Some Changes in the Triglyceride Metabolism of Rats on High Fructose or Glucose Diets

By Y. Maruhama and I. Macdonald

Mature male and female rats were given diets low in fat and high in glucose or fructose. At weekly intervals one animal of each sex was killed. Two hours prior to death each animal was given \(^{14}\text{C}\) glucose or \(^{14}\text{C}\) fructose intragastrically. The following were observed: (1) The specific activity of the glycerol moiety of liver and plasma triglyceride was higher than that in the fatty acid moiety. (2) The specific activity in the adipose triglyceride was greater after glucose than after fructose feeding, but the reverse was found in the plasma. (3) The differences found between the responses to fructose and glucose did not, however, persist.

There is evidence in man that the replacement of dietary glucose by fructose leads to an increase in the triglyceride concentration of serum.\(^1\) In experiments in rats it was found that on a diet containing fructose as the carbohydrate, the greater the length of time on the diet (up to 8 wk) the greater the incorporation of \(^{14}\text{C}\) fructose into both liver and serum triglycerides, whereas with glucose no such change was found.\(^2\) After ingestion of \(^{14}\text{C}\) fructose or \(^{14}\text{C}\) glucose by the fasting rat, the levels of the radioactivity in the triglyceride in liver, serum, and adipose tissue was mainly in the glycerol moiety of the triglyceride.\(^3\) It was therefore considered of interest to learn whether this pattern changes after several weeks on a diet containing large amounts of either fructose or glucose. To this end, rats were given after varying intervals of time on a high fructose or glucose diet, \(^{14}\text{C}\) fructose or \(^{14}\text{C}\) glucose intragastrically. The radioactivity was then measured in the fatty acid and glycerol moieties of liver, serum, and adipose tissue triglycerides.

MATERIALS AND METHODS

Mature male and female rats, weighing 180–220 g, previously fed on a rat cube diet were given, ad libitum, either a high-fructose or high-glucose diet containing no fat (Table 1). Experiments on animals on a control diet were not carried out since the purpose of the study was to compare the responses to fructose and glucose.

Intragastric Instillation

Each week, for 11 wk, after a 6-hr fast, one rat of each sex was given, by stomach tube, 5 \(\mu\text{Ci}\) \(^{14}\text{C}\)-U-fructose or 5 \(\mu\text{Ci}\) \(^{14}\text{C}\)-U-glucose with 0.5 g of unlabeled fructose or glucose in 2 ml water. The carbohydrate given was the same as that in the animal's diet. Two hours after instillation of the labeled carbohydrate, blood was taken from the abdominal aorta by a heparinized syringe while the rat was under ether anesthesia. The entire liver and a sample of perirenal adipose tissue were removed.

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Table 1. Composition of Diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose monohydrate</td>
<td>79.2</td>
</tr>
<tr>
<td>or Fructose</td>
<td>72.0</td>
</tr>
<tr>
<td>Calcium caseinate</td>
<td>20.0</td>
</tr>
<tr>
<td>Dried yeast</td>
<td>4.5</td>
</tr>
<tr>
<td>Salt mix?</td>
<td>3.5</td>
</tr>
</tbody>
</table>

The following was mixed with or sprayed on food of each animal each week:

- Vitamin A: 500 IU
- Vitamin B₁: 0.1 mg
- Vitamin B₂: 0.04 mg
- Vitamin B₆: 0.05 mg
- Vitamin C: 5 mg
- Vitamin D: 40 IU
- Nicotinamide: 0.5 mg

Analytical Procedures

The total lipid from each animal was extracted with chloroform:methanol:4 the lipid fractions were then separated by thin layer chromatography:5 and the saponification of the triglyceride was carried out by the method of Rodbell (1964):6 Radioactivity was determined in a Beckmann automatic scintillation counter.

The specific activity (counts per minute) was adjusted to the body weight by the following procedure:

\[
\text{corrected counts (cpm)} = \frac{\text{actual counts} \times \text{body weight (g)}}{200}
\]

RESULTS

No significant difference in response was found between the sexes so the values have been combined.

Table 2. Body Weight, Amount of Liver Triglyceride, and Specific Activity of the Fatty Acid and Glycerol Moieties of Triglyceride

(Each Value Represents the Mean of One Male and One Female Animal)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
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</thead>
<tbody>
<tr>
<td>Body weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose diet</td>
<td>196</td>
<td>186</td>
<td>107</td>
<td>226</td>
<td>235</td>
<td>234</td>
<td>244</td>
<td>252</td>
<td>268</td>
<td>300</td>
<td>287</td>
<td>278</td>
</tr>
<tr>
<td>Glucose diet</td>
<td>186</td>
<td>177</td>
<td>208</td>
<td>209</td>
<td>220</td>
<td>246</td>
<td>270</td>
<td>232</td>
<td>256</td>
<td>262</td>
<td>278</td>
<td>270</td>
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</tbody>
</table>

Liver triglyceride

<table>
<thead>
<tr>
<th>Fructose diet amount (mg)</th>
<th>59</th>
<th>95</th>
<th>645</th>
<th>776</th>
<th>1103</th>
<th>1335</th>
<th>640</th>
<th>974</th>
<th>411</th>
<th>356</th>
<th>267</th>
<th>279</th>
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</thead>
<tbody>
<tr>
<td>Fatty acid moiety cpm/0.9 mg</td>
<td>5</td>
<td>431</td>
<td>54</td>
<td>18</td>
<td>159</td>
<td>117</td>
<td>114</td>
<td>40</td>
<td>132</td>
<td>72</td>
<td>31</td>
<td>93</td>
</tr>
<tr>
<td>Glycerol moiety cpm/0.1 mg</td>
<td>214</td>
<td>567</td>
<td>69</td>
<td>81</td>
<td>220</td>
<td>262</td>
<td>430</td>
<td>573</td>
<td>405</td>
<td>398</td>
<td>259</td>
<td>247</td>
</tr>
<tr>
<td>Glucose diet amount (mg)</td>
<td>54</td>
<td>42</td>
<td>64</td>
<td>71</td>
<td>214</td>
<td>186</td>
<td>217</td>
<td>163</td>
<td>113</td>
<td>186</td>
<td>133</td>
<td>119</td>
</tr>
<tr>
<td>Fatty acid moiety cpm/0.9 mg</td>
<td>14</td>
<td>89</td>
<td>68</td>
<td>184</td>
<td>157</td>
<td>115</td>
<td>69</td>
<td>135</td>
<td>76</td>
<td>45</td>
<td>46</td>
<td>28</td>
</tr>
<tr>
<td>Glycerol moiety cpm/0.1 mg</td>
<td>59</td>
<td>165</td>
<td>180</td>
<td>337</td>
<td>297</td>
<td>306</td>
<td>388</td>
<td>275</td>
<td>235</td>
<td>169</td>
<td>144</td>
<td>128</td>
</tr>
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</table>
Changes in Triglyceride Metabolism of Rats

Glycerol Moiety

**Fig. 1.** Amount of fructose or glucose incorporated in liver triglyceride fatty acid and glycerol 2 hr after intragastric instillation of $^{14}C$ fructose or glucose in rats after varying periods on high-fructose or high-glucose diets.

*Body Weight (Table 2)*

This showed an overall increase while the rats were on the diets.

*Liver Triglyceride*

**Amount (Table 2):** The amount of triglyceride in the liver rose to a maximum after 5 wk on the fructose diet with the level flattening out by wk 10 to a value 4–5 times greater than the prediet value. The glucose diet also increased the amount of liver triglyceride but to a much smaller extent than the fructose diet.

**Specific Activity (Table 2):** Fatty Acid Moiety. There was a tendency for this to be raised during both fructose and glucose diets.

**Glycerol Moiety.** No overall pattern of change was found while on the fructose diet, but there was a rise in the specific activity of this moiety of liver triglyceride while on the glucose diet.

**Amount Incorporated (Fig. 1):** This is expressed as specific activity + total triglyceride fatty acid or glycerol.

**Fatty Acid Moiety.** The fructose-fed animals showed a rise after 4–5 wk on the diet. The mean amount incorporated during the whole dietary period was $54,400 \text{ cpm, SE} \pm 17,800$.

In the glucose-fed animals a fairly constant level of incorporation was found (mean = $12,600 \text{ cpm SE} \pm 2900$). This level was significantly lower than that in the animals on the high-fructose diet ($p = 0.05–0.025$).

**Glycerol Moiety.** A peak of incorporation into this moiety was seen after 5 wk on the high-fructose diet with a return at 11 wk to a value similar to those found soon after the diet started. The mean overall incorporation during the diet was $157,000 \text{ cpm (SE} \pm 33,700)$. With the animals on the high-glucose diet, a peak value was seen after 6 wk on the diet, with a return by the end of the experimental diet to values similar to those seen at the commencement of the diet. The mean overall value...
was 33,000 cpm (SE ± 6900), a value significantly less than that found in the fructose series (p = 0.005–0.001).

**Plasma Triglyceride (Table 3)**

**Concentration:** With an increasing period of time on the fructose diet, the concentration of serum triglycerides (2 hr after intragastric fructose) rose to a maximum at 4 wk and then fell during the remainder of the time on the diet. While on the glucose diet no significant alteration in triglyceride level was seen.

**Specific Activity:** **Fatty Acid Moiety (Table 3).** On the fructose diet the specific activity in this fraction of plasma triglyceride tended to be higher than the prediet value. The values from the glucose-fed animals were also raised and were not significantly different from the values obtained from animals on the fructose diet.

**Glycerol Moiety (Table 3).** There was a threefold increase in this measurement between the fifth and ninth wk after the commencement of the fructose diet, with a return to near control values by the tenth wk. After 1 wk on the glucose diet, the specific activity in the glycerol moiety rose sharply and main-

| Table 3. Concentration of Plasma Triglyceride and Specific Activity of Fatty Acid and Glycerol Moieties of Plasma and Adipose Tissue Triglyceride (Each Value Represents the Mean of One Male and One Female Animal) |
|----------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                                  | 0    | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   |
| **Plasma Triglyceride**          |      |      |      |      |      |      |      |      |      |      |      |      |
| Fructose diet concentration      |      |      |      |      |      |      |      |      |      |      |      |      |
| (mg/100 ml)                      |      |      |      |      |      |      |      |      |      |      |      |      |
| Fatty acid moiety cpm/0.9 mg     | 32   | 53   | 85   | 108  | 130  | 101  | 99   | 83   | 90   | 72   | 67   | 61   |
| Glycerol moiety cpm/0.1 mg       | 291  | 443  | 161  | 444  | 409  | 888  | 838  | 1026 | 884  | 676  | 361  | 408  |
| Glucose diet concentration       |      |      |      |      |      |      |      |      |      |      |      |      |
| (mg/100 ml)                      |      |      |      |      |      |      |      |      |      |      |      |      |
| Fatty acid moiety cpm/0.9 mg     | 43   | 36   | 63   | 53   | 58   | 35   | 44   | 43   | 33   | 62   | 54   | 48   |
| Glycerol moiety cpm/0.1 mg       | 35   | 306  | 150  | 346  | 222  | 417  | 357  | 298  | 234  | 418  | 216  | 277  |
| **Adipose tissue**               |      |      |      |      |      |      |      |      |      |      |      |      |
| Fructose diet                     |      |      |      |      |      |      |      |      |      |      |      |      |
| Fatty acid moiety cpm/9 mg       | 4    | 1120 | 870  | 690  | 812  | 630  | 225  | 430  | 430  | 256  | 83   | 476  |
| Glycerol moiety cpm/1 mg         | 120  | 425  | 1021 | 284  | 1290 | 851  | 697  | 430  | 598  | 606  | 640  | 850  |
| Glucose diet                      |      |      |      |      |      |      |      |      |      |      |      |      |
| Fatty Acid moiety cpm/9 mg       | 70   | 350  | 1280 | 1655 | 2790 | 2870 | 482  | 1350 | 910  | 902  | 333  | 365  |
| Glycerol moiety cpm/1 mg         | 388  | 220  | 1012 | 2325 | 2668 | 2503 | 900  | 1053 | 1302 | 838  | 1280 | 853  |
Fig. 2. Amount of fructose or glucose incorporated in plasma (100 ml) triglyceride 2 hr after intragastric instillation of $^{14}$C fructose or glucose in rats after varying periods on high-fructose or high-glucose diets.

tained approximately the same level during weeks 5–9, the time when the fructose-fed animals also had high values.

Amount Incorporated (Fig. 2): This is expressed as the counts per minute $\times$ amount fatty acid or glycerol in the triglyceride in 100 ml plasma.

**Fatty Acid Moiety.** In the animals on the fructose diet, the amount of $^{14}$C fructose incorporated in plasma triglyceride tended to be higher than in the two control animals, with the greatest rise at 4 and 5 wk. The mean incorporation during the whole dietary period was 14,000 cpm (SE $\pm$ 3670).

With the glucose diet the amount of this carbohydrate incorporated in the fatty acid of plasma triglyceride remained fairly constant throughout the 11 wk on the diet. The overall mean values of incorporation ($\bar{x}$ = 4000 cpm, SE $\pm$ 810) were significantly less than those found for the fructose diet ($p = 0.025-0.01$).

**Glycerol Moiety.** A striking rise in the fructose incorporation in this part of plasma triglyceride was seen from 3–9 wk after starting the high-fructose diet (overall mean of whole dietary period—50,500 cpm SE $\pm$ 8200). The values found at the end of the dietary period were very similar to those at wk 1 and 2.

On the glucose diet the incorporation into plasma triglyceride glycerol remained constant (overall mean = 13,900, SE $\pm$ 1500). The difference between this value and that for fructose was highly significant ($p = <0.001$).

**Adipose Tissue Triglyceride Specific Activity:** **Fatty Acid Moiety** (Table 3). In the animals on the glucose diet the specific activity increased rapidly, reached a plateau at wk 4–5,
and returned to much lower levels by wk 10 on the diet. A less striking rise
was found in the animals on the fructose diet.

Glycerol Moiety (Table 3). The early rise seen in this measurement in the
glucose-fed animals, leveled off at a mean value about 2–3 times greater than
the prediet value. In the fructose-fed animals, the specific activity in the
glycerol moiety rose by a less marked degree than was found in the glucose-
fed animals.

With adipose tissue it was not possible to calculate the amount of radio-
activity incorporated.

Proportion of Radioactivity in Glycerol Moiety

When the radioactivity in the glycerol moiety of a triglyceride is expressed
as a percentage of the total activity in the triglyceride molecule, it is found
that in the plasma and liver, after either glucose or fructose feeding, the range
of mean values is 74%–80%. However, in the adipose tissue the comparable
values are 52% and 59% for glucose-fed and fructose-fed animals, respec-
tively, and these values are significantly lower than those in plasma and liver.

The greater activity found in the triglyceride glycerol may be due to the
fact that fatty acid is recycled whereas glycerol is not, though it is known that
giving glycerol to men raises the serum triglyceride concentration.

The endogenous glucose pool is far bigger than the fructose pool even after
long-term fructose feeding. However, the extent of the expansion in the tri-
glyceride precursor pool is considered to be approximately the same after a
single fructose or glucose instillation as the rate of conversion of endogenous
glucose to the liver triglyceride does not fall after the fructose instillation.

Thus, the higher specific activity in the liver and plasma triglyceride after
\(^{14}\text{C}\) fructose than after \(^{14}\text{C}\) glucose could not be due to an artifact of isotope
dilution.

Further evidence against the results being due to an artifact of isotope
dilution include the following.

(A) Since the amount of \(^{14}\text{C}\) given is the same for each carbohydrate, then
it would be reasonable to assume that the dilution of the isotope in the fluid
compartments is similar for each sugar, if the compartment were of the same
size for each sugar. In fact the fructose space is greater than the glucose
space and hence the dilution of the radiofructose is greater than that of the
radioglucose. If, in the synthesis of triglycerides, there was no distinction be-
tween glucose and fructose, then the radioactivity of the triglyceride thus
formed should be less after a \(^{14}\text{C}\) fructose diet than after an equal amount of
\(^{14}\text{C}\) glucose in the diet. This was not a consistent finding.

(B) If the greater amount of unlabeled glucose is responsible for the differ-
ence in radioactivity found in these experiments, then the incorporation of
\(^{14}\text{C}\) should always be greater with fructose than with glucose if the cells were
unable to distinguish between the sugars. This was not a consistent finding.

(C) When the overall incorporation of \(^{14}\text{C}\) into the glycerides of liver,
serum, and adipose tissue is compared, it is found that there is a greater in-
corporation from \(^{14}\text{C}\) glucose than from \(^{14}\text{C}\) fructose. This result would have
been unlikely if the greater amount of unlabeled glucose was entirely respon-
sible for the difference in the findings between $^{14}$C fructose and $^{14}$C glucose.

(D) The sinks for glucose metabolism may not be available to fructose to the same extent and this would reduce the amount of the glucose given that is available for triglyceride synthesis. Even if this were so it would mean that a difference exists between fructose and glucose given by mouth, as far as glyceride metabolism is concerned.

DISCUSSION

Unlike other work in this field, the results presented here are not from a fasting animal. Nevertheless, the rise in the plasma triglyceride concentration 2 hr after giving fructose to animals that had been an a fructose diet, and the absence of any rise under comparable circumstances while on a glucose diet, is of a pattern similar to that for the response of the triglyceride level in serum from fasting animals after fructose and glucose diets.

The specific activity of the fatty acids in the liver and plasma triglycerides show a correlation that is highly significant for both the glucose-fed animals ($y = 2.6 + 0x, \text{SE of } x = \pm 0.08$), and the fructose-fed rats ($y = 39 + 1.1x, \text{SE of } x = \pm 0.28$). This finding would be compatible with the view that the fatty acid moiety of the plasma triglyceride which arises from dietary fructose and glucose originates in the liver. There is a significant correlation between the specific activity of the fatty acid of adipose tissue triglyceride and that of the triglyceride fatty acid in the plasma ($y = 37 + 3.7x, \text{SE of } x = 1.17$) in the animals that were fed glucose. No such correlation was seen in the fructose-fed animals. This difference in response could be due to the levels of insulin after ingesting glucose being higher than after ingesting fructose, resulting in a greater uptake by adipose tissue of plasma triglyceride fatty acid and would be consistent with the hypothesis that when glucose is given, much of the fatty acid formed by the liver from the glucose is stored in the adipose tissue. Although insulin estimations were not carried out, it is reasonable to assume they were higher after the glucose meal than after the fructose meal, and this increased insulin could have been responsible for the increased uptake of fatty acids by the adipose tissue. This also implies that under these circumstances there can be only small amounts of fatty acid being formed from glucose in the adipose tissue.

There is a positive and significant correlation between the specific activities of the glycerol moieties of liver and plasma triglyceride in fructose-fed rats ($y = 201 + 1.3x, \text{SE of } x = \pm 0.57$) but not in the glucose-fed animals. No correlation was found between the specific activity of the glycerol, of adipose tissue triglyceride and that of plasma. This latter may be attributed to the fact that, after hydrolysis, triglyceride–fatty acids can enter the adipose tissue cell and can be utilized but glycerol thus liberated, cannot be utilized.8

The changes in the amount of radioisotope incorporated in the liver and plasma fatty acid and glycerol moieties as a result of several weeks on a high-fructose or high-glucose diet show two distinct patterns according to the carbohydrate consumed. The response of the rat to a high-glucose diet as assessed by incorporation into triglyceride fatty acid is negligible, whereas on a high-fructose-diet there is an increased incorporation compared with glucose
in the first 6–8 wk. Similar, but much more marked, patterns were discernible in the amount of the carbohydrate incorporated in the glycerol moiety of triglyceride. Here again the striking difference between the responses to the two sugars is seen in the markedly increased values in the fructose-fed animals during weeks 4–9 after commencing the diet. In the adipose tissue the responses are reversed in that it is the glucose-fed animals that show the "peaking" of specific activity during the middle of the experiment.

A simple explanation cannot be given for the delay in achieving maximal incorporation or specific activity until 3 or more wk after commencement of the diet. It is probably not due to a difference in the amount eaten while on the diet as weight losses in the first wk had been recovered by the third wk, and some of the early weight loss was possibly due to diminished colonic contents while on this water-soluble diet. The fluctuating patterns were not due to aging as the values had returned, by the end of the experimental period, to values approaching those seen during the early part of the dietary period. It seems likely, therefore, that the changes were due to alterations in metabolism, alterations that were subsequently followed by adaptation.

From these experiments in rats on high-carbohydrate diets, it is seen that there are differences in the triglyceride response between fructose and glucose and that these differences become less apparent after 11 wk on the diets. This does not, of course, rule out the possibility that there are differences which persist in parameters that were not investigated.

ACKNOWLEDGMENT

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REFERENCES