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Recovery of Rats With Dorsomedial Hypothalamic Nucleus Lesions (DMNL Rats) From Body Weight Restriction: Effect of Duration of Postoperative Prerestriction Period[1]

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BERNARDIS, L. L., G. McEWEN, M. KODIS AND M. J. FELDMAN. *Recovery of rats with dorsomedial hypothalamic nucleus lesions (DMNL rats) from body weight restriction: Effect of duration of postoperative prerestriction period.* PHARMACOL BIOCHEM BEHAV **30**(3) 649–656, 1988.—The present study was performed to see whether somatic and underlying metabolic-adaptive responses of DMNL rats and sham-operated controls (CON) to body weight restriction and subsequent refeeding could be influenced by the duration of ad lib feeding between lesion production and start of restriction. In contrast to previous studies (42 and 55 days, respectively) this time was reduced to 25 days. Restriction was similar, i.e., 27 days. DMNL rats show the same adaptive capacity in most parameters as do restricted CON. However, this response was at a lower level of absolute body weight, appropriate, so it appears, for their DMNL-induced lower body weight. In some parameters different responses were noted, however, suggesting that the time of ad lib feeding following the DMNL does indeed affect adaptive responses. Notably, this is the case in both DMNL and CON commensurately. Linear growth was reduced by restriction in the present but not in the two previous studies. Food intake showed a pronounced "overshoot" on refeeding but did not previously. Efficiency of food utilization was normal in the present study but depressed previously. A rise in plasma free fatty acids was not evident but was so in previous experiments. We concluded that, although DMNL rats respond to food restriction and recovery like similarly-treated CON, the duration of the ad lib feeding before restriction and/or the absolute age of the animals at that time, do indeed affect some parameters. This may be related to the fact that different aspects of the DMNL syndrome declare themselves in a sequential rather than a simultaneous manner.

Dorsomedial hypothalamic nucleus lesions Food restriction Body weight restriction Realimentation Set point recovery Food intake Efficiency of food utilization Plasma insulin Plasma substrates Glucose incorporation Adipose tissue Liver "Organismic" set point

DMNL rats that had been fed ad lib for 42 and 55 days, respectively, and had been subsequently food-restricted for 28 and 24 days, respectively, recovered body weight and numerous intermediary metabolic parameters with the same competence as sham-operated-restricted controls [7,8]. Notably, this normal recovery occurred at a body weight that was absolutely lower than that of the restricted controls. We have attributed this apparent synchronization of recovery processes to the lower, DMNL-induced body weight to the change by DMNL of an "organismic" set point.

One of the factors to influence recovery after body weight restriction could be the time between DMN lesion production and start of restriction. Such a time might indeed be critical in the establishment or "settling" of such a set point. Therefore, in the present study, the postoperative-prerestriction-ad lib feeding period was reduced to half of the two previous studies [7,8], i.e., 25 days. The duration of the restriction remained the same, i.e., 27 days. In addition to numerous behavioral and endocrine-metabolic parameters we measured organ growth in both absolute terms and relative to body mass [5,14].

In general, the present data are in agreement with previous findings [7,8] and therefore strengthen our contention that DMNL make manifest an "organismic" set point. Some

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parameters, however, appear to be influenced by the duration of the ad lib feeding period that precedes the restriction regimen or by the age of the rats at that time. For instance, linear growth was reduced by food restriction, but not in the previous studies, and food intake showed a profound "overshoot" on refeeding in the present but not in previous studies. Also, the efficiency of food utilization was normal and the rise of plasma free fatty acids was not present in contrast to previous studies.

METHOD

Male Sprague-Dawley rats (Sprague-Dawley, Indianapolis, IN) were received at the age of 22 days and accommodated in a light cycle (L:D 12:12, lights on at 0600 hr) and temperature-controlled (23°C) room and given Prolab RMH 1000 lab chow and tap water ad lib. At the age of 29 days they were anesthetized with sodium hexobarbital (14 mg/100 g) and received electrolytic lesions destroying the dorsomedial hypothalamic nuclei (DMN). The detailed methodology of lesion production has been previously described [2]. The animals were returned to their cages and given lab chow and tap water as above. Food intake was measured Mondays, Wednesdays and Fridays and the data presented are the means for the various experimental periods (Period 1: postoperative-prerestriction ad lib feeding (25 days); Period 2: restriction to 80% of ad lib-fed rats (27 days); Period 3: refeeding for seven days; Period 4: refeeding for 28 days, i.e., total refeeding time: 35 days).

Following Period 1, all rats were anesthetized with Fluothane (brand of halothane) and body weight and nose-tail length were recorded. Subsequently, the animals were divided into four groups: Group 1 (DMNL-AL) consisted of DMNL rats that continued to feed ad lib until the termination of the experiment 87 days postoperatively; Group 2 (DMNL-REST) were DMNL rats that received 80% of the food intake of Group 1; Group 3 (CON-AL) comprised sham-operated controls that were fed ad lib; and Group 4 (CON-REST) were controls that were given 80% of the food intake eaten by the animals of Group 3. The rationale for arriving at this figure was that DMNL rats generally eat less than 80% of what sham-operated controls consume. In order to avoid a "meal eating" effect, i.e., enhanced lipogenesis, one-half of the restricted daily ration was fed in the morning (0800–0830 hr), the other half was presented in the afternoon (1645–1730 hr).

We are aware that this feeding schedule might nevertheless have disturbed or shifted circadian rhythms and this might conceivably be a potentially confounding factor in the interpretation of our data.

Following this period, all animals were again anesthetized with Fluothane and their body weight and length determined as above, returned so their cages and on the following day several representative animals from each of the four groups were removed from their cages and rapidly decapitated ("Kill 1"). The remaining, previously-restricted rats were refed ad lib with lab chow and six days later several representative rats from each group were again weighed and nose-tail length measured as above and killed the following day ("Kill 2"). Finally, after 34 days of realimentation, the remaining animals were weighed and measured as above and terminated on day 35 ("Kill 3").

Plasma was obtained from trunk blood. Epididymal fat pad and liver aliquots were obtained after weighing of the respective organs. Metabolic analyses and body composition were performed as previously described [7,8], as was histological preparation of the brains.

Based on the histological analysis, the final population was as follows: "Kill 1": DMNL ad lib (5), DMNL restricted (3), Controls ad lib (5), Controls Restricted (5); "Kill 2": DMNL ad lib (5), DMNL Refed (6), Controls ad lib (3), Controls Refed (5); "Kill 3": DMNL ad lib (6), DMNL refed (6), Controls ad lib (3), Controls refed (2).

Two-way analysis of variance was used to analyze the effects of lesion (DMNL vs. sham-operated controls) and diet (restricted and refed rats, respectively vs. ad lib-fed rats). After analysis for main effects, interaction effects analysis lesion × diet were performed and in addition post hoc comparisons were done using Student's *t*-test and Tukey's test to determine group significance.

RESULTS

PERIOD 1 (Postoperative Ad Lib Feeding for 25 Days)

In order to ascertain that the rats subjected to subsequent restriction and refeeding and sequential terminations ("Kills") were derived from the same uniform population, several somatic parameters that characteristically change during the development of the DMN syndrome were measured during and at the end of this period.

DMNL rats weighed less [Lesion effect: $F(1,45)=78.12$, $p<0.0001$], were shorter [Lesion effect: $F(1,45)=66.76$, $p<0.0001$] and ate less [Lesion effect: $F(1,45)=67.96$, $p<0.0001$] than sham-operated controls but utilized food energy for body weight gain (EFU) with the same efficiency (the data for Period 1 are not shown).

Evidently, the operated rats of the present study are proper representatives of the DMNL syndrome and, moreover, the animals terminated at the end of the subsequent experimental periods ("Kills") are derived from a homogeneous population.

PERIOD 2 (Food Restriction to 80% of Ad Lib-Fed Rats for 27 Days)

Somatic parameters. Irrespective of dietary manipulation, DMNL rats (DMNL ad lib: 217±24 g, DMNL restricted: 173±11 g) were highly significantly lighter [Lesion effect: $F(1,14)=19.38$, $p<0.0006$] than sham-operated controls (Controls ad lib: 290±11 g, Controls restricted: 245±8 g). Similarly, DMNL rats were highly significantly shorter (DMNL ad lib: 382±6 mm, DMNL restricted: 339±4 mm) than sham-operated controls (Controls ad lib: 405±8 mm, Controls restricted: 390±3 mm, lesion effect: $F(1,14)=30.24$, $p<0.0001$).

Both restricted DMNL and sham-operated rats showed lower body weights [Diet effect: $F(1,14)=7.26$, $p<0.0174$] and shorter nose-tail lengths [Diet effect: $F(1,14)=18.15$, $p<0.0008$] than their ad lib-fed counterparts. Carcass lipid and protein were unaffected by either lesion or dietary manipulation. Notably, none of the parameters showed a significant diet × lesion interaction effect.

Rats with DMNL were grossly hypophagic [DMNL ad lib: 19.2±0.7 g/day, DMNL restricted: 14.0±0.1, Controls ad lib: 24.0±0.8, Controls restricted: 19.0±0.1 g/day, Lesion effect: $F(1,14)=53.60$, $p<0.00001$]. [Restricted animals obviously had also highly significantly reduced food intake compared to ad lib-fed rats (Diet effect: $F(1,14)=58.55$, $p<0.00001$).]

When food intake was calculated per metabolic mass

(g/kg$^{3/4}$, [5,14] DMNL rats were normophagic (DMNL ad lib: 56.6±1.7, DMNL restricted: 52.7±1.9 g/day/kg$^{3/4}$) compared to sham-operated controls (Controls ad lib: 60.8±1.0, Controls restricted: 54.7±1.0 g/day/kg$^{3/4}$). However, restricted vs. ad lib-fed rats showed significant hypophagia [Diet effect: $F(1,14)=10.22$, $p<0.0065$]. Notably, there was no significant lesion × diet interaction effect.

DMNL rats had smaller adrenals (in mg) but showed normal adrenal growth in mg/kg$^{3/4}$. Dietary restriction caused reduced adrenal growth in both DMNL and sham-operated controls when expressed in mg [Diet effect: $F(1,14)=7.93$, $p<0.0146$, DMNL ad lib: 28.9±1.7, DMNL restricted: 26.2±1.8 mg, Controls ad lib: 34.5±0.9, Controls restricted: 30.5±0.6 mg]. Growth expressed per metabolic mass was normal, however. The same pattern was evident for kidney growth [Lesion effect: $F(1,14)=18.16$, $p<0.0008$, Diet effect: $F(1,14)=14.86$, $p<0.0018$, DMNL ad lib: 2.2±0.1, DMNL restricted: 1.6±0.1, Controls ad lib: 2.6±0.1, Controls restricted: 2.2±0.1 g].

Liver growth was highly significantly reduced in DMNL rats [Lesion effect: $F(1,14)=39.51$, $p<0.00001$] vs. sham-operated controls (in mg) and in restricted vs. ad lib-fed rats [Diet effect: $F(1,14)=55.95$, $p<0.00001$, DMNL ad lib: 7.3±0.2, DMNL restricted: 4.8±0.2, Controls ad lib: 10.3±0.6, Controls restricted: 6.8±0.3 mg]. The same obtained for liver growth per metabolic mass [Lesion effect: $F(1,14)=17.14$, $p<0.0010$, Diet effect: $F(1,14)=46.48$, $p<0.00001$, DMNL ad lib: 21.5±0.9, DMNL restricted: 17.8±0.2, Controls ad lib: 26.0±0.7, Controls restricted: 19.5±0.6 mg/kg$^{3/4}$].

Whereas testes growth was unaffected by brain manipulation and restriction alike in absolute terms, growth was enhanced per metabolic size in DMNL rats vs. controls [Lesion effect: $F(1,14)=14.78$, $p<0.0018$] and restricted rats had larger organs than ad lib-fed rats [Diet effect: $F(1,14)=7.00$, $p<0.0192$, DMNL ad lib: 8.1±0.4, DMNL restricted: 9.2±0.5, Controls ad lib: 7.0±0.2, Controls restricted: 7.7±0.3 g/kg$^{3/4}$].

Finally, DMNL rats had lighter epididymal fat pads than sham-operated controls [Lesion effect: $F(1,14)=6.09$, $p<0.0271$] and restricted rats had smaller fat pads than ad lib-fed rats both in terms of grams [Diet effect: $F(1,14)=14.72$, $p<0.0018$] and in g/kg$^{3/4}$ [Diet effect: $F(1,14)=8.38$, $p<0.0118$, DMNL ad lib: 2.0±0.2 g, DMNL restricted: 1.0±0.1 g, Controls ad lib: 2.8±0.3 g, Controls restricted: 1.9±0.2 g; in g/kg$^{3/4}$: DMNL ad lib: 5.8±0.6, DMNL restricted: 3.9±0.5, Controls ad lib: 7.1±0.6, Controls restricted: 5.5±0.4 g/kg$^{3/4}$]. Neither liver nor epididymal fat pad lipid and protein showed lesion or diet effects, and in none of the above organs was there a significant diet × lesion interaction effect.

Metabolic parameters. As one might expect, restricted rats (DMNL restricted: 12.8±1.9, Controls restricted: 13.4±1.1, DMNL ad lib: 19.3±1.9, Controls ad lib: 18.1±2.9 uU/ml) showed significant hypoinsulinemia [Diet effect: $F(1,14)=6.69$, $p<0.0215$]. Restricted animals also were hypoglycemic (DMNL restricted: 114.9±3.2, Controls restricted: 112.4±5.6, DMNL ad lib: 130.6±2.2, Controls ad lib: 131.1±5.9 mg/dl, Diet effect, $F(1,14)=17.30$, $p<0.001$] and hypotriglyceridemic [DMNL restricted: 65.5±2.2, Controls restricted: 70.8±8.2, DMNL ad lib: 74.4±8.7, Controls ad lib: 96.9±6.3 mg/dl, Diet effect: $F(1,14)=4.98$, $p<0.0425$]. Both ad lib-fed and restricted DMN groups were hypoglycerolemic (DMNL ad lib: 3.74±0.21, Controls ad lib: 4.50±0.34, DMNL restricted: 3.33±0.26, Controls restricted: 4.08±0.23 mg/dl, Lesion effect: $F(1,14)=7.42$, $p<0.0164$]. Notably, there were no significant lesion × diet interaction effects and no significant differences among the groups in cholesterol and total plasma protein concentrations.

In epididymal fat pads, oxidation (incorporation into CO_2) was greater in DMNL than in sham-operated controls [DMNL ad lib: 109712±25769, DMNL restricted: 296252±17519, Controls ad lib: 70029±4764, Controls restricted: 159018±32044 DPM, Lesion effect: $F(1,14)=12.96$, $p<0.0029$]. Similarly, restricted rats showed a higher oxidation than ad lib-fed rats [Diet effect: $F(1,14)=31.44$, $p<0.0001$]. These changes were not evident when oxidation was calculated in DPM/protein, however. There were no significant changes in other tissue fractions and, furthermore, there was no significant lesion × diet interaction in any of the parameters.

In the liver, oxidation (in DPM) was significantly enhanced in restricted over ad lib-fed animals [Diet effect: $F(1,14)=6.15$, $p<0.0265$, DMNL ad lib: 34191±4337, DMNL restricted: 45527±5356, Controls ad lib: 36189±4480, Controls restricted: 46623±3188]. Restriction also resulted in significant reduction (in DPM/protein) of glucose incorporation into total lipid [Diet effect: $F(1,14)=6.34$, $p<0.0246$, DMNL ad lib: 102±19, DMNL restricted: 42±33, Controls ad lib: 86±10, Controls restricted: 62±8] and reduced incorporation into glycogen (in DPM): Diet effect: $F(1,14)=8.08$, $p<0.0131$, DMNL ad lib: 34612±9196, DMNL restricted: 847±479, Controls ad lib: 33130±4722, Controls restricted: 27009±6349 (interaction effect: diet, lesion was not signifi cant]. Calculated for DPM/protein: Diet effect: $F(1,14)=31.03$, $p<0.0001$, DMNL ad lib: 1082±370, DMNL restricted: 30325±8197, Controls ad lib: 847±154, Controls restricted: 575±125. Incorporation was greater in refed rats but only among the DMNL rats [Interaction Diet × Lesion: $F(1,1)=32.21$, $p<0.00001$].

DMNL rats showed higher counts than sham-operated controls for glucose incorporation into total lipid (in DPM) [Lesion effect: $F(1,14)=9.55$, $p<0.0080$, DMNL ad lib: 3586±171, DMNL restricted: 5103±100, Controls ad lib: 3395±224, Controls restricted: 2779±341]. Similarly, DMNL rats incorporated more glucose into glucogen (in DPM/protein) than sham-operated controls [Lesion effect: $F(1,14)=33.24$, $p<0.00001$, DMNL ad lib: 1082±370, DMNL restricted: 30325±8197, Controls ad lib: 847±154, Controls restricted: 575±125]. In both instances there was also a lesion × diet interaction effect, i.e., DMNL rats incorporated more than controls into liver lipid among the restricted groups (Interaction diet × lesion: $F(1,1)=6.88$, $p<0.0201$]. In the case of incorporation into glycogen in DPM/protein, restricted rats incorporated more than ad lib-fed rats only in the case of DMNL rats [Interaction lesion × diet: $F(1,1)=32.21$, $p<0.00001$].

PERIOD 3 (Refeeding for Seven Days)

Somatic parameters. Following refeeding, body weights of DMNL rats (DMNL ad lib: 231±4 g; DMNL refed: 213±6 g) continued to be highly significantly lower [Lesion effect: $F(1,15)=64.92$, $p<0.00001$] than sham-operated controls (Controls ad lib: 325±7 g, Controls refed: 281±9 g). Notably, refed animals were still lighter than ad lib-fed rats [Diet effect: $F(1,15)=9.67$, $p<0.0072$]. A similar pattern was true for linear growth (DMNL ad lib: 378±5 mm, DMNL refed: 363±3 mm, Controls ad lib: 425±7 mm, Controls refed: 397±4 mm, Lesion effect: $F(1,15)=67.54$, $p<0.00001$, Diet

effect: $F(1,15)=19.65$, $p<0.0001$]. Carcass composition (percent lipid and protein) was comparable among the groups.

Although DMNL rats were still hypophagic (DMNL ad lib: 17.4±1.5, DMNL refed: 18.7±1.7 g/day) compared to controls [Controls ad lib: 23.6±1.7, Controls refed: 23.2±1.9 g/day, Lesion effect: $F(1,15)=7.62$, $p<0.0146$], food intake of previously restricted rats had normalized to the levels of their respective ad lib-fed counterparts. This was true for both absolute (g/day) and relative ($g/kg^{3/4}$) food intake. The refed animals showed a significantly greater efficiency of food utilization than the ad lib-fed groups [DMNL ad lib: 0.048±0.022, DMNL refed: 0.186±0.026, Controls ad lib: 0.133±0.034, Controls refed: 0.215±0.038 g/day, Diet effect: $F(1,15)=10.95$, $p<0.0048$]. Notably, there was no significant interaction lesion × diet in any of the above parameters.

Adrenals in DMNL rats were lighter than in sham-operated controls [Lesion effect: $F(1,15)=12.01$, $p<0.0035$] but this was true only for the absolute weight (DMNL ad lib: 32.2±1.2, DMNL restricted: 33.1±1.6, Controls ad lib: 40.9±2.5, Controls restricted: 37.0±1.8 mg). Kidney [Lesion effect: $F(1,15)=7.51$, $p<0.0146$, DMNL ad lib: 2.1±0.2, DMNL restricted: 1.9±0.1, Controls ad lib: 2.5±0.9, Controls restricted: 2.3±0.2 mg] and epididymal fat pad growth [Lesion effect: $F(1,15)=7.15$, $p<0.0174$, DMNL ad lib: 1.9±0.2, DMNL restricted: 1.6±0.2, Controls ad lib: 2.6±0.3, Controls restricted: 2.1±0.3 g] showed a similar pattern for both absolute and relative weight.

Rats with DMNL also had smaller livers, both in absolute terms [Lesion effect: $F(1,15)=43.67$, $p<0.00001$, DMNL ad lib: 7.1±0.5, DMNL restricted: 6.8±0.3, Controls ad lib: 11.0±0.3, Controls restricted: 8.9±0.5 g] and when calculated per metabolic mass [Lesion effect: $F(1,15)=22.11$, $p<0.0003$, DMNL ad lib: 21.5±0.7, DMNL restricted: 21.4±0.5, Controls ad lib: 25.8±0.9, Controls restricted: 23.2±0.6].

Both absolute and relative growth of the testes showed significant differences between DMNL rats and sham-operated controls. In absolute terms, DMNL rats had smaller organs than sham-operated controls [Lesion effect: $F(1,15)=13.07$, $p<0.0025$, DMNL ad lib: 2.8±0.01, DMNL restricted: 2.9±0.03, Controls ad lib: 3.2±0.2, Controls restricted: 3.0±0.06], whereas per metabolic mass, DMNL rats had larger testes than controls [Lesion effect: $F(1,15)=10.94$, $p<0.0048$, DMNL ad lib: 8.5±0.4, DMNL restricted: 9.3±0.3, Controls ad lib: 7.5±0.4, Controls restricted: 7.9±0.3]. Absolute testes growth also showed an interaction effect, i.e., reduced growth in DMNL rats was seen only among the ad lib-fed animals [Interaction effect lesion × diet: $F(1,11)=7.57$, $p<0.0149$]. Notably, there were no significant diet, lesion, and lesion × diet interaction effects for fat pad weight in $kg^{3/4}$, liver and epididymal fat pad lipid and protein.

Metabolic parameters. Refeeding for seven days normalized both restriction-induced hypoinsulinemia and hypoglycemia. However, DMNL rats still showed a significant hypoglycerolemia [DMNL ad lib: 3.20±0.5 mg/dl, DMNL refed: 2.65±0.20, Controls ad lib: 4.20±0.21, Controls refed: 3.76±0.45 mg/dl, Lesion effect: $F(1,15)=7.81$, $p<0.0136$]. Furthermore, refed animals, irrespective of brain manipulation, showed significant hypoproteinemia [DMNL ad lib: 7.96±0.30, DMNL refed: 7.08±0.34, Controls ad lib: 7.80±0.15, Controls refed: 6.54±0.3 g/dl, Diet effect: $F(1,15)=10.41$, $p<0.0057$]. As in most previous instances, there were no significant interaction effects diet × lesion.

Following seven days of refeeding, oxidation (in DPM) was greatly enhanced in epididymal fat pads in both refed groups [Diet effect: $F(1,15)=11.02$, $p<0.0047$, DMNL ad lib: 38051±7091, DMNL refed: 94359±5501, Controls ad lib: 39397±7537, Controls refed: 81875±24952]. This effect was not evident when oxidation was expressed in DPM/protein.

Irrespective of whether incorporation of glucose into total lipid was expressed in DPM or DPM/protein, refed groups showed higher counts [DPM: Diet effect: $F(1,15)=4.80$, $p<0.0446$,, DPM/Protein: Diet effect: $F(1,15)=5.81$, $p<0.0293$, DMNL ad lib: 1145±143, DMNL refed: 2428±438, Controls ad lib: 898±343, Controls refed: 1532±428]. The same pattern was found for glucose incorporation into saponifiable lipids [DPM: Diet effect: $F(1,15)=5.82$, $p<0.0291$, DPM/Protein: Diet effect: $F(1,15)=9.85$, $p<0.0068$, DPM: DMNL ad lib: 166±70, DMNL refed: 1369±475, Controls ad lib: 116±37, Controls refed: 801±408, DPM/Protein: DMNL ad lib: 102±36, DMNL refed: 814±185, Controls ad lib: 136±56, Controls refed: 341±132].

Incorporation of glucose into fat pad glycogen was enhanced in refed rats only when expressed in DPM [Diet effect: $F(1,15)=5.53$, $p<0.0328$, DMNL ad lib: 159±32, DMNL refed: 221±45, Controls ad lib: 106±24, Controls refed: 241±39]. Notably there were no lesion and no lesion × diet interaction effect in any of the epididymal fat pad parameters.

In liver tissue, oxidation was enhanced in refed animals, irrespective of brain manipulation, but barely significant and only when expressed in DPM/protein [DMNL ad lib: 640±154, DMNL refed: 510±26, Controls ad lib: 442±121, Controls refed: 490±45 DPM/Protein, Diet effect: $F(1,15)=4.62$, $p<0.0495$]. Incorporation into glycogen (in DPM) showed an interaction effect, $F(1,1)=5.05$, $p<0.0412$, i.e., the incorporation was greater in refed than in ad lib-fed DMNL rats (DMNL refed: 51485±7055, DMNL ad lib: 25377±6070 DPM). No such effect was seen in refed vs. ad lib-fed sham-operated controls. Most notably, neither of the liver fractions showed a lesion effect.

PERIOD 4 (Refeeding for 35 Days, Termination of Experiment)

Somatic parameters. As expected, DMNL rats continued to be highly significantly lighter (DMNL ad lib: 276±14, DMNL refed: 271±7 g) than sham-operated controls [Controls ad lib: 335±11, Controls refed: 322±22 g, Lesion effect: $F(1,15)=16.69$, $p<0.0010$] and exhibited reduced linear growth [DMNL ad lib: 396±9, DMNL refed: 391±7, Controls ad lib: 434±5, Controls refed: 435±2 mm, Lesion effect: $F(1,15)=16.37$, $p<0.0012$]. Percent carcass lipid and protein were comparable among the groups.

Food intake in absolute terms showed a similar pattern as ponderal and linear growth, i.e., the DMNL rats were grossly hypophagic (DMNL ad lib: 18.1±0.8, DMNL refed: 18.3±0.7) compared to sham-operated controls [Controls ad lib: 23.7±1.1, Controls refed: 22.9±1.2 g/day, Lesion effect: $F(1,15)=20.40$, $p<0.0004$]. Food intake relative to metabolic mass was normal among the groups, as was the efficiency of food utilization. Notable is the lack of significant diet and lesion × diet interaction effects in all of the above parameters.

Even after 35 days of refeeding, DMNL rats had in absolute terms still lower adrenal weights than sham-operated controls [Lesion effect: $F(1,15)=14.33$, $p<0.0019$, DMNL ad

lib: 40.9±1.0, DMNL refed: 37.4±1.5, Controls ad lib: 44.7±1.6, Controls refed: 45.7±0.5 mg]. Similarly, absolute liver growth was still less [Lesion effect: $F(1,15)=13.62$, $p<0.0021$, DMNL ad lib: 8.1±0.5, DMNL refed: 0.8±0.4, Controls ad lib: 10.3±0.3, Controls refed: 9.7±1.5 g] as was kidney growth [Lesion effect: $F(1,15)=14.33$, $p<0.0018$, DMNL ad lib: 2.34±0.13, DMNL refed: 1.94±0.23, Controls ad lib: 2.88±0.16, Controls refed: 2.73±0.17 g]. Notably, organ growth was *normal* in all of the above instances when calculated per metabolic mass. The only diet effect was noted in liver protein, which was significantly increased in refed rats [Diet effect: $F(1,15)=6.84$, $p<0.02$, DMNL ad lib: 12.0±0.4, DMNL refed: 13.0±0.5, Controls ad lib: 11.5±0.4, Controls refed: 13.3±0.5%]. In none of the other parameters was there either a diet or lesion × diet interaction effect.

Metabolic parameters. After 35 days of refeeding, refed animals (DMNL refed: 27.6±1.8, Controls refed: 37.5±1.5) showed significant higher plasma insulin levels than ad lib-fed groups [DMNL ad lib: 24.8±2.4, Controls ad lib: 25.1±2.3 uU/ml, Diet effect: $F(1,115)=8.79$, $p<0.0097$]. This was, however, unaccompanied by significant substrate changes.

In epididymal fat pad fractions, the only significant lesion effect, $F(1,15)=9.41$, $p<0.0078$] was seen in the incorporation of glucose into total lipid (DMNL ad lib: 2960±539, DMNL refed: 4693±407, Controls ad lib: 1781±347, Controls refed: 2553±673 DPM). Refed rats incorporated glucose at a higher rate than ad lib-fed rats into total lipid [DPM: Diet effect $F(1,15)=5.36$, $p<0.0325$], DPM/protein [Diet effect: $F(1,15)=5.03$, $p<0.0404$, DMNL ad lib: 1747±285, DMNL refed: 2572±365, Controls ad lib: 1425±171, Controls refed: 2181±301 DPM/protein]. The same pattern was evident for the incorporation of glucose into saponifiable lipid [DPM: Diet effect: $F(1,15)=7.67$, $p<0.0143$, DMNL ad lib: 438±142, DMNL refed: 3170±818, Controls ad lib: 638±278, Controls refed: 1605±546 DPM; DPM/protein: Diet effect: $F(1,15)=8.03$, $p<0.0126$, DMNL ad lib: 231±62, DMNL refed: 1904±610, Controls ad lib: 446±177, Controls refed: 1357±297 DPM/protein]. Notably, none of the fat pad parameters showed a significant interaction effect lesion × diet.

In the liver, only two out of eight parameters showed significant lesion effects. Rats with DMNL had higher oxidation, but only when expressed in DPM [DMNL ad lib: 22263±2195, DMNL refed: 23430±1202, Controls ad lib: 18579±1929, Controls refed: 15899±1224, Lesion effect: $F(1,15)=6.94$, $p<0.0188$]. Lesioned rats also incorporated glucose more avidly into total lipid [DPM: Lesion effect: $F(1,15)=6.80$, $p<0.0207$, DMNL ad lib: 3749±541, DMNL refed: 2901±209, Controls ad lib: 2395±150, Controls refed: 2221±422]. All of the above data are also summarized in a semiquantitative fashion in Tables 1 and 2.

DISCUSSION

Since the principal purpose of the present study is the comparison of changes that follow different durations of the postoperative-prerestriction ad lib feeding period, the major thrust of the present discussion will focus on comparing data from the present study with the findings from two previous experiments. For quick reference, the following table summarizes the essential differences in the experimental manipulations of these three studies.

Study	Source	Age (days) at Operation	Duration of Ad Lib Feeding	Restriction	Refeeding
1	Bernardis *et al.* [7]	28	42	28	9
2	Bernardis *et al.* [8]	27	55	24	7,22
3	present	29	25	27	7,35

Body weight loss due to food restriction was clear-cut in all three studies but appeared smallest in Study 1 and greatest in the present study, at least in DMNL rats. On refeeding, both DMNL and sham-operated control rats gained as much weight as their ad lib-fed counterparts in the present study. In contrast, both DMNL and control groups of Study 1 exhibited a conspicuous "overshoot" over their ad lib-fed counterparts; in Study 2 only the refed DMNL rats, but not the sham-operated controls showed such an overshoot response. Although one might expect from the two previous studies that carcass lipid would be significantly reduced in restricted rats, it did not reach significance in the present study, and what little reduction in carcass lipid there was became normalized upon refeeding.

These data are very important because they not only confirm previous findings of normal body composition in ad lib-fed DMNL rats [6], but also because they show that DMNL rats with their significantly lowered body weight responded like sham-operated control rats to reduced caloric intake and subsequent realimentation. Notably, carcass protein was uninfluenced in all three studies by both DMN lesions and food restriction.

Whereas linear growth (nose-tail length) was not determined in Study 1, the data show that in both the present study and Study 2 body length was highly significantly reduced in DMNL vs. sham-operated control rats, as had been reported in our original findings [1]. However, whereas in Study 2 both restricted DMNL and sham-operated control rats showed the same body length as their ad lib-fed counterparts, in the present study there was a significant body length reduction in the restricted DMNL and control groups. This effect persisted throughout the first week of refeeding but normalized by 35 days of realimentation. Notably, in Study 2, body length reduction did not become evident during restriction but made its appearance on refeeding, being still discernible 22 days thereafter. Since the only essential difference between Study 2 and the present study in the duration of ad lib feeding prior to restriction (Study 2, 55 days, present study: 25 days), it may well have to be invoked as possibly contributing to the observed effect.

It is clear from the food intake data that the hypophagic "growth-retarded" DMNL rat is capable of increasing its food intake into the hyperphagic range in attempts to reach its "true," i.e., lesion-induced, but lower-than-control, body weight and the body weight of ad lib-fed DMNL rats.

Conspicuous differences among the three studies were noticeable in the efficiency of food utilization (EFU), i.e., the amount of weight gained per amount of food eaten. Whereas in Studies 1 and 2 both restricted DMNL and sham-operated control rats utilized food more poorly than their ad lib-fed counterparts, in the present study the EFU

TABLE 1

SEMI-QUANTITATIVE OVERVIEW OF SOMATIC DATA

Parameter	Kill 1			Kill 2			Kill 3		
	L	D	LD	L	D	LD	L	D	LD
Body Weight (g)	↓	↓	NS	↓	↓	NS	↓	NS	NS
Body Length (mm)	↓	↓	NS	↓	↓	NS	↓	NS	NS
Food (g/day)	↓	↓	NS	↓	NS	NS	↓	NS	NS
Food ($g/kg^{3/4}$)	NS	↓	NS	NS	NS	NS	↓	NS	NS
EFU (g/day/g/day)	NS	NS	NS	NS	↑	NS	NS	NS	NS
Adrenal Wt. (mg)	↓	↓	NS	↓	NS	NS	↓	NS	NS
Liver (g)	↓	↓		↓	↓	NS	↓	NS	NS
Liver ($g/kg^{3/4}$)	↓	↓	NS	↓	NS	NS	NS	NS	NS
Kidneys (g)	↓	↓	NS	↓	NS	NS	↓	NS	NS
Kidney ($g/kg^{3/4}$)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Testes (g)	NS	↓	NS	↓	NS	↓*	NS	NS	NS
Testes ($g/kg^{3/4}$)	↑	↑	NS	↑	NS	NS	NS	NS	NS
Epididymal Fat Pads Fat Pads (g)	↓	↓	NS	↓	NS	NS	NS	NS	NS
Epdidymal Fat Pads ($g/kg^{3/4}$)	↓	↓	NS	NS	NS	NS	NS	NS	NS

↑↓Denotes that changes are significantly greater or smaller. In the lesion columns this means that changes in DMNL rats are different from sham-operated controls whereas in the diet column (D) it indicates that ad lib-fed rats are different from restricted-refed animals.

*Reduced organ growth in DMNL rats occurred only among ad lib-fed groups.

TABLE 2

SEMI-QUANTITATIVE OVERVIEW OF METABOLIC DATA

Parameters	Kill 1			Kill 2			Kill 3		
	L	D	LD	L	D	LD	L	D	LD
Plasma									
Insulin (μU/ml)	NS	↓	NS	NS	NS	NS	NS	↑	↓*
Glucose (mg/dl)	NS	↓	NS	NS	NS	NS	NS	NS	NS
Triglycerides (mg/dl)	↓	NS	NS	NS	NS	NS	NS	NS	NS
Glycerol (mg/dl)	↓	NS	NS	↓	NS	NS	NS	NS	NS
Total protein (g/dl)	NS	NS	NS	NS	↓	NS	NS	NS	NS
Epididymal Fat Pad									
CO_2 (DPM)	↓	↑	NS	NS	↑	NS	NS	NS	NS
Total lipid (DPM)	NS	NS	NS	NS	↑	NS	NS	↑	NS
Sap. Lip. (DPM)	NS	NS	NS	NS	↑	NS	NS	↑	NS
Sap. Lip. (DPM/Prot)	NS	↑	NS	NS	↑	NS	NS	↑	NS
Glycogen (DPM)	NS	NS	NS	NS	↑	NS	NS	NS	NS
Liver									
CO_2 (DPM)	NS	↑	NS	NS	NS	NS	↑	NS	NS
Total Lip. (DPM)	↑	NS	↑‡	NS	NS	NS	↑	NS	NS
Total Lip. (DPM/Protein)	NS	↑	NS	NS	NS	NS	NS	NS	NS
Glycogen (DPM)	NS	↑	NS	NS	NS	↑†	↑	NS	NS
Glycogen (DPM/Protein)	NS	NS	↑§	NS	NS	NS	NS	NS	NS

For general legend see Table 1.

*Reduced plasma insulin levels in ad lib-fed vs. restricted-refed rats occurred only among sham-operated controls.

†Glucose incorporation is greater in restricted-refed than in ad lib-fed rats only among DMNL animals.

‡Glucose incorporation is greater in restricted-refed rats only among DMNL rats.

§Glucose incorporation is greater in refed rats only among DMNL animals.

was normal among all groups during restriction. Notably, in all three studies refeeding for the first week resulted in significant "overshoot" over the values of the ad lib-fed groups, a response that normalized 22 days (Study 2) and 35 days (present study) thereafter.

We have known for some time [5] that DMNL rats have smaller organs in absolute terms but show normal organ growth when the latter is calculated per metabolic size [14]. In the present study, the reduced adrenal growth in absolute and normal growth in relative terms is therefore in good accord with previous findings [5]. Quite notable is that during restriction absolute adrenal growth was smaller in both DMNL and sham-operated control rats, but that it was normal when calculated for $kg^{3/4}$. This suggests that adrenal growth proceeded normally, i.e., in accordance with the reduced metabolic mass. The identical pattern—both in absolute and relative modes of presentation—was noted in kidney growth. We have previously reported that in ad lib-fed DMNL rats kidney growth was either increased or decreased in absolute terms but normal per $kg^{3/4}$.

Remarkable growth patterns emerged in the case of the testes and the liver. In the former, restriction is accompanied by normal absolute growth but refeeding for seven days showed reduced absolute testes growth in DMNL rats. Notably, testes growth expressed in relative terms was enhanced in DMNL rats over sham-operated controls, during both restriction and refeeding. In previous studies we had reported normal testes growth in DMNL rats under ad lib feeding conditions in both absolute and relative terms [5].

The present data on liver growth are in excellent agreement with previous findings [5] which show smaller livers in DMNL rats in both absolute and relative terms under ad lib feeding conditions. In addition—and as expected—restriction in the present study resulted in reduced lever growth, again both in absolute and relative terms. It is noteworthy that, in comparison to other organs, liver growth is not normalized when calculated per $kg^{3/4}$. The data show that the liver is most severely affected by both DMNL and dietary manipulation. However, as will be discussed below, these severe growth reductions are not matched by comparably severe metabolic changes.

Epididymal fat pad growth was significantly reduced in DMNL vs. sham-operated controls during both restriction and seven days of refeeding but then normalized when considered in absolute terms. It is noteworthy that, like the liver, fat pads were also smaller in DMNL rats when expressed in $kg^{3/4}$. This also holds for restricted vs. ad lib-fed groups. Although there are strong lesion effects in both absolute and relative fat pad growth, these changes are not matched by equally profound alterations in glucose incorporation into fat pad fractions; this will be discussed below.

Our data on plasma glucose and insulin for the present study are in excellent agreement with previous findings regarding normoglycemia and normoinsulinemia in ad lib-fed DMNL rats [3,6]. Since both DMNL rats and sham-operated controls showed reduced glucose and insulin levels during restriction and normalization upon refeeding, it is obvious that DMNL rats do not show a deficient adaptive response to these challenges. Nevertheless, it should be noted that in Study 1 DMNL rats were hypoinsulinemic, an anomly that we cannot explain.

Our present data on free fatty acids (FFA) and triglyceride (TG) in DMNL rats are also in excellent accord with normal levels under ad lib feeding conditions [6]. Notably, upon restriction, FFA levels increased in both Studies 1 and 2, an effect that would be expected because of enhanced lipolysis. However, the normal FFA levels in the restricted animals of the present study are reminiscent of the normal FFA levels reported by Harris and Martin [11] in severely restricted rats (40% of ad lib intake as opposed to 80% of ad lib intake in our three studies). Since the data of the present study in most parameters agree with Studies 1 and 2, it may well be that this finding is related to the only essential difference between the two studies, i.e., the time of ad lib feeding prior to restriction and/or the age of the rats at that time.

From an overall viewpoint—and in confirmation of previous data—lesion effects (DMNL rats vs. sham-operated controls) in epididymal fat pad fractions are only evident in one out of eight parameters measured [6]. Notably, diet effects (restricted-refed vs. ad lib-fed animals) are present in six out of eight parameters. In the liver, the corresponding values are two out of eight and four out of eight parameters, respectively. From these data it is evident that DMNL bring about little, if any, significant metabolic changes in both of these tissues. Moreover, the majority of metabolic alterations observed in this and past studies are due to dietary manipulation and occur commensurately in DMNL rats and sham-operated controls.

These data are important because they show that not only are the experimental effects reproducible in studies performed several years apart, but that, more importantly, DMNL do not cause significant metabolic effects. The findings also show that DMNL rats respond not only in somatic but also in presumably underlying metabolic parameters like controls to the challenge of food and body weight restriction and refeeding.

In general, a similar pattern is seen in the liver, inasmuch as restricted and subsequently refed groups incorporated glucose more avidly than ad lib-fed animals. Again this occurred in both DMNL and sham-operated control rats. As with fat pads, there were exceptions, as for instance the incorporation of glucose into liver total lipid in DPM/protein which in the present study was significantly reduced in restricted DMNL rats and controls but normal in Study 1. Nevertheless, these changes normalized on refeeding.

In their totality, both metabolic and somatic data of the present and the previous two studies suggest that DMNL rats, although in absolute terms hypophagic-hypodipsic and "growth-retarded," respond to food and body weight restriction and subsequent refeeding like sham-operated controls that have been restricted and refed. This suggests the manifestation in the DMNL rat of a well-tuned set point system that functions in a global fashion, since it extends and exerts its influence into apparently all somatic and neuroendocrine-autonomic-metabolic processes that appear necessary for orderly growth. We have for this reason termed this set point "organismic" and juxtapose it to "compartment-specific" set points, such as become manifest following experimental destruction of the ventromedial (VMH) and lateral (LHA) hypothalamic areas. Such experimental manipulation results in grossly altered changes in body composition and, at least in the VMH rat, in profound neuroendocrine-metabolic deficits [9].

REFERENCES

1. Bernardis, L. L. Participation of the dorsomedial hypothalamic nucleus in the "feeding center" and water intake circuitry of the weanling rat. J. Neurovisceral Relat. 31:387–398; 1970.
2. Bernardis, L. L. Disruption of diurnal feeding and weight gain cycles in weanling rats with ventromedial and dorsomedial hypothalamic lesions. Physiol. Behav. 10:855–861; 1973.
3. Bernardis, L. L. The dorsomedial hypothalamic nucleus in an autonomic and neuroendocrine homeostasis. Can. J. Neurol. Sci. 2:45–60; 1975.
4. Bernardis, L. L.; Goldman, J. K. Origin of endocrine-metabolic changes in weanling rat ventromedial syndrome. Neurol. Sci. Res. 2:91–116; 1976.
5. Bernardis, L. L.; Bellinger, L. L. Relative normophagia and organ growth in growth-retarded weanling rats with dorsomedial hypothalamic lesions. J. Neurosci. Res. 4:197–203; 1979.
6. Bernardis, L. L.; Bellinger, L. L. Organismic set point system in dorsomedial hypothalamic nuclei. In: Almli, C. R.; Finger, S., eds. The behavioral biology of early brain damage. New York: Academic Press; 1984:269–287.
7. Bernardis, L. L.; McEwen, G.; Kodis, M. Body weight set point studies in weanling rats with dorsomedial hypothalamic lesions (DMNL rats). Brain Res. Bull. 17:451–460; 1986.
8. Bernardis, L. L.; McEwen, G.; Kodis, M. Somatic, metabolic and endocrine correlates of set point recovery in food-restricted and ad lib-fed weanling rats with dorsomedial hypothalamic lesions. Physiol. Behav. 37:875–884; 1986.
9. Bucolo, G.; Daivd, H. Determination of serum triglycerides by the use of enzymes. Clin. Chem. 19:476–482; 1972.
10. Folch, J.; Leese, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509; 1957.
11. Harris, R. B.; Martin, R. J. Recovery of body weight from below "set point" in mature female rats. J. Nutr. 114:1143–1150; 1984.
12. Hollifield, G.; Parson, W. Metabolic adaptations to a "stuff and starve" feeding program. II. Obesity and the resistance of adaptive changes in adipose tissue and liver occurring in rats limited to short daily feeding period. J. Clin. Invest. 41:250–253; 1962.
13. Itaya, K.; Ui, J. Colorimetric determination of free fatty acids in biological fluids. J. Lipid Res. 6:16–20; 1965.
14. Kleiber, M. Body size and metabolic rate. Physiol. Rev. 27:511–514; 1947.
15. Lowry, O. H.; Rosebrough, N. J.; Randall, R. J. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
16. Tepperman, J.; Tepperman, H. Effect of antecedent food intake pattern on hepatic lipogenesis. Am. J. Physiol. 193:56–64; 1958.