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Normal Catch-Up Growth in Rats Severely Food-Restricted Prior to Lesions of the Dorsomedial Hypothalamic Nucleus: The First 48 Hours

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BERNARDIS, L. L., L. L. BELLINGER, M. KODIS AND M. J. FELDMAN. *Normal catch-up growth in rats severely food-restricted prior to lesions of the dorsomedial hypothalamic nucleus: The first 48 hours.* PHARMACOL BIOCHEM BEHAV **32**(4) 957–960, 1989.—Following a brief period of ad lib (AL) feeding, 45-day-old male Sprague-Dawley rats were either fed AL or food-restricted (REST) for 21 days to 50% of the intake of the AL rats. At this time, some AL and some REST rats received electrolytic lesions in the dorsomedial hypothalamic nuclei (DMNL), whereas other AL and REST rats were sham-operated (CON). Following this, all rats were refed (REF) AL and killed two days later. At this time, DMNL-REST+REF and DMNL-AL weighed as much as CON-REST+REF and CON-AL, whereas the body weight of the DMNL-AL group began to separate from the CON-AL group; carcass lipid and protein were normal among the groups. DMNL-AL laid down more % lipid and % protein/g food eaten than CON-AL; this was not the case in the REST+REF groups. DMNL-AL were hypophagic vs. CON-AL, but DMNL-REST+REF ate as much as CON-REST+REF. Compared to DMNL-AL, DMNL-REST+REF increased their food intake more than four-fold and also utilized food energy more efficiently than DMNL-AL rats. Epididymal fat pads and kidneys were smaller in REST+REF vs. AL groups, irrespective of brain manipulation. Plasma glucose and growth hormone were normal among the groups, but plasma insulin concentrations were higher in REST+REF DMNL and CON groups vs. DMNL-AL and CON-AL, respectively. Glucose incorporation into epididymal fat pad lipid and CO_2 and liver lipid was elevated in REST-REF groups vs. respective AL groups. DMNL rats are capable of marshaling mechanisms for catch-up growth as early as two days after lesion production following severe body weight restriction. DMNL rats are fully capable of increasing their food intake immediately after lesion production. Evidently, their hypophagia under ad lib feeding conditions is not due to the loss of a feeding system, but rather may be a stratagem to decrease their body weight to a new set point.

Catch-up growth Food restriction Lesions, dorsomedial hypothalamic nucleus

FOLLOWING DMNL, rats fed ad lib become hypophagic and show reduced linear and ponderal growth (3). On the other hand, rats that had their body weight severely reduced prior to lesion production, became hyperphagic and gain body weight rapidly following the DMNL operation. This weight gain continues until the weight of the DMNL rats approaches the body weight of previously ad lib-fed DMNL rats (1). We have suggested that DMN lesions do not inhibit the capacity of the animals to eat, and hypothesized, instead, that the hypophagia was a stratagem by the animals to lower their body weights to a reduced new set point (1).

The present investigation was conducted to study early (48 hr) metabolic (plasma glucose, incorporation of glucose-U-C^{14} carbon into carbon dioxide, lipid and glycogen of epididymal fat pad and liver fractions) and endocrine (plasma insulin and growth hormone) changes, as well as some organ weights in rats with normal and reduced body weights prior to lesion production. This was done in an effort to learn whether DMNL rats restricted to below their body weight set point prior to lesion production were capable of marshaling hormonal and metabolic mechanisms like sham-operated controls treated similarly.

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TABLE 1

SOMATIC PARAMETERS

Group/Parameter Operation/ Treatment (n)	1 DMNL-AL (8)	2 CON-AL (8)	3 DMNL-REST +REF (7)	4 CON-REST +REF (6)
Body Weight at Operation and Refeeding (g)	284.1 ± 6.7†	274.5 ± 6.2	204.7 ± 3.4	198.5 ± 1.8
Body Weight at Kill (g)	252.8 ± 4.2	266.9 ± 7.2	217.0 ± 2.5	221.8 ± 3.0
Body Weight Change From Operat. to Kill (g)	−15.4 ± 1.7	−4.00 ± 3.5	6.14 ± 1.4	11.67 ± 1.1
Food Intake During 2 Days of Refeeding (g/day)	5.44 ± 0.8	16.8 ± 1.2	23.5 ± 0.9	25.0 ± 0.7
EFU During Restriction‡ (g/day/g/day)	0.16 ± 0.01	0.23 ± 0.02	−0.11 ± 0.02	−0.13 ± 0.02
EFU During Refeeding (g/day/g/day)	−3.92 ± 1.00	−0.20 ± 0.06	0.25 ± 0.06	0.46 ± 0.04
Lipogenic Efficiency§ (%/g/day)	1.50 ± 0.03	0.39 ± 0.03	0.26 ± 0.02	0.21 ± 0.01
Proteinogenic Effic. (%/g/day)	2.67 ± 0.6	0.66 ± 0.06	0.50 ± 0.04	0.49 ± 0.05
Kidney Weight [2, (g)]	3.1 ± 0.05	3.1 ± 0.10	2.58 ± 0.05	2.58 ± 0.06
Epididymal Fat Pad Weight [2, (g)]	1.9 ± 0.09	2.25 ± 0.21	1.14 ± 0.07	1.80 ± 0.09

*DMNL-AL: Rats with DMNL fed ad lib throughout experiment. CON-AL: Sham-operated controls fed ad lib throughout experiment. DMNL-REST+REF: DMNL rats food-restricted for 21 days prior to lesion production and then fed ad lib. CON-REST+REF: Sham-operated controls food-restricted for 21 days and then refed.

†Mean ± SEM.

‡Efficiency of food utilization, i.e., weight gained/experimental period/mean food intake during same period.

§Percent carcass fat laid down divided by mean food intake during refeeding.

#Percent carcass protein laid down divided by mean food intake during refeeding.

METHOD

Forty-five male Sprague-Dawley rats were accommodated in individual cages in a light cycle- (L:D 12:12, lights on at 0600 hr) and temperature (23°C)-controlled room and given lab chow and tap water ad lib for 16 days. At the end of this period all rats were weighed (223 ± 2 g) and divided into two groups of matched body weights.

Twenty-two rats continued to feed ad lib, whereas 23 rats received one-half of the mean food intake of the ad lib-fed group. In order to minimize a "meal eating" effect (12), one-half of the daily ration was fed between 0730 and 0830 hr, the other half between 1545 and 1630 hr.

After 21 days, all rats were again weighed and anesthetized with sodium hexobarbital (14 mg/100 g body weight). Rats for Group 1 (DMNL-AL, n = 13) were taken from the ad lib-fed group and received bilateral lesions in the DMN as described earlier (4). The remaining ad lib-fed rats (Group 2, CON-AL, n = 8), received sham lesions (4). Group 3 (DMNL-REST+REF, n = 15) consisted of rats that had been food-restricted and then received DMNL. Group 4 (CON-REST+REF, n = 6) comprised the remaining restricted rats and became sham-operated controls.

During the next two days, the two groups that had been previously restricted (Groups 3 and 4) were given free access to food, as Groups 1 and 2 had been from the beginning. At the end of this refeeding period all rats were killed by decapitation. Trunk blood was collected, centrifuged and plasma stored at −20°C until assayed. Brains were treated as previously described (4) for the histological assessment of the lesions. Kidneys, livers, testes, adrenals and epididymal fat pads were excised, trimmed and weighed. Carcasses were eviscerated, shaved and frozen for the subsequent determination of total lipid (13) and protein (16).

Plasma glucose was determined according to the method of Saifer and Gerstenfeld (17), plasma insulin was assayed using a radioimmunoassay kit (Cambridge Diagnostics, Ballerica, MA), and plasma growth hormone (GH) was determined using materials [rGH-I-5, anti-rGH serum-5, rGHRP-2 (approximately 1.5 IU/mg)], generously supplied by NIADDK and NHPP.

The distal portion of the dissected epididymal fat pad (200 mg) was incubated for glucose oxidation (14), as were liver slices (500 mg). After incubation, tissues were removed and assayed for protein (16), glycogen (14) and lipid (13).

The data were analyzed by one-way analysis of variance and Tukey's test where appropriate. The GH data were analyzed using the Mann-Whitney test for nonparametric data.

RESULTS

Somatic Parameters (Table 1)

As expected, at the time of surgery, both restricted-refed groups had significantly, $F(1,25) = 180.40$, $p < 0.001$, reduced body weights vs. the two ad lib-fed groups. The same pattern persisted, $F(1,25) = 57.32$, $p < 0.001$, two days after lesion production and refeeding for the restricted groups, whereas the body weights of the ad lib-fed groups were beginning to separate.

TABLE 2

ENDOCRINE AND METABOLIC PARAMETERS

Group/Parameter Operation/ Treatment (n)	1* DMNL-AL (8)	2 CON-AL (8)	3 DMNL-REST +REF (7)	4 CON-REST +REF (6)
Plasma				
Insulin (μU/ml)	25.13† ±6.13	28.93 ±5.234	94.44 ±5.77	57.42 ±11.30
Growth Hormone (ng/ml)	41.43 ±21.51	94.19 ±45.33	30.91 ±25.65	23.77 ±13.50
Glucose (mg/dl)	147.4 ±8.26	146.8 ±8.98	144.9 ±8.06	135.8 ±9.28
Epididymal				
Glucose Incorporation Into Total Lipid (DPM)	33964 ±5001	62260 ±20413	102332 ±5858	117345 ±15936
Into Carbon Dioxide (DPM)	71278 ±15718	190092 ±59540	266749 ±27399	255909 ±41772
Liver				
Glucose Incorporation	4276 ±422	6546 ±1246	10123 ±2503	9623 ±1622

*For group identification see Table 1.
†Mean ± SEM.

In terms of body weight *changes* (g/day), during the two days of refeeding the previously restricted groups showed dramatic weight gains, whereas the ad lib-fed groups exhibited a decrease in body weight, $F(1,25) = 50.02$, $p<0.001$, with the DMNL-AL rats (Group 1) showing the greatest weight loss, $F(1,25) = 9.26$, $p<0.01$. There was no statistically significant difference among the groups in carcass lipid and protein (data not shown).

During the postoperative 2-day refeeding period, the previously restricted groups dramatically increased, $F(1,25) = 163.87$, $p<0.001$, their food intake, with both groups consuming similar amounts. The ad lib-fed DMNL rats showed the characteristic ($p<0.01$) hypophagia when compared to the ad lib-fed controls.

As expected, prior to lesion production, both restricted groups utilized food energy poorer than ad lib-fed rats, $F(1,25) = 35.00$, $p<0.001$. Upon refeeding, however, both previously restricted groups had higher EFUs, $F(1,25) = 15.9$, $p<0.01$, than the ad lib-fed groups. Notably, whereas the previously restricted DMNL rats had the same EFUs as their respective controls, the ad lib-fed DMNL rats utilized food poorer, $F(1,25) = 10.56$, $p<0.001$, than the ad lib-fed controls. On the other hand, ad lib-fed DMNL rats layed down more % protein, $F(1,25) = 10.90$, $p<0.01$, and more % fat, $F(1,25) = 14.32$, $p<0.001$, per gram food eaten than ad lib-fed controls.

In terms of absolute weights, adrenals, livers and testes are comparable among the four groups. However, kidneys and epididymal fat pads were lighter ($p<0.01$) in previously restricted rats, irrespective of brain manipulation.

Both liver and epididymal fat pads had normal composition (data not shown).

Endocrine and Metabolic Parameters (Table 2)

Neither plasma GH nor glucose were different among the groups, but insulin was significantly, $F(1,25) = 11.97$, $p<0.01$, higher in previously restricted rats compared to ad lib-fed rats.

Incorporation of glucose-U-C^{14} carbon into epididymal fat pad lipid was significantly, $F(1,25) = 13.69$, $p<0.002$, enhanced in the restricted-refed groups. The same was true, $F(1,25) = 7.98$, $p<0.01$, for incorporation into carbon dioxide (oxidation). There was no lesion effect.

Glucose incorporation into liver lipid was significantly, $F(1,25) = 6.70$, $p<0.02$, increased in the restricted-refed groups. Again, there was no lesion effect.

All other measured indices for both fat pads and liver were similar among the groups.

DISCUSSION

The present data clearly show that our earlier (9) reservation "*. . . that a more severe restriction of body weight would have uncovered some deficits in DMNL rats . . .*," was not justified: not only do DMNL rats show the same capacity for catch-up growth as sham-operated controls when food-restricted to 50% of ad lib-fed rats, but they are capable of doing this within the first 48 hours after lesion production and refeeding. The fact that such severely restricted DMNL rats become grossly hyperphagic and have a high efficiency of food utilization [see also (3)], suggests that DMN lesions do not destroy a "feeding center" or circuitry (1). If they did, severely food-restricted DMNL rats should on refeeding eat as little food as ad lib-fed DMNL rats. We proposed some time ago (1) that these previously restricted and DMN-lesioned animals are hyperphagic because they are below their lesion-induced body weight set point.

The fact that there are no lesion effects in any of the metabolic data is in excellent agreement with previous findings on the intermediary metabolism of the ad lib-fed DMNL rats (7). Regarding short-term effects, we have recently investigated the effects of DMN lesions on the incorporation of glucose-U-C^{14} carbon four hours and three and seven days after lesion production. Notably, and in excellent agreement with the findings on the ad lib-fed DMNL rats of the present study (2 days), incorporation into epididymal fat pad total lipid and glycogen as well as liver lipid, carbon dioxide (oxidation) and saponifiable fatty acids was normal in DMNL rats three days after the hypothalamic operation compared to sham-operated controls (6).

In the present study it is particularly noteworthy that the only significant effects are evident between groups on different dietary regimens, i.e., restricted vs. ad lib-fed groups. Whether the enhanced incorporation of glucose into lipid of both fat pads and liver and oxidation in epididymal fat pads of restricted-refed groups is due to a "meal eating" effect caused by the preoperative restricted feeding (12, 15, 18), or is attributable to the flooding of the organism by excess substrate upon refeeding following the 21-day restriction, is not evident from our data. Nevertheless, it is clear that whatever the underlying reasons for this enhanced incorporation of glucose carbon, DMNL rats show the same changes as sham-operated controls. The fact that all metabolic parameters were normal in the DMNL rats of the present study gives strong support to previous data (2, 10, 11) that anabolic hormones are normal in the DMNL rat. The data further indicate that a hormonal imbalance does not contribute to the lower body weight of the DMNL rat.

To summarize, the destruction of the DMN in mature male rats after severe food restriction and thus body weight reduction does not interfere with the catch-up growth that after such manipulation occurs in neurologically intact rats. The fact that DMNL rats regulate about a lower body weight set point with appropriately scaled-down food intake, but normal food efficiency, body com-

position and several metabolic and endocrine indices, strengthens our concept that DMN lesions have released an "organismic" set point (5, 7, 8). This type of set point is juxtaposed to a "compartment-specific" set point that comes to the fore after lesions in the ventromedial and lateral hypothalamic areas.

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