Dietary-induced hypertrophic–hyperplastic obesity in mice

L. Herberg, W. Döppen, E. Major, and F. A. Gries
Diabetes Research Institute, University of Düsseldorf, 4 Düsseldorf 1, West Germany

Abstract
Metabolically intact NMRI mice and genetically obese NZO mice were fed ad lib. either a high-carbohydrate diet (standard) or a high-fat diet for a period of about 11 (NMRI mice) or 38 (NZO mice) wk. In both strains of mice, body weight increased more in the groups fed the high-fat diet. However, caloric intake by NMRI mice fed the high-fat diet was less than that of the controls. In NMRI mice fed the high-fat diet, epididymal and subcutaneous fat cell volumes increased; when these mice were fed the standard diet, only epididymal fat cell volume increased. Epididymal and subcutaneous fat cell numbers increased only in the group fed the high-fat diet. In NMRI mice fed either diet, the postprandial blood glucose was lower in older animals, but plasma insulin remained unchanged. The glucose tolerance deteriorated insignificantly. In NZO mice fed either diet, epididymal fat cell volumes and fat cell numbers increased. In this strain of mice the postprandial blood glucose and plasma insulin exhibited the strain-specific pattern, independent of the diet. In older animals fed either diet the glucose tolerance decreased.

Supplementary key words adipose cell size • adipose cell number • glucose tolerance • plasma insulin

The mass of adipose tissue is determined by fat cell size and fat cell number. For a long time it was assumed that obesity in adult rats was due only to an increase in cell size (1). More recently it has been reported that an increase in cellularity also occurs with obesity in adult rats fed a standard diet (2, 3). Braun et al. (4) observed that meal eating as well as refeeding after a single fast increases the number of fat cells in epididymal and parametrial adipose tissue of adult, metabolically intact rats. Using genetically obese rats, Johnson et al. (5) noticed that fat cell proliferation in subcutaneous, perirenal, and epididymal adipose tissue continued throughout the first 26 wk of life. In genetically obese mice of the C57BL/6J–ob strain, we found an increase in cell number in the epididymal fat pads with age (6). This parallels the findings of DiGirolamo, Mendlinger, and Fertig (7) in guinea pigs, rats, and rabbits.

Since the factors leading to adipose tissue hyperplasia are still unclear, we have investigated the effects of different diets on adipose tissue cellularity and fat cell size. Obesity was induced in metabolically intact NMRI mice by a high-fat diet. In a second series of experiments, this form of dietary obesity was superimposed on genetically obese NZO mice.

MATERIALS AND METHODS

Animals and diets
Male NMRI mice (Duhr-Wuppertal, Germany) and male NZO mice (our own inbreeding colony, originating from the strain described earlier [8]) were used. The animals were housed, six to a cage, in a temperature-controlled room with a light-dark cycle and had free access to food and tap water. Although the precise eating behavior was not recorded, no preferential mode (meal eating vs. nibbling) was noticed. In all experiments the mice were weaned at 4 wk to either a high-carbohydrate (standard) or a high-fat diet. The compositions of the diets are shown in Table 1. Only in NMRI mice was the amount of ingested food determined daily by weighing the food offered and subtracting from it any uneaten food 24 hr later. The animals were weighed weekly. The preperiod is defined as the nursing time plus the time of adjustment to the diet (40 days for NMRI mice and 70 days for NZO mice), indicated by vertical lines in the figures. The experimental period is the time following the preperiod.

For NMRI mice fed the high-carbohydrate and the high-fat diets the experimental period was 11.6 and 11.0 wk, respectively; for both groups of NZO mice it was 38 wk.

Analytical tests
Intraperitoneal glucose tolerance tests (9) and insulin determinations were performed in all groups. In addition, postprandial blood glucose and plasma insulin were determined in NZO mice at monthly intervals.

Abbreviations: NMRI mice, Naval Medical Research Institute mice; NZO mice, New Zealand obese mice; EFA, esterified fatty acids; TG, triglycerides.

124 ± 1°C.
212 hr dark, 12 hr light.
TABLE 1. Composition of diets

<table>
<thead>
<tr>
<th>Percentage by Weight</th>
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<tbody>
<tr>
<td><strong>High carbohydrate diet</strong></td>
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<tr>
<td>Protein concentrate</td>
</tr>
<tr>
<td>Oatmeal</td>
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<tr>
<td>Whole meal</td>
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<tr>
<td>Rusk flour</td>
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<tr>
<td>Tapioca flour</td>
</tr>
<tr>
<td>Wheat bran</td>
</tr>
<tr>
<td>Wheat germ</td>
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<tr>
<td>Lucerne green flour</td>
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<tr>
<td>Distillers solubles</td>
</tr>
<tr>
<td>Sweet whey</td>
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<tr>
<td>Vitamin concentrates</td>
</tr>
<tr>
<td>Mineral mixture</td>
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<tr>
<td><strong>Wheat bran</strong></td>
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<td><strong>Sweet whey</strong></td>
</tr>
<tr>
<td><strong>Vitamin concentrates</strong></td>
</tr>
<tr>
<td><strong>Mineral mixture</strong></td>
</tr>
<tr>
<td><strong>Casein</strong></td>
</tr>
<tr>
<td><strong>Starch</strong></td>
</tr>
<tr>
<td><strong>Saccharose</strong></td>
</tr>
<tr>
<td><strong>Soy oil</strong></td>
</tr>
<tr>
<td><strong>Powdered cellulose</strong></td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
</tr>
<tr>
<td><strong>Minerals</strong></td>
</tr>
</tbody>
</table>

* Intermast, Soest, Germany.
* Distribution of calories: carbohydrate, 63%; fat, 13%; protein, 24%.
* 50% soy whole meal, extracted and toasted, 30% fish flour, 10% codfish flour, 10% fish solubles.
* 41.6% wheat, 25% Indian corn, 16.7% barley, 16.7% oats.
* Distribution of calories: carbohydrate, 10%; fat, 63%; protein, 18%.

Blood was collected from unanesthetized mice at 9 a.m. Glucose determinations were performed immediately on 25 μl of whole blood by the neocuproine method with an AutoAnalyzer II (10). For insulin determinations, blood was collected in heparinized plastic vessels and centrifuged, and the plasma was stored at −18°C until assayed. Plasma insulin levels were measured in duplicate by a solid-phase immunoassay (Deutsche Pharmacia, Frankfurt/Main, Germany).

**Determination of cellularity**

Both epididymal fat pads were excised. In NMRI mice the subcutaneous adipose tissue, excluding the intrascapular brown fat pads, was also removed. Wet weight, esterified fatty acid (EFA) content, and fat cell volume were determined as previously described (11). Cellularity of adipose tissue was calculated using the following formula:

\[
\frac{\mu\text{moles EFA}}{\text{wet weight of tissue}} \times \frac{289.667}{\mu\text{g TG cells}} = \frac{\text{cells}}{\text{wet weight of tissue}}
\]

**RESULTS**

**NMRI mice**

As shown in Table 2, in mice fed the high-fat diet, food intake was about 60% of that of the controls, whereas total caloric intake was nearly the same in both groups. Taking the composition of the diets into account, the high-fat group consumed 4.5 times more fat and about one-fourth of the carbohydrate as compared with the high-carbohydrate controls. Weight gains of mice fed the high-fat diet were twice those of the controls.

**Fig. 1** shows that the mice fed the high-fat diet gained weight faster than the control animals and continued gaining weight until the end of the experimental period. However, the final body weight of the control mice was reached 30 days after beginning of the experimental period. Differences in body weight were highly significant (P < 0.001) by the 20th day of the experimental period.

**Table 2. Food intake and changes in body weight and immunoreactive insulin (IRI) in NMRI mice fed different diets during the experimental period**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Average Food Intake</th>
<th>Average Weight</th>
<th>Postprandial Plasma IRI</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Carbohydrate</td>
<td>Fat</td>
</tr>
<tr>
<td>High carbohydrate</td>
<td>5.7</td>
<td>20</td>
<td>12.6</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High fat</td>
<td>3.4</td>
<td>19</td>
<td>3.6</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
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</table>

Values for body weight and plasma IRI are means ± SEM.
it is evident that the relative mass of fat cells was dependent on the site of the tissue (Table 3).

In young mice, weighing about 19 g, epididymal fat pads consisted of 66.5% fat (expressed as EFA), 76.5% fat cells, and 23.5% nonfat cell mass. With aging and weight gain these values changed to 80.7%, 92.9%, and 7.1%, respectively, for mice on the high-carbohydrate diet, weighing about 34 g, and to 78.9%, 90.8%, and 9.2%, respectively, for mice on the high-fat diet, weighing about 49 g. The subcutaneous tissue of young mice weighing about 19 g contained only 51.7% fat, 59.4% fat cell mass, and 40.6% nonfat cell mass. In the high-carbohydrate group the ratio was nearly the same, 47.6%, 54.8%, and 45.2%, respectively. Feeding the high-fat diet, however, caused an increase in fat to 66.3%, in fat cell mass to 76.3%, and a decrease in nonfat cell mass to 23.7%.

Glucose tolerance in the high-fat and the control groups was not significantly different at the beginning and the end of the experimental period. In addition, there was no significant difference in plasma insulin (Table 2).

**NZO mice**

In NZO mice fed the high-fat diet, body weight and epididymal adipose tissue mass increased (Fig. 3). At the end of the experimental period, the mean body weight was significantly higher ($P < 0.001$) than that of the controls.

During the preperiod, cell volume of the epididymal fat pads increased only slightly (Fig. 4). Within the first 40 days of the experimental period, the enlargement of fat cells in the controls was delayed when compared with that of cells in the mice on the high-fat diet. In all mice on either diet, weighing about 65 g, cell volumes were nearly the same. However, at the end of the experimental period, cell volume in mice fed the high-fat diet was significantly higher ($P < 0.001$) than in mice on the standard diet. In the group fed the standard diet, cell numbers in the epididymal adipose tissue increased rapidly during the preperi-

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**TABLE 3. Weight, fat cell number, and composition of epididymal and subcutaneous adipose tissue in NMRI mice**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Preperiod (n = 6)</td>
<td>19.1 mg</td>
<td>109.9 mg</td>
<td>162 ± 0.5</td>
<td>15.2 ± 17</td>
<td>66.5 (76.3)</td>
<td>23.5 ± 17</td>
<td>73.1 mg</td>
<td>84.1 mg</td>
<td>25.8</td>
<td>449.3 mg</td>
<td>580 ± 64</td>
</tr>
<tr>
<td></td>
<td>± 0.5 mg</td>
<td>± 15.2 mg</td>
<td>± 17</td>
<td>± 17</td>
<td>(66.5) (76.3)</td>
<td>± 25.8</td>
<td>± 84.1</td>
<td>± 25.8</td>
<td>± 449.3</td>
<td>± 580 ± 64</td>
<td></td>
</tr>
<tr>
<td>High carbohydrate</td>
<td>34.4 mg</td>
<td>683.5 mg</td>
<td>162 ± 1.2</td>
<td>± 75.3 ± 31</td>
<td>80.7 (92.9)</td>
<td>(7.1) ± 77.7</td>
<td>515.8 mg</td>
<td>635.0 mg</td>
<td>48.5</td>
<td>745.0 mg</td>
<td>627 ± 84</td>
</tr>
<tr>
<td></td>
<td>± 0.5 mg</td>
<td>± 15.2 mg</td>
<td>± 17</td>
<td>± 17</td>
<td>(80.7) (92.9)</td>
<td>(7.1) ± 77.7</td>
<td>± 515.8</td>
<td>± 635.0</td>
<td>± 48.5</td>
<td>± 745.0 ± 627</td>
<td></td>
</tr>
<tr>
<td>High fat (n = 8)</td>
<td>48.8 mg</td>
<td>2090.0 mg</td>
<td>389 ± 2.6</td>
<td>± 477.0 ± 57</td>
<td>1649.3 (1897.4)</td>
<td>192.6 ± 197.2</td>
<td>1920.9 mg</td>
<td>2360.9 mg</td>
<td>1137</td>
<td>1566.2 mg</td>
<td>1559.1 ± 232</td>
</tr>
<tr>
<td></td>
<td>± 0.5 mg</td>
<td>± 15.2 mg</td>
<td>± 17</td>
<td>± 17</td>
<td>(1649.3) (1897.4)</td>
<td>± 192.6</td>
<td>± 2360.9</td>
<td>± 1137</td>
<td>± 1566.2</td>
<td>± 1559.1 ± 232</td>
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</tbody>
</table>

Values for weight and fat cell number are means ± SEM. Composition values in parentheses are percentages.

* Experimental period.
and continued increasing at nearly the same rate until the 40th day of the experimental period, when the mean body weight was about 25 g. Thereafter, no change in cellularity occurred in these animals. In mice fed the high-fat diet, cell proliferation was almost undetectable until the animals had reached a body weight of about 45 g, i.e., 40 days after beginning the experimental period. With further weight gain, cellularity of the epididymal fat pads increased rapidly, but the final cell volume was only slightly higher than that of the controls.

Postprandial blood glucose and plasma insulin levels were analogous in both groups and exhibited the typical age-dependent pattern characteristic of this species (Fig. 4). Glucose tolerance decreased in animals on both diets.

**DISCUSSION**

During recent years, research in obesity has concentrated on the fat cell itself. In both man and animals, two types of obesity, hypertrophic and hypertrophic-hyperplastic, have been differentiated. In human subjects, however, hyperplasia may be accompanied by normal fat cell size (12).

A relation of fat cell size to the severity of metabolic disturbances has been described in mice (9) and in humans (13, 14). Based on the experiments of Hirsch and Han (15), cellularity is believed to increase only during weaning or in the immediate postweaning period. This theory seems to be supported by the observation that, in rats, malnutrition during early life results in a reduction in fat cell number that cannot be overcome by refeeding during adulthood (16-18). However, Therricauld and Mellin (19) reported that in young, cold-exposed rats adipose tissue primarily enlarges by hyperplasia and that the increase in cell number continues in the adult animals if they remain in the cold. Even in rats maintained under conventional environmental conditions, Enesco and Leblond (3) observed an increase in the number of epididymal adipose tissue cells as determined by the DNA content. This increase continued during aging. Similarly, investigations in rats, hamsters, and guinea pigs (20) revealed that fat cell number may continue to increase in the adult animal. Previous investigations by our group on C57BL/6J-06/06 mice led to the conclusion that this strain represents the type of hypertrophic-hyperplastic obesity that develops during adulthood. This has been confirmed by the morphological studies of Johnson et al. (5). Therefore, one may conclude that cellularity of adipose tissue is regulated during the entire life by various factors, including hormonal state and genotype.

It is well known that a high-fat diet favors the development of obesity in animals (21-31). Both insulin (32) and the level of fat in a diet (33, 34) are involved in the regulation of fat cell size. However, factors regulating cell number are still in question. Although there is strong evidence that under certain circumstances insulin plays a role in this process (35-37), this cannot be the only factor. We observed an increase in fat cell number in normal NMRI mice exhibiting insulin levels within the physiological range. The insulin level in the group with fat cell proliferation was even lower than in the control group, although this difference was not significant. Furthermore, in normal rats administration of even 25 mU of insulin/g body weight failed to stimulate adipocyte proliferation (38). As shown presently, in genetically obese NZO mice fed a high-carbohydrate diet, new fat cell formation clearly had ceased before plasma insulin began to rise slightly.
In our studies with NMRI mice, epididymal as well as subcutaneous adipose cell number remained unchanged after the beginning of sexual maturity when the animals were maintained on the standard diet. However, feeding the high-fat diet resulted in a significant increase in the number of cells in epididymal and subcutaneous adipose tissue. From Fig. 2 one might conclude that the greatest increase in cellularity occurred in the subcutaneous tissue, which also had exhibited the highest activity in cell formation during the preperiod. However, the factor of cell multiplication in animals on the high-fat diet was greater for the epididymal than for the subcutaneous tissue. Whether the increase in cell number was due to the multiplication of fat cells or the differentiation of potential fat cells and their consequent filling with lipid cannot be concluded from our studies. In addition, we have no data on the time course of cell proliferation because the determinations of cell numbers were performed before and at the end of the experimental period only. The calculation of the composition of the adipose tissue showed that nonfat cell mass is minor in epididymal fat pads. Therefore, when using tissue slices, epididymal adipose tissue may be more suitable for metabolic studies than subcutaneous tissue. Growth of epididymal adipose tissue is age-dependent, whereas subcutaneous adipose tissue is more accessible to exogenous factors such as dietary influences.

An increase in adipose tissue weight caused by feeding a fat-enriched diet had also been observed by Lemmonier (39-42): in epididymal adipose tissue of rats hypertrophy occurred, while in Swiss mice the reaction depended on the site of the tissue. In the perirenal site, hyperplasia was noticed; in the parametrical fat pad, hyperplasia as well as hypertrophy occurred; and the enlargement of the epididymal and subcutaneous adipose tissue was due only to hypertrophy. These observations are in contrast to our results with epididymal and subcutaneous adipose tissue in NMRI mice. Because the genotype of albino mice is very heterogeneous, it is likely that strain-specific factors are involved in adipose tissue response to dietary regimen. For example, Johnson and Hirsch (43) noticed even different types of obesity in aging yellow obese mice and ob/ob mice on identical diets, although both genotypes, the A\(^{Y}\) and the \(ob\), had been transferred to the same strain, namely the C57BL/6J.

The observation that high-fat diets are consumed in smaller amounts than low-fat diets has been reported by others (22, 44, 45). Despite a nearly identical caloric intake, it was surprising to find that the increase in body weight of mice fed the high-fat diet was twice that of mice fed the standard diet. Therefore, the degree of obesity cannot be predicted from the absolute caloric intake. The amount of energy stored as triglycerides is dependent on metabolic pathways, which in turn are predominantly influenced by the composition of the diet. Although apparent differences in physical activity were not noticed during the daytime, they may be a contributing factor to the different weight gains, as rodents are known to be physically active mainly during the night.

Our findings show that the relationship between body weight and caloric intake depends on the composition of the diet. Further investigations defining this relationship must take into account such factors as energy expenditure and energy metabolism in obese or nonobese organisms.

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