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A Sucrose-Rich Diet during Pregnancy Causes a Similar Response in Sprague-Dawley and Wistar Rats

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Key Words
Sucrose-rich diet · Pregnancy · Wistar rats · Sprague-Dawley rats · Triglycerides · Lipoprotein lipase

Abstract
Background/Aims: In order to determine whether the response to a sucrose-rich diet (SRD) during pregnancy in the rat varies depending on the strain, the responsiveness to a SRD (63 g sucrose/100 g) during pregnancy in Wistar and Sprague-Dawley rats was studied. Methods: One group of rats of each strain was fed the SRD, whereas another group received the same diet except that sucrose was replaced by an equal amount of cornstarch. Half of the rats were mated, and all animals were studied 20 days later. Results: Initial body weight did not differ among groups, but final body weight of pregnant Wistar rats was lower than in Sprague-Dawley, and this difference corresponded to a decrease in fetal body weight in the former. Feeding a SRD did not modify pregnancy outcome in either rat strain. Plasma triglycerides increased with a SRD, although this effect was milder in Wistar pregnant rats than in the other groups. Adipose tissue lipoprotein lipase activity was lower in pregnant than in virgin rats, but no differences were found as result of either diet or rat strain. Liver triglyceride concentration increased in virgin rats fed SRD, the effect being greater in Sprague-Dawley than in Wistar rats. Conclusion: Differences in the response to a SRD in pregnant and virgin rats do not depend on the strain of rats used.

Introduction
Prolonged fructose or sucrose feeding in healthy or hyperlipidemic men [1–5] and in the normal rat [6–9] causes hypertriglyceridemia and insulin resistance. Since both hypertriglyceridemia and insulin resistance also occur during pregnancy both in humans [10, 11] and rats [12–14], many studies in the rat have been addressed to determine whether gestational hypertriglyceridemia and pregnancy outcome are modified by feeding a sucrose-rich diet (SRD). The proportional effect of a SRD enhancing the already augmented triglyceride plasma levels in pregnant rats is similar to that seen in nonpregnant animals, although the mechanism may differ. Whereas this effect is followed by an accumulation of liver triglycerides in nonpregnant rats, this is however not the case in preg-
Differences in pregnancy outcome have been previously reported in rats fed a SRD. Some authors have reported decreased litter size and fetal body weight [15], whereas others did not find any effect on this variable [16, 18]; however, an enhanced number of pups per litter but decreased pups' weight have been also reported [19]. The reason for this difference is yet unknown, but it could reside in the strain of the animals, since when using similar experimental conditions, a decreased fetal weight in late pregnant Wistar rats fed SRD was detected [15], whereas this change was not found with Sprague-Dawley rats [16]. Thus, the present work was carried out to determine the comparative responsiveness to a SRD during pregnancy between Wistar and Sprague-Dawley rats, which were studied in parallel. Data show that although minor differences in the hypertriglyceridemic responsiveness between both strains were detected, no difference was found as far as pregnancy outcome was concerned, and therefore, other reasons have to be claimed to explain the reported differences.

**Material and Methods**

**Animals**

Female Wistar or Sprague-Dawley rats from our animal quarters were initially fed a nonpurified diet (B&K Universal, Barcelona, Spain) and housed under controlled light and temperature conditions (12-hour light:dark cycle; 22–23°C). The experimental protocol was approved by the Animal Research Committee of the University San Pablo-CEU in Madrid, Spain. Rats of either strain weighing 150–160 g were divided into two groups. In one group, rats were fed a semisynthetic SRD (63 g sucrose/100 g), and in the other group (control), rats were fed the same diet, except that the sucrose was replaced by cornstarch (CD; 63 g/100 g). The composition of diets and the duration of treatments have been previously reported [15, 16]. Both diets were isocaloric (15.28 kJ/g), and rats always had free access to food and tap water. Half of the rats from each group were mated with males of the same strain 5 days after the onset of diets, and the day that spermatozoids appeared in vaginal smears was considered day 0 of gestation. The other half of the rats were kept virgin and studied in parallel. Rats of the same strain and condition were housed in collective cages (4/cage). Daily food intake was only roughly estimated because a considerable loss of food was detected; however, no apparent difference was found in food intake between rats of either strain or between those fed SRD and CD (data not shown).

Pregnant and virgin rats were decapitated between 09:00 and 10:00 h at day 25 on the corresponding diet, which in the case of pregnant rats corresponded to day 20 of pregnancy. Trunk blood was collected into ice-chilled tubes containing 1 g/l of Na2-EDTA. The two uterine horns were immediately dissected and weighed with their content to obtain the conceptus weight. Liver and lumbar adipose tissue were quickly removed and placed into liquid nitrogen before freezing at –80°C until analysis. Fetuses were weighed and decapitated as indicated above, and blood from all fetuses coming from the same dam was pooled and processed in parallel to that of the adults. Plasma was separated by centrifugation at 1,500 g for 15 min at 4°C, and kept frozen until processed.

**Analytical Methods**

Triglyceride concentration in plasma was measured by an enzymatic method using a commercial kit (No. B-7648, Menarini Diagnostic, Florence, Italy). Portions of frozen liver were extracted with chloroform-methanol (2:1) [20]. Triglycerides were quantified following an image analysis after separation by one-dimensional thin-layer chromatography [21] using the GS-700 BioImage TLC scanner of Bio-Rad (Hercules, Calif., USA). Optical density of the spots was compared to standards on each plate and curves were drawn from second-order least-square regression equations on the standards. Lipoprotein lipase activity was measured in lumbar adipose tissue as previously described [22] and was expressed as pkat (picomoles of substrate transformed per second) per weight of fresh tissue.

**Statistics**

Data were expressed as means ± SEM. Data were log transformed since it was necessary to achieve equal variance among means. Statistical significance between two groups was determined by the Student’s t test or, when appropriate, data were subjected to two-way ANOVA with diet and pregnancy as the main effects for each strain of rats, using a computer software package (Systat Version 5.03, Wilkinson, Evanston, Ill., USA). When differences between treatments were significant (p < 0.05), means were tested by Tukey’s test, and linear regressions were calculated by the least-square method [23].

**Results**

At the beginning of the experiments, there were no differences in body weight of the rats (table 1), whereas at day 25 of feeding the corresponding diet (day 20 of pregnancy) all groups of virgin rats had a similar body weight while pregnant Wistar rats weighed less than Sprague-Dawley rats; however, no difference was found as result of the different type of diet fed. As also shown in table 1, the difference in body weight of pregnant rats from either strain corresponded to the conceptus weighed which was lower in Wistar than in Sprague-Dawley rats, whereas maternal own structures weight (net body weight, free of conceptus) did not differ between strains, and values were always higher than the body weight of virgins of the same strain, as expected. Within the conceptus, fetal weight was the major responsible for the lower weight in Wistar than in Sprague-Dawley rats, which was significantly lower in the former, and no statistically significant differences.
Table 1. Effect of feeding a sucrose-rich (SRD) or control (CD) diet in virgin (V) and pregnant (P) Sprague-Dawley and Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>Initial body weight, g</th>
<th>Final body weight, g</th>
<th>Conceptus weight, g</th>
<th>Net maternal weight (free of conceptus), g</th>
<th>Fetuses/litter</th>
<th>Average fetus body weight, g</th>
<th>Average placental weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sprague-Dawley</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>V, CD</td>
<td>181 ± 3a</td>
<td>250 ± 7a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V, SRD</td>
<td>188 ± 5a</td>
<td>257 ± 6a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P, CD</td>
<td>179 ± 4b</td>
<td>359 ± 9b</td>
<td>80.5 ± 2.6</td>
<td>278 ± 8</td>
<td>14.1 ± 0.9</td>
<td>4.3 ± 0.1</td>
<td>0.53 ± 0.04</td>
</tr>
<tr>
<td>P, SRD</td>
<td>188 ± 4a</td>
<td>370 ± 7b</td>
<td>78.0 ± 5.2</td>
<td>287 ± 4</td>
<td>13.8 ± 0.8</td>
<td>3.9 ± 0.1</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td><strong>Wistar</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V, CD</td>
<td>182 ± 3a</td>
<td>248 ± 4a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V, SRD</td>
<td>186 ± 2a</td>
<td>249 ± 4a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P, CD</td>
<td>189 ± 5b***</td>
<td>329 ± 5b***</td>
<td>62.9 ± 2.2***</td>
<td>267 ± 4</td>
<td>12.5 ± 0.5</td>
<td>3.7 ± 0.1***</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>P, SRD</td>
<td>189 ± 6b</td>
<td>325 ± 11b***</td>
<td>61.6 ± 2.2**</td>
<td>263 ± 9</td>
<td>12.6 ± 0.7</td>
<td>3.6 ± 0.1**</td>
<td>0.48 ± 0.02</td>
</tr>
</tbody>
</table>

1 Values are expressed as means ± SEM, n = 7–13. Tukey’s test was used to determine differences between groups after ANOVA. Different superscripts in a row indicate significant differences (p < 0.05) among the four groups in the same strain. Statistical comparisons between Wistar and Sprague-Dawley for the same variable and experimental group is shown by asterisks (* = p < 0.05, ** = p < 0.01, *** = p < 0.001). Statistical comparisons between SRD and CD within each strain were not significant for any of the studied variables (p > 0.05).

were found in the number of fetus per rat or in placental weights, despite that mean values always appeared slightly lower in Wistar than in Sprague-Dawley rats (table 1). Feeding a SRD did not modify either of these variables in Wistar or Sprague-Dawley rats (table 1). A SRD in virgin rats, either Wistar or Sprague-Dawley, increased plasma triglyceride concentration (table 2). In pregnant rats, the SRD also increased plasma triglycerides, although this increase was not as marked as in virgin rats. Under the CD, plasma triglycerides were higher in pregnant than in virgin rats, although this difference was statistically significant only in Sprague-Dawley rats (table 2). Lipoprotein lipase activity (LPL) in lumbar adipose tissue, in Sprague-Dawley or Wistar rats fed CD, was lower in pregnant than in virgin rats, and whereas feeding the SRD always decreased this variable, the change was mild and not statistically significant, and neither of these changes differed between the two strains studied (table 2). Neither diet affected plasma triglyceride concentration in fetuses of Sprague-Dawley mothers, whereas in fetuses of Wistar mothers fed SRD, plasma triglycerides were higher than in any other group (table 2).

Liver weight always appeared higher in rats fed SRD than CD, independently of the strain and of whether they were virgin or pregnant, values in the latter being even higher than in the former (table 3). Liver triglyceride concentration greatly increased in virgin Sprague-Dawley rats fed SRD, and this effect completely disappeared when

Table 2. Plasma triglycerides and lumbar adipose tissue lipoprotein lipase activity (LPL) in pregnant (P) and virgin (V) Sprague-Dawley and Wistar rats fed sucrose-rich (SRD) or control (CD) diets

<table>
<thead>
<tr>
<th></th>
<th>Plasma triglycerides mmol/l</th>
<th>Adipose tissue LPL activity pkat/g wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sprague-Dawley</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V, CD</td>
<td>112 ± 47a</td>
<td>3.002 ± 274a</td>
</tr>
<tr>
<td>V, SRD</td>
<td>326 ± 72a, c</td>
<td>2.095 ± 304b</td>
</tr>
<tr>
<td>P, CD</td>
<td>190 ± 23b</td>
<td>1.691 ± 353b</td>
</tr>
<tr>
<td>P, SRD</td>
<td>360 ± 27b</td>
<td>1.411 ± 284b</td>
</tr>
<tr>
<td>Fetuses, CD</td>
<td>58 ± 3</td>
<td></td>
</tr>
<tr>
<td>Fetuses, SRD</td>
<td>60 ± 4</td>
<td></td>
</tr>
<tr>
<td><strong>Wistar</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V, CD</td>
<td>133 ± 8a</td>
<td>2.935 ± 226a</td>
</tr>
<tr>
<td>V, SRD</td>
<td>329 ± 43b</td>
<td>2.096 ± 351b</td>
</tr>
<tr>
<td>P, CD</td>
<td>162 ± 16c</td>
<td>1.522 ± 224b</td>
</tr>
<tr>
<td>P, SRD</td>
<td>248 ± 32a, b, *</td>
<td>1.150 ± 237b</td>
</tr>
<tr>
<td>Fetuses, CD</td>
<td>64 ± 4</td>
<td></td>
</tr>
<tr>
<td>Fetuses, SRD</td>
<td>78 ± 4* x</td>
<td></td>
</tr>
</tbody>
</table>

1 Presentation of the data and statistical comparisons as in table 1. x Denotes significant differences among SRD and CD in fetuses in the same strain.
animals were studied under pregnant conditions (table 3). Liver triglyceride concentration was higher in virgin Wistar rats fed CD than in Sprague-Dawley rats, but the effect of the SRD was not as marked, and means were not statistically different (table 3). As in Sprague-Dawley rats, the SRD in pregnant Wistar did not modify liver triglyceride concentrations, and values seen in pregnant rats of either strain fed SRD were always significantly lower than those of virgin rats under the same diet (table 3).

Discussion

Results of this study show that feeding SRD during pregnancy in the rat does not modify fetal number and weight, independently of whether the animals were Sprague-Dawley or Wistar. Findings in the literature are very variable, ranging from those showing a reduction in fetal weight [15, 19] to an increase in fetal number [19] or even no effect in either variable [18]. Since the strain of rats used here and the time of treatment and diet composition are the same as those previously used [15, 16], the present results allow to conclude that the reported differences in pregnancy outcome in rats fed SRD are not a consequence of the rat strain used in the study. The present results do not allow to determine the reason for such reported variable responsiveness to the SRD. However, it could not be attributed to the amount of sucrose used, since in one case its proportion in the diet was lower (50%) [19], in other, equal (63%) [15], and in another, higher (70.6%) [18], and while the two first studies report decreased fetal weight, in the latter, no effect was found, as in the present study. It may be then claimed that uncontrolled environmental condition differences would modify the sensitivity of the animals to the same high sucrose intake stimulus.

Despite the lack of response to the SRD on fetal weight or number, the present results confirm its metabolic effects, pointing out some different responses depending on the strain of rat used. One of these differences is the increase in maternal body weight with pregnancy, which is lower in Wistar than in Sprague-Dawley rats, the difference being mainly due to the lighter fetal body weight in the former. Rats from either group used in the present study had the same body weight at the onset of the experiment, but it is known that adult age-matched Wistar rats weigh less than Sprague-Dawley rats [24–26]. Thus, the Wistar rats used here were 2 weeks older than the Sprague-Dawley rats, a difference that could be responsible for the lower body weight gain of the former throughout the experiment. When planning the experiment, we were aware of this limitation in benefit of a similar initial body weight of the animals, since there were reports in the literature on the response to SRDs by Wistar and Sprague-Dawley rats, where groups were matched by weight rather than by age [27].

According to previous experiments in our laboratory [15] and others’ [7] carried out with Wistar rats, feeding a SRD for 25 days causes hypertriglyceridemia in both Wistar and Sprague-Dawley rats, the effect being probably a consequence of an enhanced liver triglyceride production and a decreased extrahepatic clearance [6, 15]. Consistent with previous reports [15, 28], plasma triglyceride levels increased in pregnant rats, although the response in Wistar rats was smaller than in Sprague-Dawley rats. Since the additive effect of enhanced liver production of triglycerides and decreased triglyceride removal as a consequence of decreased adipose tissue LPL activity seems to be responsible for the enhanced triglycerides during late pregnancy [15], these changes may be expected to take place more moderately in pregnant Wistar than in Sprague-Dawley rats. In this study, LPL activity in lumbar adipose tissue was lower in pregnant than in virgin rats, but feeding the SRD did not further decrease this variable, and no differences could be found between both rat strains. Thus, although direct experiments are needed, the smaller hypertriglyceridemic response to the sum of pregnancy and SRD feeding in Wistar rats may be a consequence of a smaller effect enhancing liver triglyceride production.
Sucrose-Rich Diet in Pregnant Rats

Special attention should be given to liver triglyceride concentration, which was enhanced in virgin rats fed SRD in this study, although the effect was greater in Sprague-Dawley than in Wistar rats, which may be also related to the greater plasma triglyceride concentration increase found in the former. Although this effect could be a consequence of fructose enhancing liver triglyceride synthesis, it also may result from a limited capability of the liver to secrete triglycerides into the circulation. In fact, we have seen here that liver triglyceride storage does not occur in pregnant rats from either strain fed SRD, since pregnant rats have a higher ability to secrete triglycerides than virgin rats [17]. The greater amount of liver triglycerides of virgin Wistar rats fed CD as compared to Sprague-Dawley, together with the lower response of this variable to the SRD stimulus, could well be a consequence of the greater age of the former, since aging is known to enhance hepatic lipid accumulation [29], thus decreasing the sensitivity to a further increase as result of the dietary stimulus.

The specific increase in plasma triglycerides found in fetuses from Wistar rats fed SRD, but not from Sprague-Dawley rats, seems to be a consequence of a greater triglyceride synthesis by the fetus itself rather than the result of their enhanced transfer throughout the placenta. On the one hand, we know that the rat placenta is unable to secrete triglycerides into the circulation. In fact, we have seen here that liver triglyceride storage does not occur in pregnant rats from either strain fed SRD, since pregnant rats have a higher ability to secrete triglycerides than virgin rats [17]. The greater amount of liver triglycerides of virgin Wistar rats fed CD as compared to Sprague-Dawley, together with the lower response of this variable to the SRD stimulus, could well be a consequence of the greater age of the former, since aging is known to enhance hepatic lipid accumulation [29], thus decreasing the sensitivity to a further increase as result of the dietary stimulus.

In summary, the present results show that the reported differences in pregnancy outcome in rats fed SRD are not just due to differences in the strain of rats used. Besides, whereas feeding a SRD enhances both plasma and liver triglyceride concentrations in virgin rats, this response is smaller in pregnant rats.

Acknowledgements

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