Obesity and Adipocyte Abnormalities in Offspring of Rats Undernourished During Pregnancy

Abstract. *Pregnant rats underfed in the first 2 weeks of gestation had offspring with normal body weight at birth and weaning. However, starting at about 5 weeks of age the male offspring became hyperphagic and gained more weight than the controls. The female offspring did not overeat and did not become obese. Both male and female progeny showed increased fat cell size as adults. In males the epididymal and retroperitoneal fat pads were significantly enlarged and adipocytes in these pads were hypertrophied. In females the fat pads were not enlarged, but adipocytes in the parametrial pads were hypertrophied.*

During the winter of 1944 to 1945, German occupation forces attempted to undermine resistance activities in the northern Netherlands by imposing an embargo on all incoming supplies, including foodstuffs, to that area. The acute famine that resulted, known as the Dutch Hungerwinter, has been the focus of a number of epidemiological investigations. In one, Ravelli *et al.* (*1*) analyzed body weights of 300,000 male Dutch army draftees whose mothers had been exposed to the famine during pregnancy. It was found that men whose mothers had been deprived of food in the first two trimesters only had a higher incidence of obesity than the general population. Because this finding tends to contradict a number of studies of humans and animals (*2*) showing that undernutrition throughout gestation leads to permanently lowered body weights and adiposity in offspring, we examined the effects of undernutrition limited to early pregnancy in laboratory animals. We report that male rats born to mothers that were undernourished in the first 2 weeks of gestation became hyperphagic and obese as adults and that, although female offspring did not become obese, both sexes showed adipocyte abnormalities.

Female Sprague-Dawley rats (Charles River) were allowed to breed in the laboratory. The day of conception was determined by daily examination of vaginal smears for the presence of sperm. When a sperm-positive smear was found, the female was isolated in a plastic maternity cage. The animals were alternately assigned to experimental and control groups. Experimental animals were immediately restricted to 50 percent of their preconception food intake until day 14 of gestation, at which time they were given unrestricted access to food (powdered Purina Lab Chow). This level of food restriction is similar to that experienced by the victims of the Dutch Hungerwinter (*1*). Control rats were given unlimited food throughout gestation. Four experimental and seven control mothers met the acceptance criterion of giving birth within 2 days of the expected delivery date (21 days after detection of sperm); all the litters were culled to three males and three females. The offspring were weaned 21 days after birth, and males and females were housed separately in groups. Body weight was measured at birth and weaning, and body weight and daily food intake were measured every fifth day after weaning until the rats were 111 days of age. Food intake per rat was determined by dividing the intake of each group of male and female littermates by the number of rats in the group. Locomotor activity was assessed 59 and 102 days after birth by placing the rats individually for 24 hours in tilting cages that recorded the number of times the animal crossed the enclosure.

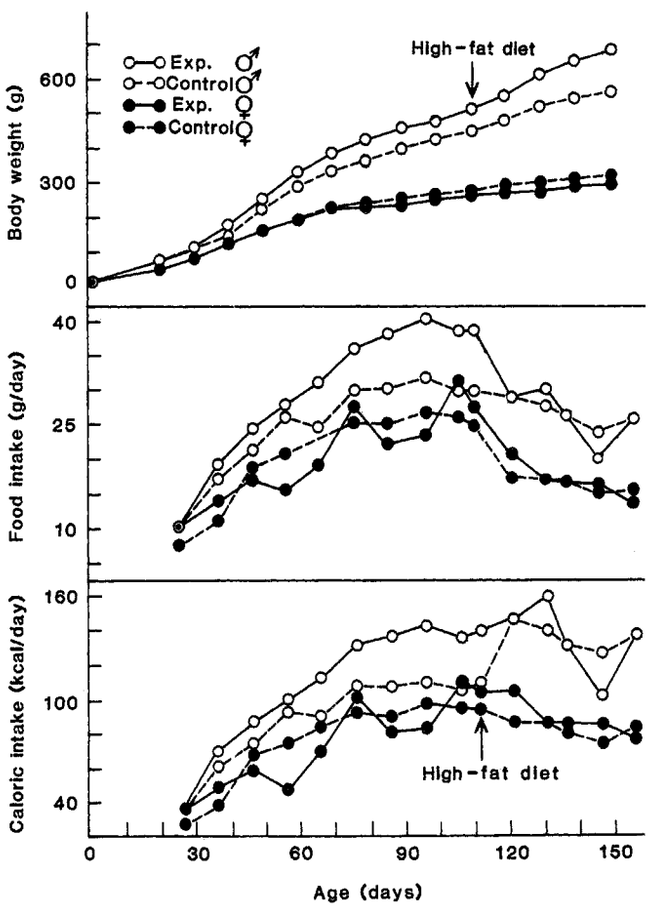

Fig. 1. Body weight, food intake, and caloric intake of rats born to mothers given unlimited food throughout pregnancy or underfed during the first 2 weeks. Each point represents the mean for 12 to 18 animals.

Control and experimental mothers gave birth to similar numbers of young, and the body weight of offspring did not differ between groups at birth (*3*) or weaning. However, male offspring of experimental mothers gained more weight after weaning than control offspring [$F(11, 220) = 5.32, P < .001$] (Fig. 1). This difference appeared around 35 days after birth, became statistically reliable ($P < .05$) at 50 days, and gradually expanded thereafter (*4*). Female offspring from underfed mothers gained the same amount of weight as control females. Similarly, food and caloric intakes of experimental male, but not experimental female, offspring were larger than those of the same-sex controls [$F(8, 160) = 3.10, P < .003$]. There was no difference between experimental and control offspring of either sex in the number of cage crossings, suggesting that changes in energy expenditure associated with locomotor activity did not account for the observed differences in body weight and food intake.

When the offspring were 111 days of age they were given a high-fat diet (*5*) in place of their powdered food. Body weight gains accelerated in experimental males after introduction of the new diet [$t(9) = 2.1, P < .05$], whereas control males gained weight at the same rate as before. Caloric intakes of the experimen-

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tal and control males converged after introduction of the high-fat diet because gram intake of this more calorically dense food decreased in experimental rats but did not change in controls. Thus, the increase in weight gain in experimental males during high-fat feeding occurred despite no increase in caloric intake. The metabolic efficiency [weight gain (grams)/caloric intake (kilocalories)] of experimental male rats on the high-fat diet was significantly higher than that of experimental males on the powdered diet [t (3) = 3.89, $P < .05$]. This increase was not seen, however, in control males or in females of either group. It appears that male offspring of underfed mothers deposit excessive amounts of fat and that the composition of the postnatal diet may contribute to development of the obesity. Experimental and control female offspring decreased food intake when fed the high-fat food; caloric intake and body weight did not change.

At the end of the experiment the animals were decapitated and their retroperitoneal and epididymal fat pads were removed (*6, 7*). The parametrial pads were bilaterally excised along the entire length of the uterine horns. Fat cells were suspended in a Krebs-Ringer solution containing phosphate, collagenase, glucose, and albumin and incubated for 1 hour at 37°C (*8*). They were then filtered through a 250-μm nylon mesh and rinsed twice with the solution. A 50-μl sample of the fat cell layer was placed on a slide and photomicrographed through a microscope fitted with a yellow filtered light source (*9*). Negative images were projected onto a translucent grid and adipocyte diameters were manually digitized with a Graf pen interfaced with a Data General Nova 3 computer (*10*). Three hundred cells were digitized per pad and mean cell diameter and variance were calculated.

Epididymal and retroperitoneal fat pads from experimental males weighed, respectively, two and three times more than those from control males (Table 1). These greater fat pad weights in experimental males were associated with greater fat cell diameters. Although retroperitoneal and parametrial fat pads from experimental females tended to be heavier than those from control females, the difference was not statistically reliable (*11*). Parametrial fat pad cells were larger in experimental females than in control females.

Thus, the effects indicated in the epidemiological study of Ravelli *et al.* (*1*) can be reproduced in laboratory animals under controlled conditions. Ravelli observed a higher incidence of obesity in young men whose mothers had been subjected to food restriction in the first two trimesters of pregnancy. Similarly, we found that male rats born to mothers that were undernourished in the first two trimesters (weeks) of gestation gain more weight than those born to mothers given ample food throughout pregnancy. In addition, we found that this excessive weight gain does not appear until after weaning and that it is associated with hyperphagia, heavier fat pads, and adipocyte hypertrophy. The results also suggest that the composition of the postnatal diet may influence the degree of obesity in adulthood. Because Ravelli *et al.* surveyed only male inductees, it is not known what impact early gestational undernutrition had on the incidence of obesity in women born to mothers who experienced the Dutch Hungerwinter. In our animal study we found that the female offspring of underfed mothers did not become overweight but that the adipocytes in their parametrial fat pads were hypertrophied.

Table 1. Fat pad weight and adipocyte size in rats born to mothers that were underfed during the first 2 weeks of pregnancy or allowed to eat freely throughout pregnancy. Each litter contained three male and three female rats. Values are means ± standard errors.

Group	Litters	Fat pad weight (g)		Adipocyte diameter (μm)	
		Retroperitoneal	Perigonadal	Retroperitoneal	Perigonadal
Males					
Control	6	9.07 ± 1.51	8.63 ± 0.77	91.3 ± 9.5	85.5 ± 3.5
Experimental	4	27.83 ± 4.45*	17.15 ± 1.86*	121.4 ± 5.3†	107.3 ± 5.3*
Females					
Control	6	5.10 ± 0.34	6.49 ± 0.49	84.44 ± 4.40	82.13 ± 1.18
Experimental	4	7.52 ± 2.48	10.04 ± 3.17	89.97 ± 1.90	111.24 ± 5.18‡

*Significantly different from corresponding control value at $P < .01$. †$P < .025$. ‡$P < .001$.

Although a history of hyperphagia may have contributed to fat cell enlargement in the male offspring, we do not believe that adipocyte hypertrophy is entirely secondary to overeating because the experimental females, which did not overeat, also had larger fat cells. In addition, we recently found that both male and female offspring of mothers that were underfed in early pregnancy show fat cell hypertrophy at weaning, before the onset of hyperphagia and frank obesity (*12*). The fact that body weight, food intake, and fat cell size are affected differently in male and female animals suggests that sex hormones modulate the consequences of early undernutrition.

A number of studies have shown that offspring of mothers that were underfed throughout pregnancy, and in some cases during lactation as well, have permanently reduced body weights (*2*). Typically, early nutrition has been found to affect adipocyte number rather than cell size (*13*), and it has been suggested that the obesity in offspring of the victims of the Dutch Hungerwinter resulted from adipose hyperplasia (*1, 14*). Our findings stand in contrast to the results of previous studies and suggest that the long-term effects of maternal undernutrition on the weight of offspring are dependent on the timing and duration of the nutritional experience and on the sex of the offspring.

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References and Notes

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