Cold-induced alterations of phospholipid fatty acyl composition in brown adipose tissue mitochondria are independent of uncoupling protein-1

Augustine Ocloo,1 Irina G. Shabalina,2 Jan Nedergaard,2 and Martin D. Brand1

1Medical Research Council Dunn Human Nutrition Unit, Cambridge, United Kingdom; and 2The Wenner-Gren Institute, The Arrhenius Laboratories, Stockholm University, Stockholm, Sweden

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Ocloo A, Shabalina IG, Nedergaard J, Brand MD. Cold-induced alterations of phospholipid fatty acyl composition in brown adipose tissue mitochondria are independent of uncoupling protein-1. Am J Physiol Regul Integr Comp Physiol 293: R1086–R1093, 2007. First published July 3, 2007; doi:10.1152/ajpregu.00128.2007.—The recruitment process induced by acclimation of mammals to cold includes a marked alteration in the acyl composition of the phospholipids of mitochondria from brown adipose tissue: increases in 18:0, 18:2(n–6), and 20:4(n–6) and decreases in 16:0, 16:1, 18:1, and 22:6(n–3). A basic question is whether these alterations are caused by changes in the concentration of uncoupling protein-1 (UCP1) or the thermogenesis it mediates—implies that they are secondary effects—or whether they are an integrated, independent part of the recruitment process. This question was addressed here using wild-type and UCP1-ablated C57BL/6 mice acclimated to 24°C or 4°C. In wild-type mice, the phospholipid fatty acyl composition of mitochondria from brown adipose tissue showed the changes in response to cold that were expected from observations in other species and strains. The changes were specific, as different changes occurred in skeletal muscle mitochondria. In UCP1-ablated mice, cold acclimation induced acyl alterations in brown adipose tissue that were qualitatively identical and quantitatively similar to those in wild-type mice. Therefore, neither the increased content of UCP1 nor mitochondrial uncoupling altered the effect of cold on acyl composition. Cold acclimation in wild-type mice had little effect on phospholipid acyl composition in muscle mitochondria, but cold-acclimation in UCP1-ablated mice caused significant alterations, probably due to sustained shivering. Thus, the alterations in brown adipose tissue phospholipid acyl composition are revealed to be an independent part of the recruitment process, and their functional significance for thermogenesis should be elucidated.

knockout mice; adaptive thermogenesis; skeletal muscle

WHEN ACUTELY EXPOSED TO COLD, mammals defend their body temperature by activating muscular shivering. However, when chronically exposed to cold, mammals successively recruit their brown adipose tissue, and the resulting ability to perform nonshivering thermogenesis leads ultimately to cessation of shivering (13). Recruitment of brown adipose tissue is a multicomponent process, apparently mainly adrenergically induced, which involves cell proliferation, mitochondrial biogenesis, and induction of the expression of many different proteins, most prominently uncoupling protein-1 (UCP1). Adrenergic stimulation also increases the activity of UCP1, which partly uncouples brown-fat mitochondria, leading to a high rate of lipid combustion, that is, nonshivering thermogenesis. One of the components in the cold-induced recruitment process in brown adipose tissue is a very marked alteration in the phospholipid acyl group composition of the mitochondria. This marked alteration was first observed in the 1970s (15, 46, 52), and since then, many studies have verified qualitatively identical alterations in animals of different species and different ages (detailed in the DISCUSSION). In particular, increases in the content of certain polyunsaturated acyl groups, especially linoleic acid, 18:2(n–6), and arachidonic acid, 20:4(n–6), have been observed consistently.

Because of the high consistency of the alterations and their relatively high amplitude, they have been suggested to be directly important for the thermogenic process in the tissue (41, 42, 51). In general, there is good evidence that membrane fatty acyl group composition affects membrane protein activity and cellular function (26, 27, 56–58, 60, 64). Phospholipid acyl composition has been discussed as being a factor in determining the native proton conductance of the mitochondrial membrane (6, 8, 10, 28, 43), although variations in adenine nucleotide translocase content may be more important (7). When superoxide reacts with phospholipid (n–6)-acyl groups, particularly 18:2(n–6) and 20:4(n–6) (the fatty acids that are increased in the recruitment process), derivatives are produced, particularly 4-hydroxy-2-nonenal, which may stimulate the activity of uncoupling proteins, including UCP1 (5, 18, 20, 55). However, an alternative possibility to direct importance of the phospholipid alterations is that they simply represent a secondary local adaptive response in the mitochondrial membrane to the increase in the concentration of UCP1, or to the increased catabolism and thermogenesis that it catalyzes. The concentration of UCP1 rises threefold in the cold in rodents, from about 3% to 9% of mitochondrial protein (3, 31, 59), and this change in abundance of a protein that occupies a significant area of the inner membrane might require compensatory changes in acyl chains to accommodate it.

Thus, two possible explanations of the change in acyl composition are that it is a coordinated, independent, and primary part of the recruitment process that is of functional significance or that it is a secondary change in response to UCP1 presence or activity, which may or may not be important for thermogenesis. Up to now, it has not been possible to distinguish between these possibilities. This is because adrenergic stimulation via the sympathetic nervous system, which causes the phospholipid acyl composition alterations (34–36), also necessarily induces UCP1 and the thermogenic process. The availability of UCP1-ablated mice (19) has now made it possible

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to distinguish between primary recruitment effects and effects secondary to UCP1 content, uncoupling, and thermogenesis.

Brown-fat cells from animals that lack UCP1 fully lose their ability to respond thermogenically to adrenergic stimulation (32), and no adaptive adrenergic thermogenesis can be induced in the intact UCP1-ablated animal (21). This means that the process of adaptive adrenergic nonshivering thermogenesis cannot take place in UCP1-ablated mice, and as these animals are unable to recruit any other form of nonshivering thermogenesis, they must continuously use shivering thermogenesis of muscular origin to defend their body temperature (22). Studies of these animals have shown that glucose metabolism (30) and lipid accumulation/loss (19) in brown adipose tissue are processes that are indeed secondary to the thermogenic process. Accordingly, it is possible that the alterations in phospholipid acyl composition are also secondary to thermogenesis.

We have used UCP1-ablated mice to establish whether the alterations in phospholipid acyl composition are independent components in the recruitment process or are secondary to UCP1 expression and thermogenesis. We have also investigated the phospholipid acyl pattern in skeletal muscle mitochondria, to examine whether the alterations are brown-fat specific, and particularly to examine whether the continuous shivering process in the muscle of cold-exposed UCP1-ablated mice results in specific alterations of phospholipid composition. We conclude that the phospholipid acyl alterations in brown-fat mitochondria represent a process that is not secondary to the presence of UCP1 or to mitochondrial uncoupling and thermogenesis.

**METHODS**

**Animals.** The UCP1-ablated mice used here were derived from those described previously (19, 54). UCP1-ablated mice on a mixed 129/SvRas and C57BL/6 background were backcrossed to the C57BL/6 strain for at least six generations. The resulting mice were intercrossed to obtain two mouse strains, UCP1<sup>−/−</sup> and UCP1<sup>+/+</sup>, which were bred at the Wenner-Gren Institute. Animals were fed ad libitum (R70 Standard Diet, Lactamin, Kimstad, Sweden); the fatty acid composition of this diet is presented in Table 1. The animals had free access to water and were bred and kept on a 12:12-h light-dark cycle at 24°C (maximum of 5 animals per cage). No differences in body weight or growth rate were found between the two strains under these conditions. At the initiation of the experiment, UCP1<sup>+/+</sup> and UCP1<sup>−/−</sup> male mice were divided into groups matched for age (7–8 wk-old) and body weight (23–24 g) and kept one per cage. Controls were acclimated for 7–8 wk at 24°C; cold-acclimated mice were obtained by first acclimating mice at 18°C for 4 wk, followed by 3–4 wk at 4°C. The intermediate 18°C step allows survival of UCP1<sup>−/−</sup> animals at 4°C (22).

**Tissue collection.** Brown adipose tissue and skeletal muscle mitochondria were isolated at the Wenner-Gren Institute (Stockholm, Sweden) in parallel from UCP1<sup>+/+</sup> or UCP1<sup>−/−</sup> mice, that is, two mitochondrial preparations each experimental day. Mice were anesthetized for 1 min by a mixture of 79% CO2 and 21% O2 and decapitated. Interscapular, periaortic and axillary brown adipose tissue of 3 or 4 UCP1<sup>+/+</sup> mice or 5–6 UCP1<sup>−/−</sup> mice was pooled, transferred to ice-cold 250 mM sucrose and used for isolation of brown-fat mitochondria. The greater number of UCP1<sup>+/+</sup> mice was needed to compensate for the reduced body weight and mass following prolonged cold exposure of these animals. In parallel, hindlimb skeletal muscles of two mice from each group were placed in ice-cold medium containing (in mM) 100 sucrose, 50 KCl, 20 K-TEA, 1 EDTA, and 0.1% (wt/vol) fatty acid-free BSA and used for isolation of skeletal muscle mitochondria. Tissues were freed of white fat and connective tissue, weighed, finely minced with scissors, and homogenized in a Potter homogenizer with a Teflon pestle. During mincing and homogenizing, the skeletal muscle tissue fragments were treated with nagarse (1 mg/g of tissue) added to the medium. Throughout the isolation process, the samples were kept between 0°C and 2°C.

**Mitochondrial isolation.** Mitochondria were isolated by differential centrifugation, principally as described previously (14), with some modifications. Tissue homogenates were centrifuged at 8,500 g for 10 min at 2°C using a Beckman J2-21M centrifuge. The resulting supernatants, containing floating fat in brown adipose tissue preparations or nagarse in skeletal muscle preparations, were discarded. Pellets from brown adipose tissue were resuspended in cold medium consisting of 250 mM sucrose and 0.2% (wt/vol) fatty acid-free BSA. Pellets from skeletal muscle were resuspended in cold medium containing (in mM) 100 sucrose, 50 KCl, 20 K-TEA, 1 EDTA, as well as 0.1% (wt/vol) fatty acid-free BSA. The resuspended tissue homogenates were centrifuged at 800 g for 10 min, and the resulting supernatants were centrifuged at 8,500 g for 10 min. The resulting mitochondrial pellet from brown adipose tissue was resuspended in 100 mM KCl containing 20 mM K-TEA, pH 7.2. The resulting mitochondrial pellet from skeletal muscle was resuspended in 100 mM sucrose, 50 mM KCl, 20 mM K-TEA, and 1 mM EDTA and centrifuged again at 8,500 g for 10 min. The final mitochondrial pellets were resuspended by hand homogenization in a small glass homogenizer in the appropriate final centrifugation medium. Mitochondrial suspensions were kept under nitrogen at 70°C until they were shipped to the Medical Research Council Dunn Human Nutrition Unit (Cambridge, UK) on dry ice.

**Fatty acid analysis.** Total mitochondrial lipids were extracted in Cambridge, as described previously (11). Approximately 2 mg of mitochondrial protein was extracted in 10 ml of 2:1 vol/vol chloroform:methanol containing 0.05% (wt/vol) butyrylated hydroxytroponine. Lipids were redisolved in 1–2 ml of chloroform. Phospholipids were isolated by silicic acid chromatography using silica cartridges (8). Briefly, the total lipid extract was loaded on a silicic acid column, the column was washed with 2 ml chloroform to elute neutral lipids, and the phospholipids were eluted with 2 ml methanol in a round-bottom flask. The methanol eluate was dried under nitrogen at 25°C and dissolved in 19/1 (vol/vol) chloroform:methanol. From the dried eluate, fatty acid methyl esters were prepared in the presence of boron trifluoride in methanol, as previously described (11). They were

### Table 1. Fatty acid composition of the diet

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<th>Fatty Acid</th>
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<td>16:3(n-4)</td>
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<td>Stearic</td>
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<td>18:2(n-6)</td>
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<td>20:4(n-6)</td>
<td>Arachidononic</td>
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<tr>
<td>20:5(n-3)</td>
<td>Eicosapentaenoic</td>
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<tr>
<td>22:4(n-3)</td>
<td>Docosatetraenoic</td>
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<tr>
<td>22:5(n-3)</td>
<td>Docosapentaenoic</td>
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<tr>
<td>22:6(n-3)</td>
<td>Docosahexaenoic</td>
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The diet of the mice (R70 Standard Diet, Lactamin) was analyzed for total fatty acyl group composition. The fatty acids listed are those shown in Figs. 1 and 3; other fatty acids made up a few percent of the fatty acids of the diet (6:0, 0.3%; 15:0, 0.1%; 17:0, 0.2%; 17:1, 0.1%; 18:4, 0.3%; 20:1, 0.5%; 20:2, 0.1%; 22:0, 0.3%; 22:1, 2.7%; 24:0, 0.2%; 24:1, 0.3%). The protein source for this diet was fish meal. Data are from Lactamin.
purified on a Florisil column, dried under nitrogen, redissolved in hexane, and analyzed on a gas chromatograph [Hewlett Packard 6890 with HP chemstation, using a SUPELCOWAX 10 fused silica capillary column (Supelco, Dorset, UK)], which was calibrated using commercial fatty acid methyl ester standards, PUFA-2, rapeseed oil, GLC-40, and GLC-50 from Supelco. Fatty acid methyl esters were detected using a flame ionization detector. Relative molar amounts were calculated by dividing the integrated peak areas after baseline correction by the molecular weight of the appropriate fatty acid methyl ester. Mol% of each fatty acid (FA) was calculated as (100 × mole FA)/(sum of mole FA for all FA). No unidentified peak contributed more than 0.1% of the total fatty acid methyl esters present. Unsaturation index (UI) was calculated as [(Σmol% monoenoic) + (2 × Σmol% dienoic) + (3 × Σmol% trienoic) + (4 × Σmol% tetraenoic) + (5 × Σmol% pentaenoic) + (6 × Σmol% hexaenoic)].

Statistical analysis. Significance was tested by multivariate analysis using the general linear model in SPSS 11.0 (SPSS, Chicago, IL). Analysis was performed on the full data set for mitochondria from each tissue to test for effects of temperature, presence of UCP1, and interactions between them. Effects of presence of UCP1 at each temperature and effects of temperature with and without UCP1 were tested in the same way on the appropriate data subset. \( P \) values < 0.05 were considered to be significant.

RESULTS

Effect of acclimation to cold on the phospholipid fatty acyl composition of brown adipose tissue mitochondria. Fig. 1A shows the fatty acyl composition of phospholipids from brown adipose tissue mitochondria of wild-type mice. In mitochondria from animals kept in the control 24°C state (open bars), the pattern was dominated by 16:0, 18:1, and 18:2 acyl groups. Cold acclimation (black bars) led to a marked alteration of this pattern, there were significant increases in 18:0 and 20:4 (6), 18:2 (also increased, but the effect did not reach statistical significance), and decreases in 14:0, 16:1(n-7), 18:1(n-9), 18:1(n-7), and 22:6(n-3). Although this is the first investigation of acyl group alterations in any inbred mouse strain, the alterations observed are in good agreement with many previous investigations in different species (see Discussion).

The absence of UCP1 in the UCP1-ablated mice leads to the inability of the mitochondria of brown adipose tissue to be uncoupled and thus to the absence of any increased lipid combustion and induced thermogenesis in the tissue. Not only could marked alterations in acyl composition still be observed after cold adaptation, but these alterations were also essentially the same as in wild-type animals (Fig. 1B). In mitochondria from the UCP1-ablated mice, as in the wild-type, there were significant increases after cold acclimation in 18:0 and 20:4 (n-6), and decreases in 16:1(n-7) and 22:6(n-3).

The absence of UCP1 had no marked effect in control mice: in a direct comparison of the pattern in brown adipose tissue mitochondria from wild-type and UCP1-ablated mice kept under control 24°C conditions (compare Fig. 1, A and B), there was no significant difference in the amount of any major fatty acyl group. The levels of three minor acyl groups were significantly different: 18:1(n-7) (vaccenyl acid) content was higher and 18:3(n-3) and 22:4(n-3) content was lower in UCP1-ablated mice. The same differences in these minor acyl groups were observed in cold-acclimated animals, indicating that they were not statistically random events, but differences that depend on the expression of UCP1.

Overall, multivariate analysis at 24°C and 4°C, with and without UCP1, revealed that there were significant effects of temperature on the levels of 16:1(n-7), 18:0, 18:1(n-9), 18:3(n-3), 20:4(n-6) and 22:6(n-3), but significant effects of UCP1 only on the minor fatty acyl groups 18:1(n-7), 18:3(n-3), and 22:4(n-3). There was only one significant interaction between the effects of temperature and UCP1 on 18:1(n-7) content. Thus, in cold-acclimated mice from either strain, the acyl composition was clearly different from that under control 24°C conditions (Fig. 1, A and B), but there were few differences in acyl composition between the two strains with either acclimation temperature.

Figure 2 compares global fatty acid indices of mitochondria from control 24°C wild-type mice with those from cold-acclimated wild-type, control UCP1-ablated and cold-acclimated UCP1-ablated mice. There were no significant differences in the proportion of saturated fatty acids (SFA), unsaturated fatty acids (UFA), or PUFA in the phospholipids, or in the ratio of unsaturated to saturated fatty acids (UFA/SFA).
The UI and the average acyl chain length were also the same. The proportion of monounsaturated fatty acid decreased in the cold, as did the balance between \((n\text{-}3)\) and \((n\text{-}6)\) fatty acids, but there were no effects of UCP1 status.

We conclude that the marked alterations in brown adipose tissue phospholipid acyl composition following cold acclimation are not secondary to the presence of UCP1 or to the uncoupling process.

Effect of acclimation to cold on the phospholipid fatty acyl composition of skeletal muscle mitochondria. The acyl composition of the phospholipids of skeletal muscle mitochondria from control animals (Fig. 3A, open bars) was markedly different from that of brown adipose tissue mitochondria (Fig. 1A, open bars). Although \(16:0\) and \(18:2\) were major acyl groups in both types of mitochondria, there was a clear difference in the preponderance of very long-chain polyunsaturated acyl groups. \(22:6\) (docosahexaenoic acid, or DHA) in the skeletal muscle mitochondria constituted about \(30\%\) of the acyl groups, compared with about \(8\%\) in brown-fat mitochondria. This echoes the well-known observation that phospholipid composition is actively regulated in different tissues and species (29).

As seen above, the composition of brown adipose tissue mitochondrial phospholipids was markedly altered as an effect of cold acclimation (Figs. 1 and 2). Cold exposure is associated with a large increase in food intake (about threefold) and thus in lipid metabolism. If the alterations in brown adipose tissue were caused by systemic changes in fatty acid metabolism, such alterations should also be present in other tissues. However, except for a small decrease in \(18:1\text{–}9\) content, cold acclimation did not lead to any significant alteration of the acyl composition of skeletal muscle phospholipids in wild-type mice (Fig. 3A). In contrast, when UCP1-ablated mice were acclimated to cold, small (but significant) effects of cold acclimation were seen in muscle: decreases in the relative amounts of \(18:2\) and \(20:4\) and an increase in the relative amount of palmitoleic acid \((16:1\text{–}7)\) (Fig. 3B). Note that the changes in \(18:2\) and \(20:4\) are augmentations of trends also visible in wild-type mice (Fig. 3A). The changes were very different from those occurring in brown-fat mitochondria due to cold acclimation (Fig. 1), the changes in \(18:2\) and \(20:4\) even being in the opposite direction, again pointing to the tissue-specific control of phospholipid composition. That significant effects of cold acclimation were observed in muscle mitochondria from UCP1-ablated mice and not in those from wild-type mice indicates that the function of skeletal muscle mitochondria is different in these two types of mice.

Under control conditions, the absence of UCP1 in brown adipose tissue was without effect on the acyl composition of the phospholipids of skeletal muscle mitochondria. As effects of cold acclimation were found in UCP1-ablated mice, but not in wild-type mice, the acyl composition of the phospholipids in the skeletal muscle mitochondria of cold-acclimated UCP1-ablated mice was different from that of wild-type mice. However, statistically, this was only the case for \(16:1\text{–}7\) and \(18:1\text{–}9\), which were both higher in the UCP1-ablated mice (compare Fig. 3, A and B).

Overall, multivariate analysis at \(24\text{°C}\) and \(4\text{°C}\), with and without UCP1, revealed that in skeletal muscle mitochondria,
there were significant effects of temperature on the levels of 14:0, 16:3(n–4), 18:1(n–7), 18:2(n–6) and 20:4(n–6), and significant effects of UCP1 on 16:1(n–7) and 18:1(n–9). There were no significant interactions between the effects of temperature and UCP1.

The global acyl composition parameters indicated few changes between the muscle mitochondria in the different conditions; one or two indices were significantly altered, but the changes were minor (Fig. 4), and not the same as in brown adipose tissue (Fig. 2).

**DISCUSSION**

In the present study, we used UCP1-ablated mice to examine the classical question of whether the marked alterations in mitochondrial phospholipid acyl composition observed during acclimation to cold are a secondary effect of UCP1 expression or the uncoupling process or whether they are independent events of the recruitment process. We found that acclimation to cold induced similar changes in acyl composition in the mitochondrial phospholipids in brown adipose tissue, irrespective of whether the mice possessed UCP1 or not. In skeletal muscle mitochondria, acclimation to cold hardly altered the acyl composition in wild-type mice, but in mice that lacked UCP1, small but significant changes were observed. This would be in agreement with the involvement of skeletal muscle mitochondria in the sustained shivering process responsible for maintaining the heat balance of UCP1-ablated mice in the cold.

**Effect of cold acclimation on acyl composition of the phospholipids of brown-fat mitochondria.** We found marked alterations in the fatty acyl composition of the phospholipids of brown-fat mitochondria in the C57BL/6 mouse strain, consisting in upregulation of the relative content of 18:0, (18:2) and 20:4(n–6) and downregulation of 16:1(n–7) and 18:1(n–9). As seen in Table 2, our data are not only in qualitative agreement with earlier data obtained with another mouse strain but also accord with an extensive array of earlier observations on cold-induced alterations in acyl composition in brown-fat mitochondria. Indeed, as summarized in Table 2, the effects of cold acclimation reported are qualitatively extremely consistent, despite the use of different species and strains of animals, diverse diets, exact degree of cold exposure, different investigators, and differences in time in several aspects (age of animals, time in cold, year of investigation). Thus, this alteration of phospholipid acyl composition is a very reproducible effect of cold acclimation, associated with the recruitment process of brown adipose tissue, and its cause and effects require explanation.

We observed two further cold-induced alterations that have not been widely reported earlier, probably due to technical limitations in the detection systems. One is a decrease in 22:6(n–3) (DHA), also observed by (42). The other is a decrease in 18:1(n–7) (vaccenic acid), which may be specifically associated with cardiolipin (63).

Alterations in acyl composition are not secondary to the presence of UCP1 or to mitochondrial uncoupling. The main question raised here, that is, whether the alterations in acyl composition were secondary to either the presence of UCP1 in the membrane or the uncoupling process or were independently regulated, could be clearly answered: almost all the fatty acyl changes that occurred in the cold in wild-type mice were duplicated in the UCP1-ablated mice. Thus, the alterations were not secondary to the mere presence of UCP1 in the

**Table 2. Effect of cold acclimation on the fatty acyl composition of (mitochondrial) phospholipids in brown adipose tissue**

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Published data show downward (d) or upward (u) shifts in the amount of the indicated fatty acyl group in response to cold acclimation. (---) indicates no change. (-d) and (-u) a trend or small change; blanks indicate absence of information. Some studies report on total phospholipids from brown adipose tissue but as mitochondrial phospholipids are the dominant fraction, such studies are included here. Species examined were m, mouse; r, rat; h, hamster; and gp, guinea-pig. v, vaccenic acid (18:1(n–7)).
membrane (which would not be impossible as UCP1 may be 5–10% of total mitochondrial membrane protein), nor were they secondary to the uncoupling process as such (e.g., a lower membrane potential), nor were they secondary to the increased catabolism in the tissue.

There must, therefore, be other mechanisms that cause the alterations in acyl composition. There are two obvious candidates, not mutually exclusive. First, upregulated 18:2(n–6) and 20:4(n–6) are both essential fatty acids that are provided by the food; the former may be seen as a precursor of the latter. Table 1 shows that the diet contained a very high amount of linoleic acid [18:2(n–6)], and as food intake in the cold is about threefold that under control conditions, the food-supplied fatty acids may have a greater effect on mitochondrial composition in the cold. However, the lack of changes in skeletal muscle acyl groups in the cold suggests that this alone cannot explain the compositional differences in brown adipose tissue. Brown adipose tissue is under constant adrenergic stimulation in the cold, leading to increased lipoprotein lipase gene expression (17) and lipoprotein lipase activity (16). UCP1-ablated mice also exhibit strongly increased lipoprotein lipase gene expression in the cold (12). This would lead to an increased uptake of circulating lipids, including the higher amounts of dietary 18:2, which, in itself, might cause acyl composition to change in brown adipose tissue. Secondly, cold acclimation also leads to an increased expression of fatty acid anabolic enzymes. The fatty acid elongase elov13 ("cig30") (62) and certain desaturases (66) have been demonstrated to increase markedly in brown adipose tissue, and other elongases and desaturases may behave similarly, although this has not been examined. Such cold-induced increases in elongase and desaturase activity may effect, for example, the transformation of 18:2 to 20:4 and cause the large increases that are observed in the 20:4 content of brown adipose tissue mitochondria. Thus, the alterations observed may be consequences of either or both of these dietary and enzymatic alterations. As the recruitment process is associated with general mitochondriogenesis in the tissue (13), the alteration in phospholipid acyl composition may not have to await the turnover of phospholipids in preexisting mitochondria but may reflect the insertion of altered phospholipids into the new population of mitochondria that is synthesized (33). A further possibility is that the alterations in total mitochondrial phospholipid fatty acyl composition reflect changes in the proportion of outer and inner membranes in the extracts. However, this explanation is unlikely, since the area of outer membrane is small compared with the area of the highly invaginated inner membrane in mitochondria from brown adipose tissue and skeletal muscle. In any case, any difference in fatty acyl group composition between the inner and outer membranes is generally small (10).

Although adrenergic regulation of the enzymes involved may explain the alterations in acyl composition, the mere existence of the alterations does not mean that they are functionally important; they may just be nonfunctional consequences of the enzymatic effects. Although many studies imply that the alterations in acyl composition are of thermoregulatory significance, there are only a few studies that have attempted to examine this. Animals fed diets deficient in linoleic acid (18:2), which in the long term should lead to less 20:4, demonstrated only marginal and unspecific effects on brown adipose tissue (not a diminished thermogenic capacity) (23, 38, 45, 65). Thus, the significance of the alterations remains enigmatic.

Changes in skeletal muscle acyl composition correlate with shivering. We found that the acyl composition of skeletal muscle mitochondria was clearly different from that of brown-fat mitochondria. In particular, the muscle mitochondria exhibited a high level of 22:6 (nearly 1/3 of all acyl groups). The 22:6 content of skeletal muscle varies dramatically with body mass in homeotherms, but it is generally about 20% in mice (29). One explanation for the slightly higher concentration found here could be that the diet of the present mice had fish meal as a protein source, with high levels of DHA precursors (Table 1); diet composition has been demonstrated to influence acyl composition of skeletal muscle (and brown-fat) mitochondria (41, 61).

In wild-type animals, cold acclimation did not lead to any large changes in acyl composition in skeletal muscle mitochondria. There would only seem to be one earlier study (performed in rats) examining the effect of cold acclimation on phospholipid acyl composition in skeletal muscle (40). That study found very small effects on acyl composition and concurs with the present study in observing a clear difference between the alterations in brown adipose tissue and those in skeletal muscle. Similarly, the effect of cold on total mitochondrial fatty acyl groups in liver mitochondria (9) is clearly different from that found for brown-fat mitochondria. Together, these studies demonstrate that the observed alterations in brown adipose tissue are specific and do not represent a general, systemic effect of cold acclimation.

In contrast, cold acclimation does cause statistically significant alterations in phospholipid acyl composition in skeletal muscle mitochondria from UCP1-ablated mice. This is understandable in that the muscles in such mice are constantly shivering. This is because the absence of UCP1 is associated with a total absence of adaptive adrenergic nonshivering thermogenesis (22), and as these animals are unable to recruit any alternative form of nonshivering thermogenesis (21), all necessary extra heat in the cold has to come from sustained shivering thermogenesis. Interestingly, the decreases in 18:2 and 20:4 due to cold acclimation (Fig. 3) are parallel to what has been observed as an effect of training, both in rodents (4, 24, 44) and in man (1, 2, 25). However, the effects on phospholipid acyl composition of training in skeletal muscle mitochondria are not as consistent as the effects of cold acclimation in brown-fat mitochondria. The similarity between the effects of constant shivering and training agrees with other observations on the properties of skeletal muscle mitochondria in cold-acclimated UCP1-ablated mice (I. G. Shabalina and J. Nedergaard, unpublished observations).

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REFERENCES


