Effects of Dietary Fat on Uptake Rate of Palmitic Acid and Lipoprotein Lipase Activity in Growing Chicks

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Abstract Three experiments were undertaken to investigate effects of dietary fat on the in vitro uptake rate of $^{14}$C-palmitic acid into the lipid soluble fraction of the liver and the adipose tissue, and on lipoprotein lipase activity in growing chicks. Three kinds of diet, a low fat diet (containing soybean oil 5.5% of total ME intake), a lard diet (containing soybean oil and lard, 5.5% and 27.3% of total ME intake, respectively) and a coconut oil diet (containing soybean oil and coconut oil, 5.5% and 27.3% of total ME intake, respectively), were used in these experiments. The chicks were given one of these diets to be isocaloric and isonitrogenous for 14 days. Lipoprotein lipase activity in the adipose tissue was not changed by the amount and the type of dietary fat. The in vitro uptake rate of palmitic acid into the liver and the adipose tissue was not affected by feeding the low fat, the lard or the coconut oil diet. This rate, however, increased with the rise of palmitic acid concentration in the medium. These results suggested that the in vitro uptake of fatty acids into the lipid soluble fraction of liver and adipose tissue was influenced by the substrate concentration rather than the amount and the type of dietary fat.

It is well known that a high fat diet increases the rate of metabolizable energy utilization and promotes fat deposition in poultry\textsuperscript{1-3}. Our previous paper\textsuperscript{4} suggested that the chicks fed a lard diet gained more fat than those fed a glucose or coconut oil diet. We also observed that the in vitro rate of fatty acid synthesis in the liver, the adipose tissue and the muscle was not changed by feeding a glucose, lard or coconut oil diet\textsuperscript{5}. Therefore, it will be considered that the increase in fat deposition by feeding a lard diet derived from the increase in the incorporation rate of dietary fatty acids into body fat.

The present study was undertaken to investigate effects of dietary lard or coconut oil on the incorporation of dietary fatty acids into the lipid soluble fraction of the liver and the adipose tissue in growing chicks. The incorporation rate of dietary fatty acids into these tissues was measured by the uptake rate of $^{14}$C-palmitic acid into the lipid soluble fraction of the liver and the adipose tissue, and by lipoprotein lipase activity in the adipose tissue.

Methods

Experiment 1. Four-week-old White Leghorn male chicks were used in the experiment. They were reared in the cage with wire floor, located in a laboratory
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room under automatically controlled temperature (25°C). They were divided into 3
groups of 4 chicks each and given a low fat (5.5% soybean oil of total ME intake),
lard (5.5% soybean oil and 27.3% lard of total ME intake) or coconut oil (5.5% soybean oil and 27.3% coconut oil of total ME intake) diet for 14 days as described
in the previous paper⁴). The experimental diets were used in the same compositions as
described in our previous paper⁵). At the end of the experiment, the chicks were
killed by heart puncture. The liver and the abdominal fat tissues were removed and
were placed in a ice-cold saline solution. The preparation procedures of the tissues
were described in our previous paper⁶). A hundred mg of the tissue was incubated
at 37°C for 2 hours in 3 ml of Krebs-Ringer bicarbonate buffer. The buffer contained
10 mM glucose, 1 or 2 mM palmitic acid and 1 µCi 1-¹⁴C-palmitic acid. Palmitic acid
was used as palmitic acid—bovine serum albumin complex which prepared by the
method of HAWKINS and HEALD⁶). At the end of the incubation periods, the tissues
were rinsed with the non-labeled palmitic acid—bovine serum albumin complex until
radioactivity was not counted. Total lipid in the tissues was extracted according to
the methods of FOLCH et al.⁷). Radioactivities in the lipid and CO₂ trapped Hyamine
were counted as described in the previous paper⁸).

Experiment. 2. Animals and feeding procedures were the same as described in
Exp. 1. Lipoprotein lipase activity in the adipose tissue was measured as follows:
enzyme solution prepared by the incubation of heparin and the adipose tissue (37°C, pH
7.4) was incubated with an activated substrate, chick serum and emulsified triglyceride
(Intralipid) (1:1), and 20% bovine serum albumin (37°C, pH8.4) for one hour. Lipo-
protein lipase activity was expressed as the amount of fatty acid released from the
activated substrate.

Experiment. 3. This experiment was undertaken to investigate the relationship
between the substrate concentration and the in vitro uptake rate of palmitic acid.
Animals and feeding procedures were the same as described in Exp. 1, except that
the low fat diet was the only experimental diet used. The preparation of the liver
and the adipose tissue, incubation procedure and tissue analysis were also described
in Exp. 1. The buffer contained 0.5, 1.0, 2.0 and 4.0 mM palmitic acid.

Results were analyzed statistically by the methods of SNEDECOR and COCHRAN⁹).

Results

Table 1 shows the uptake rate of palmitic acid into the lipid soluble fraction of
the liver and the adipose tissue, and into CO₂.

Under the condition of 1 mM palmitic acid, CO₂ production from palmitic acid
after feeding the lard or the coconut oil diet was not significantly different in both the
liver and the adipose tissue, but under the condition of 2 mM palmitic acid, CO₂ pro-
duction from palmitic acid in the adipose tissue in the chicks fed the coconut oil diet
was lower in comparison with that in the chicks fed the low fat diet. Under the
condition of 1 mM palmitic acid concentration, the in vitro uptake rate of palmitic acid
into the lipid soluble fraction of the liver and the adipose tissue was not affected by

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Table 1. Effects of dietary fat on the rate of palmitic acid uptake and CO₂ production from palmitic acid into lipid soluble fraction of liver and adipose tissue (Exp. 1)

<table>
<thead>
<tr>
<th>Palmitic acid concentration</th>
<th>Low fat diet</th>
<th>Lard diet</th>
<th>Coconut oil diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitic acid uptake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mM</td>
<td>85.4±20.1</td>
<td>81.7±10.1</td>
<td>79.0±2.8</td>
</tr>
<tr>
<td>2 mM</td>
<td>152.8±40.9</td>
<td>134.9±44.8</td>
<td>121.9±43.9</td>
</tr>
<tr>
<td>CO₂ production</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mM</td>
<td>10.8±0.3</td>
<td>10.2±0.8</td>
<td>8.1±0.6</td>
</tr>
<tr>
<td>2 mM</td>
<td>16.9±2.0</td>
<td>19.9±4.4</td>
<td>22.1±2.6</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitic acid uptake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mM</td>
<td>136.4±18.8</td>
<td>144.1±7.2</td>
<td>183.5±52.0</td>
</tr>
<tr>
<td>2 mM</td>
<td>300.8±89.3</td>
<td>229.8±23.0</td>
<td>167.6±16.7</td>
</tr>
<tr>
<td>CO₂ production</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mM</td>
<td>13.8±2.1</td>
<td>16.4±0.4</td>
<td>15.5±1.8</td>
</tr>
<tr>
<td>2 mM</td>
<td>30.9±14.2</td>
<td>61.8±3.7</td>
<td>49.1±11.1</td>
</tr>
</tbody>
</table>

1) Expressed as n moles/100 mg tissue/2 hrs. 2) Mean±SE for 4 chicks. Mean values within a row followed by different superscript letters differ significantly (P<0.05).

Table 2. Effects of dietary fat on lipoprotein lipase activity in adipose tissue (Exp. 2)

<table>
<thead>
<tr>
<th>Low fat diet</th>
<th>Lard diet</th>
<th>Coconut oil diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein lipase activity</td>
<td>5.76±0.54</td>
<td>6.68±0.72</td>
</tr>
</tbody>
</table>

1) Expressed as μ eq fatty acid released/mg protein/hr. 2) Mean±SE for 6 chicks.

the amount and the type of dietary fat. However, under the condition of 2 mM palmitic acid concentration, this rate in the adipose tissue after feeding the coconut oil diet was reduced as compared with that after feeding the low fat diet.

Table 2 shows the lipoprotein lipase activity in the adipose tissue. Lipoprotein lipase activity per mg protein was not changed by the amount and the type of dietary fat.

Figure 1 shows the relationship between the uptake rate of palmitic acid into the lipid soluble fraction of the liver or the adipose tissue and palmitic acid concentration in the medium.

The in vitro uptake rate of palmitic acid into the lipid soluble fraction of the liver or the adipose tissue was increased with the rise of palmitic acid concentration in the medium.

Discussion

It is well known that fatty acids in the site of the deposition are provided by fatty acid synthesis from carbohydrate and lipoprotein, especially from very low density lipoprotein (VLDL)⁹. It is also known that the liver is a main site of fatty acid syn-
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Fig. 1. Relationship between palmitic acid uptake and palmitic acid concentration in lipid soluble fraction of liver and adipose tissue. Each spot represents pools of 4 chicks.

thesis in the poultry and VLDL formation in the rat. On the other hand, hepatic fatty acid synthesis in the chicken was depressed by feeding a high fat diet. We estimated that about 50% of fat deposition by feeding a lard diet were achieved by the direct incorporation of dietary fatty acid into body fat. These results may indicate that the source of fatty acid in the site of fat deposition would be derived from VLDL when chicks were fed a high fat diet.

It has been reported that the uptake rate of palmitic acid in the rat liver increased with the increase in fatty acid concentration in the medium. In the present study, the in vitro uptake rate of palmitic acid into the lipid soluble fraction of the liver increased with the increase in palmitic acid concentration, though this rate was not changed by the low fat or the lard or the coconut oil diet (Table 1 and Figure 1). Wiegand et al. reported that the activity of hepatic glycerophosphate acyltransferase in the rat fed a diet containing 2.5–15% safflower oil or cocoabutter diet was not changed. On the other hand, Iritani and Fukuda reported that α-glycerophosphate acyltransferase and diglyceride acyltransferase levels were reduced to 75% of that of controls fed 0.5% corn oil diet when rats were fed a diet containing 10% corn oil. Nicolosi et al. observed that triglyceride secretion in the liver of gerbils fed a safflower oil diet was approximately twice as much as that of the coconut oil diet. Therefore, the results above mentioned and our results suggested that the fatty acid supply to compose VLDL was, at least, not changed by feeding dietary fat, though it was not known whether VLDL formation itself was changed or not.

Many researchers reported that lipoprotein lipase activity in the adipose tissue of animals fed a high fat diet was not changed as compared with that of animals fed a low fat diet. In the present experiment, lipoprotein lipase activity in the adipose tissue was not affected by feeding the lard or the coconut oil diet (Table 2). Those results suggested that lipoprotein lipase activity did not change in response to dietary fat.
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We reported that carcass fat deposition by feeding a coconut oil diet was not increased as compared with that by feeding a lard diet\(^4\). Our previous paper suggested that a cause of above observation was due to the increase in lipolytic activity in the chicks fed the coconut oil diet. In the present experiment, when the chicks were fed a diet containing coconut oil, the \textit{in vitro} rate of palmitic acid in the lipid soluble fraction of the adipose tissue was not changed due to the rise of palmitic acid concentration in the medium. Therefore, this lack of response to the substrate concentration in the adipose tissue of the chicks fed the coconut oil diet might be also a cause of our earlier observation\(^4\).

We suggested that the increase in carcase fat deposition by feeding a lard diet was due to the increase in the amount of dietary fatty acids directly incorporated into body fat\(^4,12\). If the increase in the amount of fatty acids directly incorporated into body fat is due to the tissue adaptation to the lard feeding, the \textit{in vitro} uptake rate of palmitic acid into the lipid soluble fraction in the liver or the adipose tissue, and/or the lipoprotein lipase activity must be increased by feeding a lard diet. However, in the present experiment, the \textit{in vitro} uptake rate of palmitic acid into the lipid soluble fraction of the adipose tissue was not changed by the amount and the type of dietary fat, though this rate markedly increased with the rise of palmitic acid concentration in the medium. These results suggested that the uptake rate of dietary fatty acids into lipid soluble fraction of the tissue was affected by substrate concentration and/or VLDL turnover rate rather than the tissue adaptation after feeding the dietary fat.

References

鶏ヒナのパルミチン酸取り込み能とリポ蛋白質リバーゼ活性におよぼす給与脂肪の影響

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鶏ヒナの食餌性脂肪の組織への取り込み能を調べる目的で，肝臓と脂肪組織における14C-パルミチン酸取り込み能と脂肪組織におけるリポ蛋白質リバーゼ活性に対する低脂肪飼料（摂取 ME の5.5%の大豆油を含む），ラード飼料（摂取 ME の5.5%の大豆油と27.3%のラードを含む），ヤシ油飼料（摂取 ME の5.5%の大豆油と27.3%のヤシ油を含む）給与の影響を検討した。これら3種の飼料を4週齢の白色レグホン雑種ヒナに，摂取代謝エネルギー量と摂取蛋白量が同一となるように2週間給与した。脂肪組織におけるリポ蛋白質リバーゼ活性は給与脂肪の量と種類により影響されなかった。パルミチン酸の肝臓および脂肪組織への取り込み能も給与脂肪の量と種類により大きく変化しなかった。しかし，培養液中のパルミチン酸濃度の増加に伴い，肝臓および脂肪組織におけるパルミチン酸の脂質可溶性画分への取り込み能は著しく増加した。これらの結果は，食餌性脂肪中の脂肪酸の肝臓および脂肪組織の脂質可溶性画分への取り込みが，給与脂肪の量や種類によって，組織の能力が変化するためではなく，むしろ組織への基質流入量の度の変化によって変動することを示唆している。

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