Fatty Acid Profiles of Phospholipids in Brown Adipose Tissue from Rats during Cold Acclimation and Repetitive Intermittent Immobilization: With Special Reference to Docosahexaenoic Acid

Tomie Ohno, Hiroshi Ohinata*, Koji Ogawa†, and Akihiro Kuroshima*

Medical Sciences Laboratory, Asahikawa Campus, Hokkaido University of Education, Asahikawa, 070 Japan; *Department of Physiology I, Asahikawa Medical College, Asahikawa, 078 Japan; and †Research Institute for Higher Education Programs, Hokkaido Tokai University, Asahikawa, 070 Japan

Abstract: The effects of cold acclimation and repetitive intermittent immobilization were examined on fatty acid (FA) compositions in phospholipids of rat interscapular brown adipose tissue (BAT) and plasma. As previously reported, cold acclimation and intermittent immobilization increased the degree of unsaturation as a whole in FAs of BAT but not in plasma. N-3 polyunsaturated docosahexaenoic acid (22:6; DHA) decreased in cold acclimation but increased in intermittent immobilization in phospholipids of BAT. DHA was decreased in phospholipids of plasma in both groups. Considering our previous findings that the in vitro thermogenic response of BAT was suppressed in cold acclimation and enhanced in intermittent immobilization, it was inferred that DHA in BAT is involved in the regulation of thermogenic function of this tissue. [Japanese Journal of Physiology, 46, 265–270, 1996]

Key words: brown adipose tissue, docosahexaenoic acid, cold acclimation, intermittent immobilization.

Brown adipose tissue (BAT) serves as a specific thermogenic tissue not only to enhance nonshivering thermogenesis during cold acclimation to maintain body temperature, but also to dissipate excess energy as heat in overfeeding and a metabolism-enhancing thermogenesis for adaptation to nonthermal stress such as repetitive intermittent immobilization [1].

Although the regulation of BAT function is mainly mediated by noradrenaline (NA) released from the sympathetic nerve terminals and further several hormones such as adrenaline and glucagon have been shown to be involved [2], the details of the regulatory steps by these factors have not yet been completely elucidated. In homeotherms, desaturation of fatty acids (FAs) in phospholipids has been shown to serve as an important moment in the integration of cellular membranes, improving cell functions such as ion transport, membrane permeability, activity of membrane-bound enzymes and resistance to cell damage through increased membrane fluidity [3–4]. This is also the case for poikilotherms; at low temperatures, the increase in the degree of FAs desaturation in tissue phospholipids is closely associated with the maintenance of biomembrane functions of fish [5]. In BAT, increased unsaturation of FAs in phospholipids has also been reported during its higher thermogenic activity in the neonatal period [6] and during cold acclimation [7] and repetitive intermittent immobilization stress [8]. Enhanced nonshivering thermogenesis during cold acclimation is closely associated with increased responsiveness to NA as well as increased NA secretion from the sympathetic nerves [9]. In this context, it is interesting to note the previous studies that feeding unsaturated fatty acid–rich diets alters sensitivity to catecholamines [10, 11] and similarly n-3 polyunsaturated FAs promote stimulation of lipolysis by isoproterenol in adipocytes [12]. Docosahexaenoic acid (C22:6, DHA), one of n-3 polyunsatu-
rated FAs, is found in the membrane phospholipids of
nervous tissue, muscle and several other tissues. This
specific FA markedly influences membrane fluidity,
although its full significance in cell membrane func-
tions is not well understood. Moreover, there is a very
strong inverse relationship between DHA and body
size in mammals, ranging from the mouse to the
whale, with more metabolically intense species show-
ing greater DHA content [13], and the metabolically
more intense mammalian tissues have a greater DHA
content than the less metabolically intense reptilian
tissues [14]. These findings about DHA strongly sug-
gest a significant role in the metabolic activity of the
cell. However, there have been few reports of DHA in
relation to the function of BAT. It was, therefore,
considered to be of interest and significant to study the al-
terations of DHA in BAT-phospholipids during stimu-
lation of this tissue. In the present experiment, we
studied the changes of DHA from BAT in cold-accli-
imated and repetitively immobilized rats. We also ex-
amined the changes in plasma phospholipid-FAs,
since phospholipids in plasma are catabolized as com-
ponents of their respective lipoproteins and further ex-
changed readily between lipoproteins and cellular
membranes.

MATERIALS AND METHODS

Animals. Young adult male Wistar rats, weigh-
ing about 200 g, were obtained from Shizuoka Labo-
ratory Animal Center, Hamamatsu. They were housed
individually in the metal cages. Cold-acclimated rats
were kept at 5 ± 1°C for 4 weeks. Immobilized rats
were subjected to 3 h daily immobilization with soft
wire mesh on a wooden board as described elsewhere
[15]. Control and immobilized rats were kept at 25 ±
1°C and about 50% relative humidity for 4 weeks.
All the experimental animals were placed under artificial
lighting from 7:00 to 19:00 h and provided ad libitum
with laboratory rat biscuits (Oriental MF, Oriental
Yeast, Tokyo) and tap water. Table 1 shows the chemi-
cal and FA compositions of the diet used.

Sampling. After anesthesia with pentobarbital
(Nembutal, 5 mg/100 g, i.p.), the blood was obtained
from the abdominal aorta into a heparinized syringe
and the interscapular BAT was removed and cleaned
of the adhering tissues. BAT and the separated plasma
were kept at −70°C until analyzed. The plasma tri-
glycerides and phospholipids were determined with
TG-Test Wako and PL-Test Wako reagent kits (Wako
Pure Chemical Industries), respectively. Lipids were
extracted from about 100 mg of BAT or 1 ml of
plasma according to the method of double extraction
[16] with 5 ml or 6 ml of chloroform–methanol (2:1,
v/v), respectively. BAT extract was used for the tissue
triglyceride and phospholipid measurements.

Lipid analysis. A quantity of 6 ml of each ex-
tract was evaporated to dryness under nitrogen gas in
a water bath at 30°C, then taken up in 50 μl of ex-
traction solution. This solution was applied to thin-layer
chromatography for separating phospholipids [17],
and the scratched fraction of phospholipids was trans-
esterified [18]. The transesterified solution was ex-
tracted twice with pentane and dried under nitro-
gen gas in a water bath at 40°C each time, then taken
up in extraction solution. FA analysis was performed
using a Hitachi Model 663-30 gas chromatography
equipped with a data processor (Hitachi Model
883A), using a bonded, flexible, fused silica col-
umn (30 m × 0.25 mm I.D., polyimide film thickness
<0.25 μm, J & W Scientific, USA). The temperature of
the injection inlet and the detector FID was set at
250°C. The temperature program of the column was
10°C/min from 140°C at the initial injection to 240°C.
The hydrogen carrier flow rate was 1.2 ml/min, and
the nitrogen make up gas was 15 ml/min. Air flow was
400 ml/min. One microliter of sample was injected
with a split ratio of about 25:1. The hold time was
30 min and the cooling time 3 min. Quantitative stan-
dardization of the chromatogram was based on analy-
sis of FA methyl ester standard mixtures from Funakoshi (Tokyo). FA compositions are expressed in terms
of mol%. Calculated indexes were as follows. Unsat-
uration index: the average number of double bonds
per FA molecule as indicated (ΣM/N)/100 (Mn, mol% of
each FA; Nn, number of double bonds of each FA).

Statistics. The results are expressed as means
± SE. Statistical significance was determined by
ANOVA and Fisher’s test was used as post hoc analy-
sis of individual group. A difference was regarded as
statistically significant when p was less than 0.05.

RESULTS

Body and tissue weights (Table 2)

The initial body weights did not differ among the
control, cold-acclimated and immobilized rats. The
final body weights were similarly smaller in cold-accli-
imated and immobilized rats than control ones, indi-
cating the suppressed weight gains in the former
groups as previously reported [8]. BAT weight was
significantly greater in both cold-acclimated and im-
mobilized rats, either in terms of whole tissue pad or
per 100 g body weight. However, the extent of the in-
crease was significantly greater in cold-acclimated
rats than immobilized ones.
Table 1. Chemical and fatty acid compositions of diet (Oriental MF).

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Fat</th>
<th>TG (3.89 g/100 g)</th>
<th>(weight %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(cal %)</td>
<td></td>
<td></td>
<td>C14</td>
<td>C16</td>
</tr>
<tr>
<td>59</td>
<td>27</td>
<td>14</td>
<td>0.03</td>
<td>0.63</td>
</tr>
<tr>
<td>PL (0.88 g/100 g)</td>
<td>0.02</td>
<td>0.21</td>
<td>0.02</td>
<td>0.05</td>
</tr>
</tbody>
</table>

TG, triglycerides; PL, phospholipids; C14, myristate; C16, palmitate; C18-1, palmitoleate; C18, stearate; C18-2, oleate; C18-3, linoleate; C20-1, linolenate; C20-2, eicosapentaenoate; C20-3, bis-homo-γ-linolenate; C20-4, arachidonate; C22, behenate; C22-5, docosapentaenoate; C22-6, docosahexenoate; C24, lignocerate; —, not detected.

Table 2. Effects of cold acclimation and repetitive immobilization on brown adipose tissue weight, and tissue and plasma lipid compositions.

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Weight (mg) /whole pad /100 g</th>
<th>Brown adipose tissue</th>
<th>Weight (mg) /whole pad /100 mg</th>
<th>Plasma</th>
<th>TG (mg/dl)</th>
<th>PL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>C14</td>
<td>C16</td>
<td>C18-1</td>
<td>C18-2</td>
</tr>
<tr>
<td>WC (10)</td>
<td>209±3.1</td>
<td>285±4.7</td>
<td>194±12.3</td>
<td>68±4.6</td>
<td>109±8.9</td>
<td>56±1.8</td>
</tr>
<tr>
<td>CA (10)</td>
<td>206±2.2</td>
<td>253±2.8</td>
<td>479±22.4</td>
<td>189±8.3</td>
<td>157±9.3</td>
<td>33±1.4</td>
</tr>
<tr>
<td>IM (10)</td>
<td>209±2.7</td>
<td>240±6.7</td>
<td>248±8.6</td>
<td>104±5.1</td>
<td>125±6.7</td>
<td>50±1.7</td>
</tr>
</tbody>
</table>

Mean±SE WC, warm control rats; CA, cold-acclimated rats; IM, repetitively immobilized rats. Numbers in parentheses indicate number of animals. *Significantly different vs. WC. $^\dagger$Significantly different vs. CA. Legends are the same as in Table 1.

Table 3. Fatty acid (FA) compositions (mol%) of phospholipids from cold-acclimated rats (CA) and repetitively immobilized ones (IM).

<table>
<thead>
<tr>
<th>FA</th>
<th>C14</th>
<th>C16</th>
<th>C18-1</th>
<th>C18-2</th>
<th>C20-1</th>
<th>C20-2</th>
<th>C20-3</th>
<th>C20-4</th>
<th>C22</th>
<th>C22-5</th>
<th>C22-6</th>
<th>C24</th>
<th>SA</th>
<th>MU</th>
<th>PL</th>
<th>UI</th>
<th>PU/SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: BAT</td>
<td>WC</td>
<td>1.76</td>
<td>25.25</td>
<td>2.71</td>
<td>18.93</td>
<td>15.58</td>
<td>21.00</td>
<td>0.35</td>
<td>0.47</td>
<td>0.48</td>
<td>0.79</td>
<td>7.68</td>
<td>—</td>
<td>1.26</td>
<td>4.27</td>
<td>0.06</td>
<td>45.41</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>0.67</td>
<td>18.94</td>
<td>0.46</td>
<td>26.33</td>
<td>8.58</td>
<td>26.11</td>
<td>0.19</td>
<td>0.32</td>
<td>0.28</td>
<td>0.36</td>
<td>11.46</td>
<td>0.12</td>
<td>1.10</td>
<td>2.92</td>
<td>0.15</td>
<td>48.22</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>1.19</td>
<td>21.13</td>
<td>0.69</td>
<td>19.41</td>
<td>11.11</td>
<td>27.74</td>
<td>0.31</td>
<td>0.41</td>
<td>0.35</td>
<td>0.70</td>
<td>4.46</td>
<td>—</td>
<td>1.55</td>
<td>5.76</td>
<td>0.18</td>
<td>41.92</td>
</tr>
<tr>
<td>B: Plasma</td>
<td>WC</td>
<td>1.41</td>
<td>30.73</td>
<td>0.70</td>
<td>20.55</td>
<td>4.16</td>
<td>22.63</td>
<td>0.18</td>
<td>0.41</td>
<td>0.47</td>
<td>0.73</td>
<td>13.88</td>
<td>—</td>
<td>0.84</td>
<td>3.48</td>
<td>0.23</td>
<td>52.92</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>1.43</td>
<td>29.24</td>
<td>0.26</td>
<td>22.00</td>
<td>4.42</td>
<td>24.10</td>
<td>0.19</td>
<td>0.37</td>
<td>0.57</td>
<td>0.50</td>
<td>13.30</td>
<td>—</td>
<td>0.75</td>
<td>2.67</td>
<td>0.18</td>
<td>52.85</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>1.54</td>
<td>31.35</td>
<td>0.23</td>
<td>20.88</td>
<td>4.71</td>
<td>23.27</td>
<td>0.18</td>
<td>0.25</td>
<td>0.41</td>
<td>0.66</td>
<td>12.31</td>
<td>—</td>
<td>0.89</td>
<td>3.13</td>
<td>0.23</td>
<td>53.97</td>
</tr>
</tbody>
</table>

SA, saturated FAs; MU, monounsaturated FAs; PU, polyunsaturated FAs; UI, unsaturation index; —, not detected. Legends are the same as in Tables 1 and 2.
TRIGLYCERIDES AND PHOSPHOLIPIDS IN BAT (Table 2)

Triglyceride content in whole tissue pad increased in both cold-acclimated and immobilized rats, but its level per 100 mg BAT was lower in cold-acclimated rats and did not differ in immobilized ones. Phospholipid content in whole tissue pad increased in both cold-acclimated and immobilized rats, and its level per 100 mg BAT was greater in cold-acclimated rats, but did not differ in immobilized ones.

TRIGLYCERIDES AND PHOSPHOLIPIDS IN PLASMA (Table 2)

The plasma triglyceride level was markedly decreased in cold-acclimated rats, while it did not change in immobilized ones. The plasma phospholipid level did not differ between the groups.

FA COMPOSITIONS IN PHOSPHOLIPIDS OF BAT (Table 3A)

As a whole, the polyunsaturated FA level was higher in cold-acclimated and immobilized rats as compared with that in control rats, resulting from the increases in C18-2 and C20-4. Polyunsaturated FAs/saturated FAs was also significantly higher in cold-acclimated and immobilized rats. However, DHA decreased in cold-acclimated rats, whereas it increased in immobilized ones. It was also noted that C22-5 n-3 decreased in cold-acclimated rats, while it increased in immobilized ones. The saturated FA level was higher due to the increase in C18 in cold-acclimated rats, while it was lower in immobilized ones due to the decrease in C16. The unsaturation index increased in immobilized rats due to the greater increase in polyunsaturated FAs and smaller decrease in monounsaturated FAs, but it did not change in cold-acclimated rats. It was noted that the extent of unsaturation as assessed by polyunsaturated FAs, unsaturation index and polyunsaturated FAs/saturated FAs was significantly greater in immobilized rats than cold-acclimated ones, being in agreement with the previous study [8].

FA COMPOSITION IN PHOSPHOLIPIDS OF PLASMA (Table 3B)

FA compositions taken collectively as saturated FAs, monounsaturated FAs and polyunsaturated FAs did not show any changes among groups. However, DHA was significantly lower in both cold-acclimated and immobilized rats than in control ones. C22-5 n-3 was also lower in cold-acclimated rats and tended to be lower in immobilized ones. The unsaturation index in immobilized rats was slightly smaller as compared with that in control rats, but did not differ in cold-acclimated ones.

DISCUSSION

We have previously found that long-term exposure to nonthermal stress such as immobilization leads to an activation of BAT and an improved cold tolerance as does cold acclimation [19]. It is inferred that common regulating factors such as sympathetic noradrenaline, glucagon and glucocorticoids to both cold and stressful stimuli [20] are involved in the development of this cross adaptation. Such stress-induced activation of BAT thermogenesis may serve to raise the metabolic rate and cope with a stressful situation. We have also reported that such a cross-adaptive phenomenon was seen in the FAs composition of BAT phospholipids, that is, an increase in the unsaturation in immobilized rats as in cold-acclimated ones. The present study confirmed the increased unsaturation of FAs in BAT phospholipids in immobilized rats. However, it was noted that n-3 polyunsaturated FA, DHA, increased in immobilized rats, while it decreased in cold-acclimated ones. As mentioned in the introduction, DHA in the cell membrane may favorably influence the metabolic activity of the cell [13, 14]. The in vivo BAT thermogenic response to noradrenaline, as assessed by the changes of tissue temperature, suggest that BAT function is enhanced in both cold-acclimated and immobilized rats by means of an enhanced responsiveness to thermogenic action of noradrenaline [21]. In this connection, it should be noted that an increase in the proportion of DHA in phospholipids of cardiac tissue has been proposed to enhance the sensitivity of this tissue to noradrenaline stimulation through an as yet unknown mechanism [22]. However, an in vivo enhanced thermogenic response to noradrenaline seems not to be related to the DHA level in the phospholipids of BAT. Here we could refer to the previous studies of in vitro thermogenic response of BAT. Although in vivo findings and further in vitro biochemical parameters related to mitochondrial thermogenic machinery such as uncoupling protein [23] and mitochondrial respiration [24] indicate enhanced metabolic activity of BAT in cold-acclimated rats, it has been unexpectedly reported that BAT and isolated BAT cells from cold-acclimated animals exhibit rather the suppressed thermogenic responses to noradrenaline or glucagon [25-27]. On the other hand, the in vitro thermogenic response of BAT is enhanced in immobilized rats in accordance with in vivo findings [21]. It is inferred that BAT from cold-acclimated animals is endowed with some mechanism(s) as an adap-
DHA in Brown Adipose Tissue

tive strategy to protect tissue with high thermogenic capacity against excessive heat production in cold and consequent cell destruction [1]. An enhanced in vivo thermogenesis of BAT in cold-acclimated rats may ensue from an extensive hyperplasia [28] and an increased blood flow through this tissue [29–31]. Accordingly, the present findings suggest that the DHA decrease in BAT-phospholipid is associated with a suppressed thermogenic response of BAT to noradrenaline or glucagon during cold acclimation [27]. The thermogenic response to exogenous cAMP is also reduced in BAT and BAT cells from cold-acclimated rats [32, 33]. It is, therefore, suggested that the site of above-mentioned physiological desensitization is located at the post-second messenger signaling system beyond the receptor itself. Considering that DHA is a component of membrane phospholipid, it could modify the activity of carnitine palmitoyltransferase in the mitochondrial inner membrane, by which energy substrate FA is able to penetrate mitochondria and gain access to the β-oxidation system of enzymes. Further study is warranted to elucidate this problem. In the present study, C22-5 n-3 exhibited similar changes in the experimental groups; it decreased in cold-acclimated rats and increased in immobilized ones. C22-6 n-3 is direct precursor of DHA, but besides that its role in cellular function has not been evidenced. Its increase may be related to an increase in DHA. Similar findings were reported whereby chronic administration of triiodothyronine or adrenalectomy increased both C22-5 and DHA in BAT of the genetic obese Zucker rat [34].

The present study confirmed the previous study that cold acclimation decreased the plasma triglyceride level, but did not change the phospholipid level [35], possibly due to an accelerated utilization of triglycerides as an energy substrate for nonshivering thermogenesis in cold. Nonshivering thermogenesis has been shown also to increase [21] and the mass of white fat decrease [19] in immobilized rats as in cold-acclimated ones. The absence of a change in plasma triglycerides in immobilized rats might result from less triglyceride utilization and/or a concomitant increase in lipogenesis in immobilized rats. In cold-acclimated and winter-acclimated trout, the increase in the unsaturation of phospholipid-FAs of plasma, mainly due to an increase in DHA, was observed [36], but no appreciable changes in the unsaturation of phospholipid-FAs of plasma in cold-acclimated and immobilized rats. DHA rather was observed to decrease in the present study. This implies that DHA produced in the liver does not contribute to the changes in DHA of BAT-phospholipids in the rat. The changes in DHA of BAT-phospholipids may, thus, occur at the tissue level. However, DHA in the diet could incorporate itself into phospholipids of liver, white adipose tissue and BAT [37]. Accordingly, it is interesting to study the effect of addition or deficiency of DHA in the diet on DHA in phospholipids of BAT and its thermogenic responses as well. Table I shows that the diet used in the present study was not especially rich in DHA and other polyunsaturated FA. It was previously reported that both cold acclimation and intermittent immobilization increased the food intake [19], while the changes in DHA and C22-5 were in the opposite direction to that in the food intake in cold-acclimated rats. It is, therefore, inferred that the changes in DHA and C22-5 of BAT are not influenced directly by dietary factors.

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