ME=median eminence; III=third ventricle; FX=fornix. (Cresyl Violet 13.5×).

TABLE 2

SOMATIC PARAMETERS OF WEANLING DMNL AND CONTROL RATS FED LAB CHOW AND JUNK FOOD

Parameter/ Group	1 DMNL† (12)	2 CON† (8)	3 DMNL‡ (10)	4 CON‡ (6)	Effect of			Tukey's $p<$			
					Diet	Lesion	Diet × Lesion	1 vs. 2	1 vs. 3	3 vs. 4	2 vs. 4
Body weight at sacrifice (g)	328±7.89	421.3±11.84	377.6±12.03	451.0±18.26	12.18* 0.001	54.02 0.001	N.S.	0.01	0.01	0.01	N.S.
Δ Body weight (operation-sacrifice) (g)	221.3±7.88	343.6±11.38	301.3±12.32	373.8±18.28	23.61 0.001	73.79 0.001	N.S.	0.01	0.01	0.01	N.S.
Nose-tail length at sacrifice (mm)	417.0±2.44	442.6± 3.37	442.2± 2.60	470.3± 6.19	67.54 0.001	69.59 0.001	N.S.	0.01	0.01	0.01	0.01
Δ Nose-tail length (operation-sacrifice (mm)	170.6±3.13	199.4± 3.03	197.5± 3.33	221.5± 6.33	48.27 0.001	56.04 0.001	N.S.	0.01	0.01	0.01	0.01
Carcass Fat (%)	13.43±0.68	18.99± 1.40	7.25± 0.51	7.18± 0.97	110.2 0.001	10.1 0.01	10.9 0.01	0.01	0.01	N.S.	0.01
Total Energy Intake (kcal/day)	71.52±2.11	89.11± 2.97	77.53± 3.22	96.92± 2.21	6.64 0.05	47.58 0.001	N.S.	0.01	N.S.	0.01	N.S.

*F(1,32).
†High-fat diet, 32% sucrose solution, chocolate chip cookies and junk food (Table 1).
‡Lab chow (Table 1).
N.S.=not significant ($p>0.05$).

TABLE 3

WEIGHT, PERCENT WEIGHT, PROTEIN/PANCREAS AND PANCREATIC ENZYME ACTIVITY IN WEANLING RATS WITH DORSOMEDIAL HYPOTHALAMIC LESIONS AND THEIR CONTROLS FED CHOW AND JUNK FOOD

Parameter/ Group	1 DMNL† (12)	2 CON† (8)	3 DMNL‡ (10)	4 CON‡ (6)	Effect of Diet	Lesion	Diet × Lesion	Tukey's $p<$ 1 vs. 2	1 vs. 3	3 vs. 4	2 vs. 4
Pancreas Weight (mg)	734.2±42.29	887.5±42.68	1467.0±51.97	1568.3±154.22	119.97* 0.001	N.S.	N.S.	N.S.	0.01	N.S.	0.01
Pancreatic Weight/Body Weight (mg/g)	2.31±0.141	2.12±0.111	3.91±0.167	3.45±0.234	87.16 0.001	N.S.	N.S.	N.S.	0.01	N.S.	0.01
Protein/ Pancreas (mg/mg)	111.43±10.69	133.69±7.82	229.59±11.53	245.52±28.91	81.58 0.001	N.S.	N.S.	N.S.	0.01	N.S.	0.01
Enzyme Concentrations:											
Trypsin (U/mg Prot)	49.25±3.07	43.03±3.17	35.15±2.73	34.85±2.48	13.97 0.001	N.S.	N.S.	N.S.	0.01	N.S.	N.S.
Amylase (U/mg Prot)	24.94±3.70	18.58±1.91	89.945±16.07	93.48±15.18	46.18 0.001	N.S.	N.S.	N.S.	0.01	N.S.	0.01
Lipase (U/mag Prot)	158.94±19.08	147.08±14.36	123.49±7.847	102.65±16.07	6.53 0.05	N.S.	N.S.	N.S.	N.S.	N.S.	0.05
Enzyme Contents:											
Trypsin (kU/panc)	5.51±0.548	5.80±0.589	8.00±0.606	8.47±0.789	18.13 0.001	N.S.	N.S.	N.S.	0.05	N.S.	0.01
Amylase (kU/panc)	2.94±0.568	2.44±0.249	20.04±3.24	22.58±3.81	74.32 0.001	N.S.	N.S.	N.S.	0.01	N.S.	0.01
Lipase (kU/panc)	18.83±3.66	19.46±1.95	28.17±1.79	24.40±3.417	5.81 0.05	N.S.	N.S.	N.S.	0.05	N.S.	0.05

*F(1.32).
†High-fat diet, 32% sucrose solution, chocolate chip cookies and junk food (Table 1).
‡Lab chow (Table 1).
N.S.=not significant ($p>0.05$).

their respective sham-operated controls. Comparable to the pattern of body weight and length changes, junk-fed DMNL rats ate less than chow-fed DMNL rats (Table 2).

Pancreatic Parameters

Table 3 indicates that both absolute and relative (per body weight) pancreas weight and protein/pancreas were not affected by the DMN lesions but were significantly reduced in both high-calorie-junk-fed groups in comparison with their chow-fed counterparts.

Both concentrations and contents of pancreatic trypsinogen, amylase and lipase were not affected by DMN lesions. Total activities of all three enzymes were dramatically reduced in high-calorie-junk-fed compared with chow-fed DMNL rats. Similarly, total activity of trypsinogen and amylase but not lipase were reduced in junk-fed vs. chow-fed sham-operated controls.

Specific activities of trypsin were significantly elevated and of amylase were significantly reduced in junk-fed DMNL compared with chow-fed DMNL rats. Lipase, however, remained the same. Both amylase and lipase specific activities were reduced and elevated, respectively, in junk-fed sham-operated controls in comparison with chow-fed sham-operated controls.

Duodenal Parameters

Table 4 shows that mucosal weight, total protein and specific activity of enterokinase, leucine aminopeptidase, lactase and maltase were uninfluenced by lesions and diet alike.

Jejunal Parameters

Table 5 indicates that the only parameter that was unaffected by lesion and diet alike was mucosal weight. Jejunum total protein was significantly reduced in junk-fed DMNL rats compared with junk-fed sham-operated controls and was significantly elevated in junk-fed controls vs. chow-fed controls. Specific activity of enterokinase was significantly altered by diet only: junk food-fed DMNL rats had dramatically less enterokinase activity than chow-fed DMNL rats and the same pattern and magnitude obtained for the sham-operated control groups. In addition, the chow-fed DMNL rats had lower activity than the chow-fed sham-operated

TABLE 4

MUCOSAL WEIGHT (mg), TOTAL PROTEIN (mg) AND SPECIFIC ACTIVITIES OF DUODENAL ENZYMES IN WEANLING RATS WITH DMN LESIONS AND SHAM-OPERATED CONTROLS FED LAB CHOW AND JUNK FOOD

Parameter/ Group	1 DMNL† (12)	2 CON† (8)	3 DMNL‡ (10)	4 CON‡ (6)	Effect of			Tukey's $p<$			
					Diet	Lesion	Diet × Lesion	1 vs. 2	1 vs. 3	3 vs. 4	2 vs. 4
Mucosal Weight (mg/15 cm)	305.0±21.93	375.0±17.98	320.0±17.21	316.7±32.76	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Total Protein (mg/15 cm)	42.08±5.36	44.58±3.71	37.49±2.05	37.55±4.22	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Enterokinase (U/g protein)	1.29±0.16	1.30±0.126	1.71±0.235	1.63±0.395	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Leucine Amino peptidase (U/g protein)	22.2±2.22	20.52±2.44	25.98±2.43	21.81±3.21	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Lactase (U/g protein)	8.20±1.50	10.35±1.39	8.87±1.92	7.20±2.40	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Sucrase (U/g protein)	63.29±5.409	54.28±7.124	85.69±9.423	100.58±9.410	19.17 0.001	N.S.	N.S.	N.S.	N.S.	N.S.	0.01
Maltase (U/g protein)	158.9±20.85	170.9±27.14	207.6±21.43	211.17±30.04	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*F(1,32).
†High-fat diet, 32% sucrose solution, chocolate chip cookies and junk food (Table 1).
‡Lab chow (Table 1).
N.S.=not significant ($p>0.05$).

TABLE 5

MUCOSAL WEIGHT, TOTAL PROTEIN AND SPECIFIC ACTIVITY (U/mg PROTEIN) OF ENZYMES IN *JEJUNUM* OF WEANLING RATS WITH DMN LESIONS AND SHAM OPERATIONS FED LAB CHOW AND HIGH-PALATABILITY JUNK FOOD

Parameter/ Group	1 DMNL† (12)	2 CON† (8)	3 DMNL‡ (10)	4 CON‡ (6)	Effect of			Tukey's $p<$			
					Diet	Lesion	Diet × Lesion	1 vs. 2	1 vs. 3	3 vs. 4	2 vs. 4
Mucosal Weight (mg)	292.5±28.84	330.0±38.24	279.0±16.44	270.0±92.14	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Total Protein (mg)	38.83±3.51	50.49±5.13	35.57±2.36	34.47±4.74	6.86 0.05	N.S.	N.S.	N.S.	N.S.	N.S.	0.01
Enterokinase U/g protein	0.050±0.0094	0.050±0.0189	0.147±0.0121	0.207±0.0444	49.79 0.001	N.S.	N.S.	N.S.	0.01	0.05	0.01
Leucine Amino peptidase U/g protein	38.80±4.04	42.46±4.66	50.57±4.72	58.63±8.00	8.16 0.01	N.S.	N.S.	N.S.	N.S.	N.S.	0.05
Lactase U/g protein	15.65±3.42	10.40±1.97	33.00±2.43	33.42±4.77	26.95 0.001	N.S.	N.S.	N.S.	0.01	N.S.	0.01
Sucrase U/g protein	73.43±10.88	64.00±3.77	198.64±13.77	217.35±11.29	120.08 0.001	N.S.	N.S.	N.S.	0.01	N.S.	0.01
Maltase U/g protein	238.8±26.20	203.5±12.17	478.8±30.39	468.67±43.05	115.02 0.001	N.S.	N.S.	N.S.	0.01	N.S.	0.01

*F(1,32).
†High-fat diet, 32% sucrose solutiion, chocolate chip cookies and junk food (Table 1).
‡Lab chow (Table 1).
N.S.=not significant ($p>0.05$).

controls. Leucine aminopeptidase was reduced in junk-fed vs. chow-fed sham-operated controls. Jejunal lactase, sucrase and maltase were not affected by DMN lesions, however, both DMNL rats and sham-operated controls fed junk food evinced lower specific activities than their chow-fed counterparts (Table 5).

DISCUSSION

The histological and somatic data of the present study confirm previous findings and indicate that weanling DMNL rats are shorter and weigh and eat less than their sham-operated controls [5,8]. However, the present data show that DMNL rats fed high-calorie-junk food diets, although showing the body weight and length changes of their chow-fed counterparts, exhibit reduced carcass fat compared with their controls. It should be noted that chow-fed DMNL rats have the same body composition as their chow-fed controls [5].

A striking finding of the present study is that body weight and weight gain and body length and length gain in the high-calorie-junk food-fed DMNL rats are less than in their chow-fed counterparts. This is all the more astounding as both DMNL rats and controls fed the high-calorie-junk food diets ate the same calories as their respective chow-fed counterparts.

It should also be noted that both DMNL and control rats fed the high-calorie junk food diets were significantly shorter than their chow-fed counterparts. Since their calorie intake was lower but since they nevertheless deposited more fat than the chow-fed animals, the energy required for lipogenesis may have been used at the expense of growth.

Although our calculations show that the junk-fed groups were not below the lower margin of the protein requirement for the growing rat [40, 41, 42], it cannot be entirely ruled out that their poor linear growth is related to a marginal protein intake.

The most surprising finding regarding the pancreas is that neither the high-calorie-diet-fed nor the chow-fed DMNL rats showed significant differences from their sham-operated controls in any of the nine parameters measured. This indicates that the lesions that caused such dramatic changes in body weight, linear growth and food intake did not disturb pancreatic function, at least as measured by the parameters of the present study. This is in contrast to findings in weanling rats with ventromedial hypothalamic lesions (VMNL rats). The latter animals, when fed synthetic diets that contain sucrose, showed decreases in most of the above parameters when compared with their controls fed the same diets. Evidently, as in so many other parameters, VMNL and DMNL are followed by opposite responses [8,11]. Regarding the duodenum, the only parameter in which a diet effect was noted was sucrase activity; in this instance the high-calorie-diet-fed controls showed a lower activity than their chow-fed counterparts.

Although weanling VMNL and DMNL rats show opposite effects in regard to pancreatic parameters, they are comparable regarding duodenal changes: chow-fed VMNL rats showed no change whatsoever in any of the above parameters measured, therefore, both types of lesions appear to have no effect on duodenal function.

The lack of a lesion effect was also evident in the jejunum, inasmuch as in none of the seven parameters measured in this study was there a significant difference between DMNL rats and controls. However, a diet effect was noted which in four out of seven parameters involved both DMNL rats and control groups.

It is well established that the acinar cell can alter its enzyme composition in response to dietary composition [21]. Since our junk food diets had a high proportion of fat, the findings of reduced amylase and high lipase activities are in accordance with what might be expected in the intact rat [27]. The data are thus in agreement with the general pattern of pancreatic enzyme adaptation and in addition indicate that despite the profound somatic alterations DMN lesions do not compromise pancreatic adaptive mechanisms.

Since our junk diets also contained high amounts of carbohydrate, and since pancreatic amylase synthesis is increased by feeding of carbohydrate-rich diets [20], it is difficult to interpret the sharp drop in pancreatic amylase in the junk-fed rats. One explanation might be that the lipid component of the diet overshadows the action of the carbohydrate part. An alternative interpretation is that not only diet but perhaps the obese state affects enzyme activity. As yet unpublished findings from our laboratories indicate that in obese Zucker rats amylase is decreased and lipase and trypsinogen is increased. This enzyme activity profile resembles that of our present junk-fed groups.

We are not aware of any studies in the effects of obesity—dietary and otherwise—on duodenal enzymes. However, preliminary data on obese Zucker rats indicate no changes in duodenal enzymes (Lee *et al.*, unpublished observations).

The data show that production of DMN lesions in weanling rats, although followed by dramatic retardation of ponderal and linear growth and food intake, causes little, if any, effect on a number of small intestine parameters, including digestive enzymes. This suggests that DMNL rats respond to dietary factors like their sham-operated controls. The only significant changes in most parameters are those related to diet effects.

Our failure to obtain greater weight gains in junk food-fed than in chow-fed rats could be due to several factors such as the young age of the animals, diet variability, duration of availability of the diets and type of food items used.

Although weanling rats have been reported to be resistant to dietary and sucrose-solution-induced obesity [28], they do become obese after the age of 70 days. This was evidently not the case in the weanling rats of the present study and we cannot offer an explanation for this.

Others who have been successful in producing dietary obesity have used a great variety of food items [35]. In our case only four different food items were available. This could conceivably be a reason for our failure to produce greater body weight gains in our rats. On the other hand, it is questionable whether the quality of the diet, i.e. the type of food used, played a role. The items used in our experiment were high in calories and contained great amounts of carbohydrate and fat. High-fat diets have been shown to make animals more efficient in converting dietary energy into fat [36].

Regarding the duration of feeding, we have previously fed the same high fat diet as used in the present study, for 101 days followed by a variety of junk foods such as used in the present experiment for an additional 34 days, and were successful in producing higher body weights and carcass fat. Since the feeding regimen in the present study lasted 145 days, it is questionable that the duration of the diet presentation was a crucial factor.

Another possible factor could be the temporal variability of the diet. Rothwell and Stock [35] changed their dietary

items every day whereas we did so every third day. On the other hand, in our 1980 study in which we were able to produce dietary obesity we had changed the diet items less frequently as in the present experiment. Thus, whether the time of changing the diet would make a great difference is questionable.

Finally—and this comparison may not be entirely appropriate because of the age of the animals—we were unsuccessful in producing increased body weight and carcass fat in mature male rats fed the identical junk food items as used in the present study. Quite notably, mature female rats fed these items became heavier and fatter than their chow-fed counterparts [24].

Irrespecitve of whether or not the junk food-fed rats became heavier than their chow-fed counterparts in the present study, they did become obese in terms of body composition. In this regard one may compare the monosodium glutamate (MSG)-induced obesity in the presence of decreased body weight and food intake as neonates [32,34] and mature rats [29] and the normophagic-normoponderal hypothalamic obesity in weanling rats [4]. In view of these findings Kanarek *et al.* [29] have questioned ". . . the utility of body weight as a measure of obesity."

The present data, in conjunction with previous findings, suggest that changes in digestive function cannot be involved as possible causes for the growth retardation and lowered food intake in weanling DMNL rats. The data also strengthen our initial "resetting" hypothesis [6] and the recently proposed "organismic" hypothesis that address the remarkable fact that weanling DMNL rats exhibit normal responses to a variety of homeostatic challenges, normal GH, insulin, T_3 and somatomedin [3,8]. Apparently, intestinal parameters reflecting digestive function are proportionally "scaled down" to accommodate an animal of smaller size that functions competently and "normally" despite its reduced size and (absolute) food intake.

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