Lipogenesis in the pigeon: in vivo studies


Glucose-\[^{14}\]C was injected into fasted-refed pigeons and the amount of \[^{14}\]C incorporated into the fatty acids of liver, adipose tissue, and blood was determined at intervals thereafter up to 2 hr. Blood glucose and its radioactivity were monitored throughout the experiment. Significant incorporation of glucose carbon into the fatty acids of liver occurred within 3 min and their concentrations then rose continuously throughout the experimental period. Incorporation of glucose carbon into glycercide-glycerol remained close to unity in plasma throughout the experiment but rose progressively to a value of 7% at 2 hr in adipose tissue. It is concluded that in the pigeon the liver is the chief site of fatty acid synthesis from glucose and may account for as much as 96% of the total body lipogenesis. It would appear that adipose tissue derives its glycercide-fatty acids from plasma triglyceride and its glycercide-glycerol from plasma glucose.

The question of how an animal meets present and future caloric requirements while utilizing food sources that vary in abundance and nature has not been satisfactorily resolved. One aspect of this problem involves the regulation of the conversion of dietary carbohydrate to fatty acids. This aspect of the problem cannot be attacked, however, unless the major tissue or tissues that synthesize fatty acids have been identified.

In mammals, adipose tissue is considered the most important site for the synthesis of fatty acids with liver playing a less important role (4, 5, 12, 15, 16). In birds noted for their remarkable ability to synthesize and deposit large amounts of fat just prior to migratory flights (3, 17), the relative roles of liver and adipose tissue have not been clearly defined.

We have reported that the activity of several enzymes associated with lipogenesis was very low in homogenates of pigeon adipose tissue and very high in homogenates of pigeon liver (16). At the same time, we reported a low rate of incorporation of glucose-\[^{14}\]C and pyruvate-\[^{14}\]C into fatty acids in pigeon adipose tissue in vitro. On the basis of these results we suggested that pigeon adipose tissue might have only a very minor role in the de novo synthesis of fatty acids and might serve largely as a depository for fat synthesized elsewhere. We suggested that liver might be the chief site of fatty acids synthesis in the pigeon.

The in vivo experiments reported in this paper provide further evidence of a major role for liver and a minor role for adipose tissue with respect to the synthesis of fatty acids in the pigeon.

MATERIALS AND METHODS

Immature (6- to 8-week-old) silver king pigeons (440-700 g body wt) were housed in a temperature (22-24 C) and humidity-controlled room on a 14:10-hr daily photoperiod. The pigeons were fed Purina pigeon grains ad lib. All animals were fasted for 72 hr and then refed their normal diet for 48 hr before an experiment. They had free access to water at all times.

Unanesthetized pigeons were injected intravenously (alar vein) with 0.9 ml of a 0.9% NaCl solution that contained about 12 \(^{14}\)C glucose and 100 mg unlabeled glucose. The pigeons were then placed in small darkened cages in a ventilated hood. After varying amounts of time had elapsed (30 sec-120 min) the animals were killed by decapitation. Blood was collected from the neck in heparinized tubes. Liver and tracheal adipose tissue were quickly removed from the pigeons and placed in iced saline.

One- to two-gram pieces of liver and adipose tissue and 2-ml aliquots of whole blood were placed directly in 5 ml of 10% alcoholic KOH. After saponification for 45-60 min at 90-100 C, nonsaponifiable lipids were extracted with two 10-ml portions of petroleum ether. The combined petroleum ether extracts were backwashed...
with alkaline 50% ethanol. The alkaline ethanol wash was added to the saponification mixture. The saponification mixture was acidified to a thymol blue end point with 12 N H2SO4. Fatty acids were extracted with two 10-ml portions of petroleum ether. The combined fatty acid extracts were washed once with acidified 33% ethanol, evaporated to dryness, and dissolved in a known volume of petroleum ether. Aliquots of the fatty acid extract were plated in duplicate on tared nickel-plated steel planchets. The petroleum ether was evaporated with the aid of a fan and the planchets reweighed and counted (SE = ±2%) in a gas-flow Geiger counter equipped with a thin window. The results were corrected for self-absorption (to infinite thinness).

Adipose tissue samples (1-2 g) for glycogen analysis were defatted with chloroform:methanol, 2:1. The defatted tissue was digested in 30% KOH at 100°C for 30 min. Carrier glycogen was added to the KOH digest and total glycogen precipitated with 2 volumes of 95% ethanol. The ethanol:KOH mixture was allowed to stand overnight at 7°C. The precipitate was collected by centrifugation. The supernatant fluid was discarded and the collected precipitate dissolved in water without further purification. (Water-insoluble material was removed by centrifugation.) Aliquots of the water solution were plated on tared nickel-plated steel planchets, dried under an infrared lamp, reweighed, and counted as described above. Liver and adipose tissue nitrogen was determined by a micro-Kjeldahl method (7).

Lipid from several adipose tissue and plasma samples was extracted by the technique of Folch et al. (6). The total lipid extracts were saponified and the fatty acids isolated as described above. The isolated fatty acids were dissolved in toluene scintillation fluid (0.4% 2,5-diphenyloxazole and 0.005% p-bis [2-(5-phenyloxazole)]-benzene) and counted in a Packard Tri-Carb scintillation spectrophotometer. Glycerol in the aqueous phase of the saponification mixture was oxidized to formaldehyde with periodate (unlabeled glycerol was added to the saponification mixture) and counted in a Packard Tri-Carb scintillation spectrophotometer.

All samples counted in the scintillation spectrophotometer were corrected for quenching and efficiency of the spectrophotometer.

Blood samples for glucose analysis were deproteinized by the addition of 0.1 ml blood to 5.0 ml of a mixture containing 4 parts 5% ZnSO4 and 15 parts of 0.08 N NaOH. The precipitate was removed by centrifugation. Glucose content of the supernatant material was determined by a glucose oxidase method (Glucostat, Worthington Biochemical Corp.). Radioactivity of the glucose was determined in an aliquot of the supernatant fluid to which 40.0 mg of unlabeled glucose had been added. The glucose was converted to the insoluble phenyl osazone derivative (14), washed twice with water, suspended in acetone, and plated on a tared planchet. Evaporation of the acetone was accelerated by a stream of air from a fan. The drying process was completed under an infrared lamp. The planchets were reweighed and counted in a gas-flow counter as described above. Radioactivity of the blood glucose was calculated by multiplying the observed specific activity (counts/min per µmole) of the recovered glucosazone by the number of micromoles of added carrier glucose and appropriate dilution factors. All of the radioactivity data were corrected for differences in body weight and number of counts injected.

Glucose-U-14C was purchased from New England Nuclear Corporation.

RESULTS

Figure 1 shows the blood glucose concentration and specific activity of the blood glucose during the experimental period. The glucose was given to pigeons in the first 30 sec of the experimental period was due to the
100 mg of unlabeled glucose injected with the glucose-U-14C.

The specific activity of the blood glucose decreased very rapidly during the first 15 min of the experimental period. From 15 to 120 min specific activity of the blood glucose was low and decreased slowly. When plotted in semilog form the data show practical linearity from 5 to 120 min indicating a constant turnover of blood glucose with a t₁/₂ of 16 to 18 min.

Figure 2 shows the accumulation of counts in liver fatty acids, adipose fatty acids, and adipose glycogen (nitrogen values are listed in Table 1). Glucose carbon was rapidly incorporated into liver fatty acids. The maximum value (430 counts/min per mg N) was achieved in 15 min and maintained throughout the remainder of the experimental period. Cessation of accumulation of labeled fatty acids at 15 min probably represented a balance between the rate of newly synthesized ¹⁴C fatty acids and their release from the liver to the blood.

In adipose tissue there was a delay of about 7 min before a significant amount of radioactivity was recovered in fatty acids. This corresponds to a similar delay in the accumulation of counts in blood fatty acids (Fig. 3). From 15 to 60 min there was a rapid accumulation of counts in adipose fatty acids. Virtually no radioactivity was found in adipose glycogen during the first 30 min of the experimental period.

The rate of accumulation of counts in adipose fatty acids was very slow during the first 15 min. During this same time period we observed a very high rate of accumulation of counts in liver fatty acids and a very rapid decline in the specific activity of the blood glucose. This leads to the conclusion that the rate of incorporation of glucose carbon into fatty acids occurred at a very slow rate in adipose tissue. On a nitrogen basis, liver fatty acids had 26 times more counts than adipose fatty acids at 7 min. From 15 to 120 min after the isotope injection there was a 28-fold increase in the radioactivity of adipose tissue fatty acids. During this same interval the specific activity of the blood glucose fell to about 2.5% of the 15-min value. Under such circumstances it seems unlikely that the labeled fatty acids of the adipose tissue were derived from blood glucose. Their source would thus appear to have been the labeled fatty acids of the plasma which rise concomitantly with adipose tissue during this interval.

The results of the experiments in which both the glycerol and fatty acid fractions of plasma and adipose tissue total lipids were radioassayed are shown in Table 2. In each case, the data were converted to a ratio of the counts in glyceride-fatty acids to the counts in glyceride-glycerol. In adipose tissue this ratio increased progressively throughout the entire experimental period. In plasma, there was a 10-fold increase in the ratio between 7 and 15 min. From 15 to 120 min, however, the ratio stayed close to 1.

**DISCUSSION**

An estimate of the relative importance of liver and adipose tissue with respect to the de novo synthesis of fatty acids can be obtained by comparing their total capacities to incorporate glucose-U-14C into fatty acids before appreciable translocation of fatty acids has occurred. In our experiments the 7-min samples are appropriate for this comparison. In making the following calculations we have assumed that our pigeons contained 10 g of liver and 100 g of adipose tissue. At 7 min, liver fatty acids contained 160 counts/min per mg N or 4,000 counts/min per liver (see Table 1 for nitrogen data). Adipose tissue fatty acids contained 6.2 counts/min per mg N or 160 counts/min per pigeon. The liver, therefore, was converting glucose to fatty acids 25 times faster than all adipose tissue. This would mean that adipose tissue could be responsible for no more than 4% of the pigeon's entire capacity to synthesize fatty acids (it could be 4% only if liver and adipose tissue were the only tissues which synthesized fatty acids de novo). It should be noted that these animals had been fasted for 3 days and refed for 2 days. They were in a rapid weight-recovery phase when tested and, therefore, should have been making fat at a relatively high rate. It is conceivable that tracheal adipose tissue, which we assayed, is abnormal with respect to its capacity to synthesize fatty acids. This is unlikely, however, since abdominal adipose tissue, which was

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Liver</th>
<th>Adipose</th>
<th>No. Birds</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>2.37 ± 0.14</td>
<td>0.47 ± 0.14</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>2.17 ± 0.04</td>
<td>0.36 ± 0.025</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>2.51 ± 0.14</td>
<td>0.26 ± 0.024</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>2.43 ± 0.25</td>
<td>0.21 ± 0.014</td>
<td>9</td>
</tr>
<tr>
<td>15</td>
<td>2.56 ± 0.15</td>
<td>0.22 ± 0.016</td>
<td>6</td>
</tr>
<tr>
<td>30</td>
<td>2.47 ± 0.16</td>
<td>0.28 ± 0.056</td>
<td>9</td>
</tr>
<tr>
<td>60</td>
<td>2.57 ± 0.12</td>
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<td></td>
</tr>
<tr>
<td>120</td>
<td>2.51 ± 0.06</td>
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</tbody>
</table>

Values are expressed in mg nitrogen/100 mg wet wt ± se.

**TABLE 1. Tissue nitrogen**

**FIG. 2. Incorporation of glucose carbon into total fatty acids of liver and adipose tissue and glycogen of adipose tissue in vivo.** Vertical lines represent se. Number of pigeons in each group is given in Table 1 (except only 4 were analyzed for adipose glycogen at 15 min).
assayed in some experiments, also was found to behave in a similar manner. Small intestine, which may play a significant role in the synthesis of fatty acids in mammals (4, 15), also was found to synthesize fatty acids at a negligible rate in pigeons, as judged by the technique used in this study (data not presented). The whole liver vs. total adipose tissue calculations thus suggest that the liver may have formed as much as 96% of the fatty acids synthesized in our pigeons.

Another approach to the relative importance of liver and adipose tissue in the de novo synthesis of fatty acids is provided by adipose tissue glyceride-glycerol and glyceride-fatty acid data at the 7-min interval. If all the radioactivity in the glyceride-fatty acids and glyceride-glycerol of adipose tissue at this time was derived directly from blood glucose, we can estimate the relative rates at which glyceride-glycerol and glyceride-fatty acids were being synthesized. The average specific activity of the blood glucose from 30 sec to 7 min was about 1.7 dp/m/m mole of glucose carbon (Geiger counter efficiency was 21%). At 7 min adipose glyceride-fatty acids contained 43 ± 11 dp/m/mg N and the glyceride-glycerol contained 150 ± 28 dp/m/mg N. These values represent 25 mmol of glucose carbon for the glyceride-fatty acids or, assuming an average fatty acid chain length of 17 carbons, 1.5 mmoles of fatty acid. The corresponding figures for glyceride-glycerol were 89 mmoles of glucose carbon or 30 mmoles of glycerol. Therefore, out of the 90 mmoles of fatty acid which would have been esterified to 30 mmoles of glycerol, only 1.5 mmoles would have been synthesized de novo from blood glucose. Thus, only 1 out of every 60 fatty acids incorporated into the triglyceride of adipose tissue at this stage would have been derived from blood glucose (3-min data indicated a ratio of 1:80). It should be noted that though the absolute values calculated here are affected by the value assumed for the mean specific activity of blood glucose the ratio of fatty acids incorporated into triglyceride is independent of this value.

Favarger (4) estimated that the liver of the young adult mouse was responsible for about 3–5% of the mouse’s total capacity to synthesize fatty acids. The data of Hutchens et al. (15), Jansen et al. (16), and Bates et al. (1) are in agreement with this approximate figure. We have estimated the relative abilities of liver and adipose tissue to make fat in fasted-refed rats using the technique described in this paper. After a 15-min experimental period, total adipose tissue had eight times more fatty acid counts than total liver. Thus data obtained by our procedure support the concept of adipose tissue being the major site of fatty acid synthesis in rats.

If the liver does synthesize most of a pigeon’s fatty acids, lipid must be released from the liver and taken up by adipose tissue to a greater extent in the pigeon than in the rat. The pigeon, therefore, might be an excellent animal in which to study the transport of lipid from liver to blood and from blood to adipose tissue. In the latter step two possibilities exist, the passage of intact triglyceride from blood into adipose tissue or the release of fatty acids from blood lipids with their subsequent absorption by the tissue and reesterification to triglyceride. The latter of these two possibilities appears to be the more plausible one. The data shown in Table 2 indicate that during the interval 15 to 120 min the ratio of counts in glyceride-fatty acid to glyceride-glycerol in plasma remained close to unity whereas the same ratio in adipose tissue steadily increased from 0.8 to 7.0. This is evidence that most fatty acid transport into adipose tissue from blood does not occur as intact triglyceride.

The source of the fatty acids absorbed by adipose tissue would appear to be largely the triglyceride fraction of the blood. This conclusion is supported by the radioassay of the partially fractionated plasma lipids. In the plasma samples obtained at the 15-min interval, 81% of the total counts in plasma fatty acids were derived from the triglyceride fraction (61–90%, N = 4). At 60 min the value was 76% (74–79%, N = 3) and at 120 min it was 91% (88–94%, N = 2). Fatty acid counts from the cholesterol ester fraction varied from 0% at 15 min to 2.5% at 120 min. The remainder of the counts were in fatty acids from a single fraction composed of all other fatty acids derived from plasma lipids.

If cleavage of plasma triglyceride into fatty acids and glycerol precedes the entrance of fatty acids into adipose tissue then lipoprotein lipase might be expected to play

### TABLE 2. Ratio of counts in glyceride-fatty acids to counts in glyceride-glycerol

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Plasma</th>
<th>Adipose Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.13 ± 0.02 (5)</td>
<td>0.22 ± 0.06 (4)</td>
</tr>
<tr>
<td>7</td>
<td>1.3 ± 0.1 (3)</td>
<td>0.30 ± 0.06 (5)</td>
</tr>
<tr>
<td>15</td>
<td>1.0 ± 0.1 (3)</td>
<td>0.30 ± 0.06 (5)</td>
</tr>
<tr>
<td>30</td>
<td>1.0 ± 0.4 (5)</td>
<td>0.8 ± 0.4 (2)</td>
</tr>
<tr>
<td>60</td>
<td>0.8 ± 0.1 (3)</td>
<td>5.7 ± 0.8 (4)</td>
</tr>
<tr>
<td>120</td>
<td>1.3 ± 0.3 (4)</td>
<td>7.0 ± 0.5 (4)</td>
</tr>
</tbody>
</table>

Values are expressed in dp/m/mg N (or per ml plasma) in glyceride-fatty acids divided by dp/m/mg N (or per ml plasma) in glyceride-glycerol ± se. Values in parentheses are no. of birds.
REFERENCES


