Some metabolic effects on lactating rats of a low-energy diet restricted in good-quality protein

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Adult female Sprague–Dawley rats were fed ad libitum during pregnancy and lactation a control diet (CD; 16·1 kJ/g) or a low-energy diet with wheat gluten as the main protein source (LED; 13·3 kJ/g). Body weight, food intake, resting energy expenditure, respiratory quotient and substrate use by the mammary gland were measured. After the animals had been killed, the parametrical and retroperitoneal fat pads were weighed. The mean food intake (g) of the two groups of rats was similar, resulting in a lower energy intake by the LED rats, significantly different during the last 2 weeks of lactation. The mean body weight of both dams and pups in the LED group was lower, starting at day 9 of lactation. The resting energy expenditure increased gradually during lactation in the control group, whereas this increase was not seen in rats of the LED group in the last week of lactation. Rats that had fasted overnight had a respiratory quotient of 0·7 or less, whereas for rats that had been fed, the mean respiratory quotient was over 1·0. Under both conditions, rats showed ketonuria. The arteriovenous difference in 3-hydroxybutyrate level was higher and those for glucose, lactate and triacylglycerol were lower across the mammary glands of LED rats. The parametrial fat depot weighed less in LED rats. Reduction in the increase in resting energy expenditure and using ketone bodies to a greater extent as fuels may represent important mechanisms in the LED dams to cover the energy cost of milk production.

Restricted diet: Lactation: Resting energy expenditure: Respiratory quotient

In small female mammals, pregnancy and, even more so, lactation are temporary conditions characterised by high energy expenditure (Trayhurn, 1989). According to Broady (1945), the extra energy cost of pregnancy is estimated to be 18 MJ/kg body weight of the litter at birth. The energy contained in the milk at the peak of lactation, i.e. from postpartum days 10–17 in the rat, has been reported to be 1000 kJ/d per kg⁰·⁷⁵ maternal weight for a litter size of eight pups (Blaxter, 1989). The extra energy has to be provided by an increase in the energy intake of the dam during both gestation and lactation, each of which lasts for 3 weeks in the rat. For optimum development of the fetuses and newborn, the dam’s food must provide not only the energy requirements, but also an adequate balance of the three macronutrients. Most important is the protein supply, necessary for the growth of the fetuses and the milk yield, and macronutrient balance for the newborn (Del Prado et al. 1997). During lactation, maternal metabolism needs to be reprogrammed to channel substrates for milk synthesis to the mammary gland (Williamson, 1990).

Related to these nutritional needs, many women in parts of the world suffer energy and/or protein malnutrition that may affect the development of their offspring. Typical diets throughout developing countries are derived primarily from vegetable sources, high in carbohydrate and fibre and low in fat, plants being the main protein source. The protein and energy use of a Guatemalan rural diet, which is similar to the Mexican rural diet (Rosado et al. 1992), has been investigated by Calloway & Kretsch (1978). Compared with egg-protein digestibility in a fibre-free diet, fibre in the Guatemalan diet reduced the apparent protein digestibility to 78 %. Energy intakes and expenditures in women living in rural villages in Guatemala showed that the energy expenditure during lactation was no different from that of non-lactating, non-pregnant women, but weight was lost (Schutz et al. 1980). In a rural Mexican community, malnourished mesoamerindian Otomi women gave birth to small infants. Although their weight and length were adequate through the first 4 months, both were thereafter less than those of the reference breast-fed infants (Butte et al. 1993). The low-energy diet (LED) used in our study was designed to resemble these rural diets. Excluding the weight of the fetuses, rats gain weight during pregnancy (Sohlström et al. 1994), in contrast with hamsters, which lose weight (Quek & Trayhurn, 1990). The pregnant rat is hyperphagic but does not increase its diet-induced thermogenesis. The extra intake is thus retained with maximum efficiency (Trayhurn, 1989). As in pregnancy, lactating rats show an increase in the efficiency of energy use regardless of the level of energy intake (Roberts & Coward, 1984).

Abbreviations: CD, control diet; LED, low-energy diet; REE, resting energy expenditure.

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Because of this metabolic adaptation of the female rat to more or less adverse alimentary conditions, we measured the changes in body weight of the dams and their pups and certain variables in dams when these were fed a low-fat, low-quality protein diet starting 2 weeks before mating and continued during gestation and lactation. We hypothesised that, under these conditions, the dams would show important differences compared with control dams in terms of resting energy expenditure (REE) and type of fuel use.

Materials and methods

Animals

Adult female Sprague–Dawley rats initially weighing 240 (se 20) g were kept in a room with a controlled temperature (22 (se 2)°C) and artificial dark–light cycles (lights on from 07.00 to 19.00 hours). The rats were housed in individual cages with free access to water and were randomly assigned to a control or a wheat protein LED (ten animals per group). After 2 weeks of adaptation to diets, the rats were mated with males of the same strain. Females that did not become pregnant were not included in the experiment.

The rats received the same diets during gestation and lactation. On the first day after birth (considered as the first lactation day) only eight pups (four females and four males when possible) were kept per dam. One group of rats for each diet was not mated but was kept as a group of non-lactating rats. The experiments were made according to the Mexican national committee recommendations for animal care.

Dietary compositions

The control diet (CD; protein 20.7 % energy, fat 11.7 % energy, carbohydrate 67.6 % energy, digestible energy 16.1 kJ/g) was prepared in accordance with recommended standards for rapid growth in rodents (Reeves, 1997). The wheat protein LED (protein 18.6 % energy, fat 7 % energy, carbohydrate 74.4 % energy, digestible energy 13.3 kJ/g) had, compared with the CD, a 50 % reduction in lipid and 91 % carbohydrate content and wheat gluten as the major protein source instead of the 100 % casein used in the CD. The compositions of the diets are shown in Table 1.

The rats were weighed three times weekly, and food intake was measured daily during the study. Litter weight was also measured three times weekly.

Measurement of metabolites

On day 14 of lactation, the uptake of substrates by the mammary gland was calculated by subtracting substrate levels in the venous plasma of the inguinal mammary glands from those in the aortic plasma. The metabolites determined by the venous plasma of the inguinal mammary glands from the mammary vein, from which 0.5 ml blood was obtained with a heparinised syringe. The abdominal cavity was opened, and a 0.5 ml blood sample was obtained from the aorta. Subsequently, the retroperitoneal and parametrial adipose tissues were excised, weighed and compared with those of non-lactating rats. The animals were killed by injecting more pentobarbital into the abdominal cavity. The blood samples were centrifuged, and plasma was kept at −20°C until assay.

Qualitative determinations of metabolites in the urine were carried out using Combur-test strips (Boehringer-Mannheim, Toluca, Mexico).

Calorimetry

Indirect calorimetry studies were made in the same six CD and six LED fed or 15-h-fasted dams on days 1 and 2, 13 and 14, and 20 and 21 of lactation, always starting with the fed condition, by using an Oxymax System (Columbus Instruments International Corporation, Ohio, OH, USA). The energy expenditure of six CD and six LED non-lactating rats was also measured. Non-lactating rats received the same diet for the same number of days as the dams.

Our apparatus used open-circuit airflows through two airight, respiration chambers where the animals were housed. The flow rate was established by the investigator according to the animal’s body weight and expected metabolic rate. The composition of the outlet air was measured by gas sensors to compute V02 and CO2 production, and was compared with the sequential analysis of the inlet air composition. RQ and energy expenditure were also computed by the system. The relative locomotor activities of the rats were also estimated as the number of pulses per time interval by connecting two Opto-Varimex Mini devices (Columbus Instruments) to the system. The collected data were stored and could be printed within a designated data file.

Dams were separated from their pups and were put in the chambers at about 11.00 hours and 12.00 hours. The O2 and CO2 sensors were calibrated each day after 90 min warming.

### Table 1. Composition of the diets

<table>
<thead>
<tr>
<th>Ingredients*</th>
<th>Control diet g/kg approx. kJ/g</th>
<th>Low-energy diet g/kg approx. kJ/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>3.25</td>
<td>44.5</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>–</td>
<td>138-14</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>5.44</td>
<td>295.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>6.8</td>
<td>295.5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>6.8</td>
<td>23.5</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.1</td>
<td>0.67</td>
</tr>
<tr>
<td>Choline</td>
<td>0.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin mix†</td>
<td>0.1</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mix†</td>
<td>0.3</td>
<td>35.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>0.5</td>
<td>159.17</td>
</tr>
<tr>
<td>Total</td>
<td>10.00</td>
<td>16.11</td>
</tr>
</tbody>
</table>

### Macronutrient composition (%)

- **Protein:** 20.7%
- **Fat:** 18.6%
- **Carbohydrate:** 67.6%
- **Digestible energy:** 16.1 kJ/g

*The ingredients used in the preparation of diets were acquired from Sigma-Aldrich, Arancia, Harlan-Tecklad and Universal Flavors of Mexico.
†Energy content as protein digestibility.
‡Vitamin (Tecklad 40060) and mineral (Rogers-Herper, Tecklad 170760) mixtures met the American Institute of Nutrition AIN-93G recommendations for rodent diets.
up the system. The experiments started after 30 min, during which the rats became calm as estimated by recording at least four periods of stable $V_{\text{O2}}$ and almost zero motor activity. Data for subsequent intervals were then obtained over 3·5 h or more, and the data for intervals with zero motor activity were averaged. The REE was calculated as $\text{kJ/kg}^{0.75}$ per hour. The locomotor activity was summed for the entire period and calculated as counts/min.

Statistics

The data are reported as means with their standard errors. Differences in food intake, body weight and plasma metabolite level and uptake through the mammary gland between the two groups were analysed by a two-tailed Student’s $t$ test, between fat pad weight by one-way ANOVA, and between REE and RQ by two-way ANOVA followed by a post hoc Bonferroni test. The level of significance was set at $P<0.05$ for all comparisons.

Results

Gestation

The mean body weight of the two groups of rats showed no significant differences during the first 2 weeks of gestation (Fig. 1). In the last week of gestation, the LED group gained less weight than the controls, weighing 406 (SE 13) g v. 440 (SE 10) g ($P=0.064$) the day before parturition. On the day of parturition, all animals had lost about 125 g. Food intake during gestation was slightly greater in LED rats (Table 2).

Lactation

From lactation day 9 on, LED rats weighed significantly less than CD rats ($P<0.05$). During lactation, all rats increased their daily intake reaching, at the end of the period, an increase of approximately 200% times greater than that of the adaptation period. No statistical differences were seen between the food intake of the two groups (data not shown). However, because the LED contained 17.5% less energy/g than the CD, the energy consumption of the LED rats was lower than that of the control group during the last 2 weeks of lactation ($P<0.05$; Fig. 2). Table 2 shows the food and energy intake of the two groups of rats during each experimental period. LED rats consumed more food before lactation than the CD controls but not enough to compensate the energy deficit during gestation and even less during lactation.

The diet did not affect the number of pups per litter (10 (SE 1) for LED v. 11 (SE 1) for CD) or their body weight at birth (7.2 (SE 0.5) g per pup for LED v. 7.8 (SE 0.4) g for CD). The lower increase in body weight in the LED pups was significant ($P<0.002$) after day 9 of lactation (Fig. 3).

Milk production and macronutrient composition in the CD and LED groups of rats were measured in another experiment (M Cervantes and H Hernández-Montes, unpublished results) between days 12 and 14 of lactation. Milk production in 24 h and protein and lactose content were 7·4%, 47% and 28% lower, respectively, in LED dams ($P<0.002$). No significant difference was shown in lipid content. As a result, the total energy in milk was 14% lower in the LED rats ($P<0.05$).

The fasted dams from both groups showed an increase in REE on days 14 and 21, but the value was lower in LED rats than in the CD group at the end of lactation (Fig. 4(A)). The same trends were shown when the rats were fed, but in each group and at the same time interval, REE values were higher when fed than when fasting (Fig. 4(B)). The differences between the two groups of rats were not caused by a greater relative activity of the CD rats, which actually showed fewer counts per min than the LED rats both when fasted and when fed (data not shown).

The RQ values of fasting rats at the beginning of lactation were significantly lower than those of non-lactating rats, whereas CD rats reached values similar to those of non-lactating controls, over 0·7, on day 14. The LED rats showed this increase only at the end of lactation. Ketonuria was present in all groups (Fig. 5(A)). When the rats were fed, RQ on day 1 was significantly lower in dams than in non-lactating rats but reached values over 1·0 on days 13 and 20 in both groups of dams (Fig. 5(B)); non-lactating fed rats had an RQ of 0·84–0·90. Ketonuria was present at each time interval only in dams, suggesting that it is a characteristic of lactation.

The weights of the parametrial and retroperitoneal (Table 3) adipose-fat pads were lower in dams than in non-lactating rats receiving the same diet during the same number of weeks. Only the parametrial pads of the LED dams weighed less than those of the CD rats.

Table 4 shows the plasma levels and arteriovenous differences through the mammary gland for the four substrates measured on lactation day 14. The glands of LED rats took up more D-3-hydroxybutyrate ($P<0.01$) and less glucose, lactate and triacylglycerol than the glands of CD rats ($P<0.001$).

Discussion

The rats fed the LED consumed the same amount of food as the control group during the 8 weeks of the experiment, thus resulting in a lower energy intake. The difference did not become statistically significant until the last 2 weeks of lactation. During lactation, the difference in mean energy intake and body weight between the two groups did not
become significant until the end of the first week. During the last week, LED lactating dams were unable to compensate for the energy loss by increasing their food intake as they did when they were pregnant. This might have been caused in part because energy requirements are much larger during lactation. In reality, the LED rats ingested less than the CD rats (309.7 (SE 17.0) g; v 362.9 (SE 14.3) g; P<0.05), showing that the difference in energy intake was not caused by the difficulty in managing the extra volume of food necessary to match the calories ingested by CD rats.

Taylor et al. (1986) offered diets of different energy value and different protein and fat contents between days 7 and 14 of lactation. They reported that energy restriction reduced body weight and body water, and affected body energy content, more than did the amount of fat or protein in the food. The results of Roberts & Coward (1984) showed that lactating rats fed ad libitum expended less energy in activity and maintenance, whereas in lactating rats fed restricted amounts of food, the reduction in energy expenditure could not prevent a negative energy balance. This suggests that the metabolic adjustments imposed by lactation represent a specific energy burden on rat dams. This may be related to the reduced growth of our LED pups after day 7, possibly caused by a lower milk yield than occurred in the CD rats. The milk yield is normally increased during the second week of lactation (Williamson et al. 1984). A low-lipid diet has been noted to cause a lower milk volume and energy output, and lighter pups, from day 6 onwards (Del Prado et al. 1997). On day 2 of lactation, non-lactating and lactating rats on both diets, both fasting and fed, showed similar energy expenditures. On days 14 and 21, the energy expenditure increased significantly in both groups but more in the CD group, in both conditions. This shows that, in spite of the reported decrease in thermogenic activity of the brown adipose tissue and in diet-induced thermogenesis (Trayhurn, 1989), the burden of lactation causes a rise in metabolic expenditure. The lower rise in LED rats suggests a metabolic ‘attempt’ to reduce energy cost to lose fewer endogenous reserves. This could diminish milk production and be the cause of the pups’ lower body weight gain. As expressed by Leon & Woodside (1983), ’rat dams apparently do not monitor and defend a maximal pup growth rate. Rather dams seem to continue to defend their own homeostasis... allowing the pups to grow and survive under a wide variety of circumstances’.

On all measurement days and with both diets, except for fed, non-lactating animals, the rats showed ketonuria. Fed dams on day 1 showed prevalent fat oxidation, and on days 13 and 20 both dietary groups had an RQ of over 1.0, which indicates that the lipogenic rate surpassed fat oxidation (Hellerstein et al. 1996). The concentration of ketone bodies in the blood represents the balance between their production by the liver and their utilisation by the peripheral tissues (Robinson & Williamson, 1980). The presence of ketone bodies in the urine shows that the rate of hepatic production of these compounds from fatty acids exceeded their uptake. This was even more evident in the fasting condition when non-lactating rats also showed ketonuria.

Fasting LED rats showed an RQ of under 0.7 on days 2 and 14, indicating an overproduction of these compounds from fatty acids. This is because ketogenesis uses O2 but does not produce CO2 (e.g. the production of four molecules of acetocacetate from one molecule of palmitate uses seven molecules of O2 without producing CO2) and results in an RQ of 0 (Simonson & DeFronzo, 1990). The O2 consumption and RQ were related to energy intake. During the first week of lactation, the energy intake of both groups was greater than that of non-lactating rats, whereas on day 1 there was no difference in O2 consumption, although fed dams showed prevalent fat oxidation (a low RQ) and an overproduction of ketone bodies. This changed dramatically during the second week, the energy intake of dams being much higher than that of non-lactating rats, and also being higher in CD than in LED rats. These changes in energy intake, without changes in body weight, were accompanied by a similar increase in metabolism in both groups of dams when fed. The high RQ, indicating a relatively elevated rate of lipogenesis, was similar in both groups of dams. That LED dams ingested fewer calories during this period of lactation than CD dams indicates a larger participation of endogenous sources in the high O2 consumption and the high RQ, which accounts for the rats’ lower body and fat-pad weight.

### Table 2. Weekly food and energy intake of control diet and low-energy diet rats during each experimental period

<table>
<thead>
<tr>
<th></th>
<th>Control diet rats</th>
<th>Low-energy diet rats</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Adaptation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed (g)</td>
<td>111</td>
<td>3</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>1788</td>
<td>48</td>
</tr>
<tr>
<td>Gestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed (g)</td>
<td>133</td>
<td>4</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>2142</td>
<td>64</td>
</tr>
<tr>
<td>Adaptation + Gestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed (g)</td>
<td>124</td>
<td>3</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>1997</td>
<td>48</td>
</tr>
<tr>
<td>Lactation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed (g)</td>
<td>300</td>
<td>12</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>4833</td>
<td>193</td>
</tr>
<tr>
<td>All experiments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed (g)</td>
<td>190</td>
<td>5</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>3060</td>
<td>81</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those of the control group: *P<0.05, **P<0.01.

Fig. 2. Energy intake for each week of dams fed a low-energy diet (●) or a control diet (○) during adaptation, pregnancy and lactation. Values are means with their standard errors shown by vertical bars for six rats per group. *P< 0.05 by Student’s t test.
During the last week, the only difference from the second week was seen in the higher REE of the fed CD dams without any change in energy intake or RQ. Because only REE was measured, this result could mean that the difference was caused by an increase in prandial thermogenesis, in milk synthesis or in both.

After fasting overnight, the dams had to synthesise milk out of their own reserves. Both lactose and lipid synthesis in the mammary gland have glucose as their common precursor (Williamson et al. 1984). After 15 h of fasting, lipogenesis in the mammary gland, 500% greater than in the liver, is strongly inhibited, at least in part because of the decrease in plasma insulin concentration (Robinson et al. 1978) and the 70% decrease in blood flow (Vinya et al. 1987). This might explain the lower energy expenditure of dams in this condition. Non-lactating rats showed the same metabolic saving when fasting, confirming previous results (Villanueva et al. 2002).

Ketogenesis is higher during early lactation than in non-lactating rats but decreases at peak lactation (Whitelaw & Williamson, 1977). These results agree with those of the present study showing the RQ on day 2 to be less than 0.7 in fasting and less than 0.75 in fed rats. The authors cited...
interpreted these temporal differences as being caused by the activation during lactation of the synthesis of fatty acids and triacylglycerols, a process that increases RQ, and a decrease in the \( \beta \)-oxidation of fatty acids, which decreases RQ.

The ketone bodies can be used for lipogenesis in the mammary gland (Robinson & Williamson, 1978). Our results on day 14 show that the mammary glands of the LED group took up significantly more D-3-hydroxybutyrate and less glucose, lactate and triacylglycerols than those of the control group. Ketone bodies are the result of lipid catabolism, and it should be preferable for rats consuming less protein and carbohydrate to use lipid byproducts instead of glucose or lactate for synthesising milk lipids. After 20 h starvation, the uptake of all substrates is decreased by at least 90% except for ketone bodies, for which the decrease is 65% (Williamson, 1990). In the present study, the high RQ in both groups of fed dams on the last week of lactation may have been caused by a large participation of ketone bodies in the synthesis of milk lipids, larger still for the dams of the LED group. Ketone bodies may serve as fuel for the brain, sparing glucose, which is a better lipogenic precursor because in the pentose phosphate pathway it reduces NADP\(^+\) to NADPH, a key factor in lipid biosynthesis.

The LED was made to resemble the rural diets in developing countries. Although its deficiencies were a slightly lower energy content, and fibre and wheat gluten as the main protein source, these differences from the CD were sufficient to alter the REE and diminish the maternal fat pads and the body-weight gain of the pups. This suggests that similar diets in women during pregnancy and lactation may have some detrimental effects on their infants.

In conclusion, female rats fed a low-energy, low-quality protein diet before pregnancy and during gestation and lactation adjusted their food intake to obtain the same amount of energy as rats fed a CD, except for the last 2 weeks of lactation, resulting in a lower body weight of both dams and pups during this phase. Indirect calorimetry used after an overnight fast showed the prevalence of lipid oxidation, suggesting an excess of ketone-body production in both groups of rats. The same test used on fed dams showed that, in the last week, both groups had an increase in metabolism and RQ, implying a high lipogenic rate. At the start of the third week of lactation, the mammary glands of dams on the restricted diet took up more ketone bodies and less glucose than the rats on the CD.

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