High Level of Uncoupling Protein 1 Expression in Muscle of Transgenic Mice Selectively Affects Muscles at Rest and Decreases Their Iib Fiber Content*

Received for publication, July 8, 2002, and in revised form, September 4, 2002
Published, JBC Papers in Press, September 6, 2002, DOI 10.1074/jbc.M206726200

The mitochondrial uncoupling protein of brown adipose tissue (UCP1) was expressed in skeletal muscle and heart of transgenic mice at levels comparable with the amount found in brown adipose tissue mitochondria. These transgenic mice have a lower body weight, and when related to body weight, food intake and energy expenditure are increased. A specific reduction of muscle mass was observed but varied according to the contractile activity of muscles. Heart and soleus muscle are unaffected, indicating that muscles undergoing regular contractions, and therefore with a continuous mitochondrial ATP production, are protected. In contrast, the gastrocnemius and plantaris muscles showed a severely reduced mass and a fast to slow shift in fiber types promoting mainly Iia and IIx fibers at the expense of fastest and glycolytic type Iib fibers. These observations are interpreted as a consequence of the strong potential dependence of the UCP1 protonophoric activity, which ensures a negligible proton leak at the membrane potential observed when mitochondrial ATP production is intense. Therefore UCP1 is not deleterious for an intense mitochondrial ATP production and this explains the tolerance of the heart to a high expression level of UCP1. In muscles at rest, where ATP production is low, the rise in membrane potential enhances UCP1 activity. The proton return through UCP1 mimics the effect of a sustained ATP production, permanently lowering mitochondrial membrane potential. This very likely constitutes the origin of the signal leading to the transition in fiber types at rest.

Uncoupling protein 1 (UCP1)\(^1\) is expressed exclusively in brown adipose tissue (reviewed in Refs. 1 and 2). Its presence in brown fat mitochondria is responsible for heat production by the mitochondria in brown adipocytes. UCP1 allows return of protons into the matrix without ATP synthesis, and therefore dissipates the proton electrochemical gradient built up after proton pumping by the respiratory complexes. When this gradient reaches high values this makes proton pumping and thus substrate oxidation less easy and therefore slows down respiration. Activity of UCP1 prevents this rise of the proton gradient and therefore allows respiration to occur at a high rate, without phosphorylation of ADP into ATP, and therefore energy is instantaneously released as heat. The essential role of the UCP1 in thermogenesis is illustrated by the cold intolerance of mice whose ucp1 gene has been disrupted (3). Recently, two genes coding for proteins highly homologous to UCP1 have been described (reviewed in Refs. 4–6). Although there are experimental evidence supporting the hypothesis of an uncoupling activity of these proteins (7, 8), their physiological relevance is still incompletely resolved (9–11). We intended to obtain transgenic mice overexpressing the UCP1 in skeletal muscles, with the aim of examining the effects of the presence of this uncoupling protein on the pattern of myosin expression and metabolic characteristics of locomotor muscles. Two other reports published describe transgenic mice either overexpressing UCP3 (12) or expressing relatively modest amounts of UCP1 in muscle (13). In both cases increased energy expenditure resulted in a lower body weight. Few or no functional data about the activity of the uncoupling protein in vivo or in vitro have been produced. In our model of transgenic mice we have obtained a high expression level of UCP1 in skeletal muscles and heart. We produce data concerning the activity of the UCP1 in isolated mitochondria and in vivo using NMR of phosphorylated intermediates. UCP1 expression in skeletal muscle induced many alterations in muscle mass, myosin composition, and metabolic characteristics, which were related to the func-

\(^{1}\) The abbreviations used are: UCP1, uncoupling protein 1; MCK, muscle creatine kinase; MHC, myosin heavy chain; PCr, phosphocreatine; CCCP, carbonyl cyanide p-chlorophenylhydrazone; Ap, A, P, P-\(\text{d(adenosine 5')-pentaphosphate.} \)
tion and contractile activity of muscles. We examined how these alterations could be explained by the functional characteristics of UCP1.

**EXPERIMENTAL PROCEDURES**

**Generation of Transgenic Mice**—By the use of classical molecular biology techniques, the cDNA coding for the rat UCP1 associated with the SV40 polyadenylation sequence was ligated at the BatEIII site of the mouse muscle creatine kinase (MCK) promoter from the ~3000MCRCAT construct, kindly provided by Dr. S. Levak Franck and Dr. S. D. Hauschka (14). The BatEIII site is located seven bases downstream of the transcription start site. Therefore, the MCK promoter drives the transcription of the rat UCP1 mRNA. A PstI site was added after the SV40 polyadenylation sequence by site-directed mutagenesis. This plasmid, called pMCK-UCP1, was used to prepare the DNA injected into mouse eggs. The integrity of the UCP1 cDNA sequence was checked by DNA sequencing.

Digestion with PstI released the transgene (MCK promoter, UCP1 cDNA, SV40 polyadenylation site) free of vector sequences. This fragment called MCK-UCP1 was isolated from an agarose gel by electrophoresis. The DNA was microinjected into pronuclei of single cell embryos obtained from B6D2F1 parents (Ifla-Credo l’Arbresle, France) and the injected embryos were transplanted into the oviduct of pseudopregnant females. After preparation of DNA from tail, transgenic offspring were identified by PCR amplification of the rat UCP1 cDNA. Positives thus identified were checked for the integrity of the transgene by performing Southern blot analysis. Our attempts to establish stable homozygous lines of both lines have hitherto failed. Although mating of homozygotes could yield progeny, subsequent mating failed. Consequently, the animals used were the offspring of matings of hemizygotes, and control animals were littermates of the transgenics, which provide the best control available with these progeny of hybrid mice.

**Phenotypical Characterization of Mice**—The body weights of the mice were determined weekly. As controls we used offspring of the same strain (B6D2F1) that were not transgenic for the MCK-UCP1 gene included among the mice. Mice (10–50 weeks of age) were housed individually, and their body weight and food intake was measured at 100 g) consumed by the mice. Mice (10–46 weeks of age) were sacrificed and dissected to measure the fresh weight of different organs.

Dual energy x-ray absorptiometry allows the determination of fat mass (15). The measurements were performed with a PIXimus mouse densitometer (Lunar France, Lambesc, France). Mice (10–46 weeks of age) were anesthetized with an intraperitoneal injection of pentobarbital (0.6 mg/kg body weight) administered intraperitoneally. Gastrocnemius, plantaris, and soleus muscles were excised, cleaned of adipose and connective tissue, and wet weighed. Muscles were slightly stretched to recoil passively to roughly resting length, mounted in an embedding medium (TEK O.C.T. compound), and frozen in isopentane cooled to its freezing point (~160 °C) by liquid nitrogen. Changes in the relative amounts of MHC isoforms were assessed using immunohistochemical detection of MHCs within single muscle fibers, and SDS-PAGE to separate MHC isoforms in whole muscle.

Serial transverse sections (10 μm thick) were cut from the mid belly portion in a cryostat maintained at ~20 °C and were incubated in a humid chamber in working solutions of mouse monoclonal antibodies that reacted either with slow type I (number NCL-MHCS, Novocastro, Newcastle upon Tyne, United Kingdom, or fast type IIa MHC (SC-71), or all adult fast and developmentally regulated epitopes but not with slow myosin (MY-32, Sigma). The avidin-biotin immunohistochemical protocol included the use of antibodies against antigens for immunostaining (Vector Laboratories, Burlingame, CA). A sample of ~400 fibers that were free from artifacts were randomly selected from fields equally distributed over the biopsy for single fiber MHC composition. Fibers were classified according to their staining profile as comprising type I, type IIa or type IIx, and/or type IIb MHC with the aid of a microscope linked to a computer-based image analysis system (Visiobab 200, Nikon-France). Negative control slides with omission of the primary antibodies were randomly included in the immunostaining procedures. Four to five areas were randomly selected on each sample and the mean fiber cross-sectional area was determined for fibers containing type I, type IIa or type IIx, and/or type IIb MHC with the aid of a microscope linked to a computer-based image analysis system (Visiobab 200, Nikon-France).

**RESULTS**

**Transgenic Lines Expressing UCP1 in Muscle and Heart**—Two independent transgenic lines were obtained. Both express UCP1 mRNA in skeletal muscles and heart (Fig. 1). No expression of the UCP1 mRNA was found in liver or brain, indicating that the tissue specificity of the MCK promoter was maintained in these transgenic lines. However, the expression level of the UCP1 mRNA varies from one muscle to another, and these variations were not consistent in the two transgenic lines: the MCK-UCP1–13 showed highest expression in the heart, and the MCK-UCP1–20 in the gastrocnemius. The other muscles
UCP1 Expression in Muscle Decreases IIb Fiber Content

Fig. 1. Expression of the transgene. Top, 10 μg of total RNA was loaded in each lane. This blot was prepared with RNA from female mice. The muscles from 6 hemizygous animals were pooled for mRNA preparation. The blot was probed with a cDNA containing the entire coding sequence for rat UCP1 produced by PCR, and labeled with 32P by random priming. Mitochondrial transcription factor 1 (Tfam) probe was prepared from the plasmid pDG5 (20), containing the mouse mitochondrial transcription factor A gene (GenBank™ accession number U57939). The MEF2C probe (GenBank™ accession number U30823) was obtained from Dr. M. Buckingham, Institut Pasteur (Paris). The 18 S rRNA probe was a gift from David Tuil, Institut Cochin (Paris). The rat cyclophilin probe (GenBank™ accession number M19533) was prepared from the p1B15 plasmid (34). These two latter probes were used to assess the actual quantity of RNA transferred in each lane. Bottom, Western blot of mitochondrial preparations. 2 μg of protein in each lane. L, liver; H, heart; M, skeletal muscle (leg and upper leg); B, brown adipose tissue. The transgenic line is indicated (MCK-UCP1–13 or MCK-UCP1–20). Control, non-transgenic mice.

examined (soleus and plantaris) showed relatively similar mRNA levels in both transgenic lines. The expression of the mitochondrial transcription factor 1 (20, 21) was examined as well as the expression of the MEF2C transcription factor (22, 23) (Fig. 1). A quantitative analysis showed no consistent correlation between UCP1 mRNA and mitochondrial transcription factor 1 mRNA expression levels nor was there a correlation between UCP1 mRNA and mitochondrial transcription factor 1 mRNA expression levels. The expression of the MEF2C transcription factor (22, 23) was examined (soleus and plantaris) showed relatively similar mRNA levels in both transgenic lines. The expression of the mitochondrial transcription factor 1 (20, 21) was examined as well as the expression of the MEF2C transcription factor (22, 23) (Fig. 1). A quantitative analysis showed no consistent correlation between UCP1 mRNA and mitochondrial transcription factor 1 mRNA expression levels nor was there a correlation between UCP1 mRNA and mitochondrial transcription factor 1 mRNA expression levels (data not shown).

The amount of UCP1 in the mitochondria isolated from leg muscles or heart was estimated by Western blotting (Fig. 1). A similar amount of UCP1 was found in the skeletal muscle mitochondria of both lines, and this amount compares well with the level of UCP1 in brown fat mitochondria. As expected, the two transgenic lines differ with respect to the amount of UCP1 found in the heart mitochondria with a significantly higher level found in the MCK-UCP1–13 line.

Phenotypic Alteration of Mice—The body weight of mice was measured (see Fig. 2). After 5 weeks transgenic mice showed an almost stable deficit in body weight of 30%. Animals were dissected, and the weight of different organs was measured. For most organs (Table I) the relative weight was unchanged. Brain and liver preserved their absolute weight and consequently showed a higher relative weight in transgensics (data not shown). The comparison of the lean and fat mass measured by x-ray tomography (Fig. 2, bottom) indicated that transgenic mice and 64% (7) of their controls shared an identical percent-age of body fat of about 15%. On the other hand, in this experiment 36% (4) of the control animals had a significantly higher percentage of body fat. Therefore two types of animals

Table I

| Organ        | Control | MCK-UCP1 |
|--------------|---------|----------|
| Leg          | 38.9 ± 0.37 (12) | 24.2 ± 0.29 (19) |
| Heart        | 5.3 ± 0.04 (12)  | 5.2 ± 0.07 (19)  |
| Brain        | 15.1 ± 0.22 (9)   | 21.8 ± 0.24 (13) |
| Liver        | 51.1 ± 0.63 (12)  | 62.1 ± 0.32 (19) |
| Kidneys      | 15.2 ± 0.21 (12)  | 14.7 ± 0.11 (19) |
| Lung         | 6.3 ± 0.12 (12)   | 6.6 ± 0.12 (12)  |
| Spleen       | 3.5 ± 0.08 (10)   | 4.1 ± 0.11 (16)  |
| Uterus       | 4.4 ± 0.10 (6)    | 4.9 ± 0.16 (11)  |

a p ≤ 0.001.

Fig. 2. Body weight and percent of fat. Top, the relative body weight values for females are indicated for even weeks, whereas the values for males are indicated for odd weeks. □, control mice; females: 3 < n < 26 mean = 11; males: 7 < n < 19 mean = 12. The 100% value is the body weight of control mice at 10 weeks of age (22.4 g ± 0.24, n = 26 for females; 27.9 ± 0.34, n = 27 for males). These control mice are brothers and sisters of transgenic mice. ○ refers to the values determined for the B6D2F1 genotype by the supplier of these hybrid mice (Ifas Credo), n = 10; ▲, MCK-UCP1–20, females, 10 < n < 28, mean = 13; males, 6 < n < 22, mean = 13; ■, MCK-UCP1–13, females, 3 < n < 12, mean = 8; males, 3 < n < 10, mean = 7. Only three values of the S.E. are above 5%, therefore error bars are omitted for clarity. Gray diamonds represent the relative body weight (mean value, 4 < n < 15) of animals bearing four insertion sites of the transgene. These mice were obtained by mating homozygotes of the two transgenic lines. Bottom, individual values of body weight and percent fat mass determined by x-ray tomography. Females are symbolized by circles and males by squares, transgensics (hemizygotes and homozygotes) are indicated by black symbols, and controls by white symbols.
Alterations in Fiber Type and Myosin Composition—Skeletal muscles of transgenic mice showed a reduced weight, which is illustrated by the significant decrease in the relative mass of the total leg (Table I). The normalized weights of the plantaris and gastrocnemius, two fast twitch skeletal muscles, from both MCK-UCP1 lines were markedly lower than those from control mice, whereas the relative mass of the soleus, a slow twitch skeletal muscle, and heart remained unchanged (Table III). The changes in myofiber size were examined in skeletal muscle. No significant alteration in the mean fiber cross-sectional area of fiber types I, Ila, or IIb/IIx was shown in soleus muscle and gastrocnemius, two fast twitch skeletal muscles, from both MCK-UCP1 lines were markedly lower than those from control mice, whereas the relative mass of the soleus, a slow twitch skeletal muscle, and heart remained unchanged (Table III). The changes in myofiber size were examined in skeletal muscle. No significant alteration in the mean fiber cross-sectional area of fiber types I, Ila, or IIb/IIx was shown in soleus muscle and gastrocnemius, two fast twitch skeletal muscles, from both MCK-UCP1 lines were markedly lower than those from control mice, whereas the relative mass of the soleus, a slow twitch skeletal muscle, and heart remained unchanged (Table III).

### Table II

**Food intake**

| Age (weeks) | Body weight (g) | Food intake (g/day) | KJ/day/BW | KJ/day/BW (a) |
|------------|-----------------|---------------------|-----------|--------------|
| Females    |                 |                     |           |              |
| Control n = 14 | 19.5 ± 1.3  | 22.7 ± 1.1  | 3.0 ± 0.2  | 2.67 ± 0.05  | 1.97 ± 0.18  | 4.25 ± 0.36  |
| Hemizygote n = 12 | 17.0 ± 1.8  | 17.9a ± 0.5  | 3.2 ± 0.1  | 2.59 ± 0.05  | 2.62b ± 0.08  | 5.26a ± 0.12  |
| Homozygote n = 11 | 19.4 ± 1.5  | 18.0b ± 0.4  | 3.3 ± 0.1  | 2.57 ± 0.05  | 2.62b ± 0.08  | 5.41b ± 0.17  |
| Males      |                 |                     |           |              |
| Control n = 12 | 24.3 ± 2.1  | 33.1 ± 1.5  | 3.6 ± 0.1  | 2.46a ± 0.07  | 1.62 ± 0.10  | 3.85 ± 0.20  |
| Hemizygote n = 18 | 20.8 ± 2.3  | 22.3b ± 0.7  | 3.8 ± 0.1  | 2.46a ± 0.07  | 2.49b ± 0.13  | 5.32b ± 0.13  |
| Homozygote n = 7  | 21.0 ± 3.2  | 24.2b ± 1.8  | 4.0 ± 0.2  | 2.49b ± 0.13  | 5.30b ± 0.23  |

* p < 0.05.
* p < 0.01.
* p < 0.001.

### Table III

**Muscle relative weight**

| Muscle                | Control n = 5 | Hemizygote n = 8 | Homozygote n = 10 |
|-----------------------|---------------|------------------|-------------------|
| Plantaris             | 1.26 ± 0.09   | 0.82 ± 0.07      | 0.69 ± 0.03       |
| Gastrocnemius         | 8.57 ± 0.35   | 4.98 ± 0.54b     | 4.31 ± 0.42       |
| Soleus                | 0.54 ± 0.03   | 0.55 ± 0.04      | 0.55 ± 0.03       |
| Myocard               | 4.67 ± 0.03   | 4.69 ± 0.18      | 4.95 ± 0.22       |

* p ≤ 0.01.
* p ≤ 0.001.

The relative content of MHC isoforms, as estimated by SDS-PAGE analysis, was affected both in plantaris and gastrocnemius muscles of MCK-UCP1 mice (Table IV). A fast to slow shift in myosin isoforms was observed in plantaris muscle from transgenic mice, with a slight increase in type I MHC (p < 0.05), a marked increase in type Ila MHC, type IIX MHC, and a concomitant decrease in the percentage of type IIB MHC (p < 0.001). Similar results were noted in gastrocnemius, whereas the MHC composition of the soleus muscle was unaffected in transgenic mice.

Changes in the fiber type distribution were consistent with...
alterations in the relative content of MHC isoforms. A significant increase in the percentage of fibers containing type I or type IIa MHC was observed in fast twitch muscles of transgenic mice, in comparison with control mice, whereas the percentage of type Ix/Ib fibers was decreased. No modification in fiber type distribution was observed in soleus muscles.

Fig. 4 compares several enzymatic activities in muscles. Citrate synthase activity and cytochrome oxidase (data not shown) were used as an index of mitochondrial abundance. With the notable exception of gastrocnemius, there was a tendency toward a slightly lower activity of these enzymes in skeletal muscles and heart of transgenics. On the other hand, creatine kinase, adenylate kinase (data not shown), and lactate dehydrogenase, considered as markers of the fast glycolytic fibers, were significantly decreased in plantaris and gastrocnemius muscle of transgenics. These results clearly indicate that the difference in the PCr/ATP ratio cannot be explained by a decrease in the creatine content relative to ATP. Quantitative data are presented in Table V. The creatine content of muscles was also measured, in fact we found slightly higher creatine concentrations in transgenics. These results, clearly indicate that the difference in the PCr/ATP ratio cannot be explained by a decrease in the creatine level. Nor is it explained by different maximal enzymatic activities because NMR experiments were done at rest when a steady state was reached, and when hydrolysis of ATP in muscle was minimal.

Activity of the UCP1 in Muscle or Heart Mitochondria—Fig. 6 shows the influence of known activators/inhibitors of UCP1 uncoupling activity on muscle mitochondria of control mice and transgenics. After inhibition of the ADP/ATP translocase by carboxyatractylate, addition of palmitic acid had no effect on the membrane potential or on the respiratory rate of muscle mitochondria from control mice. Unlike with mitochondria from transgenics, addition of the same amount of palmitic acid produced a marked decrease in membrane potential accompanied by an increase in respiratory rate, both effects being completely reversed after addition of GDP (Fig. 6A). These antagonistic effects of fatty acid and GDP revealed the presence of a fully active UCP1 in the mitochondrial inner membrane (18). Similar results were obtained with heart mitochondria of MCK-UCP1–13 mice. In Fig. 6B, the response of muscle mitochondria to ADP is shown before or after GDP addition. GDP addition had no effect on control muscle mitochondria, because the two “ADP cycles” occurred identically. In contrast, before GDP addition transgenic muscle mitochondria showed a deteriorated ADP response with respect to controls; the respiratory control was much lower and more time was needed to

### Table IV
**Myosin isoforms**
The mean ± S.E. of the percentage of the different isoforms of myosin heavy chain. The statistical significance was evaluated by means of analysis of variance.

|                | Control n = 5 | Heterozygote n = 8 | Homozygote n = 10 |
|----------------|--------------|--------------------|-------------------|
| **Soleus**     |              |                    |                   |
| MHC I          | 54.1 ± 5.0   | 45.2 ± 3.7         | 41.0 ± 2.1        |
| MHC Ia         | 21.1 ± 4.4   | 26.1 ± 4.7         | 26.5 ± 3.2        |
| MHC Ix         | 8.1 ± 2.1    | 8.7 ± 2.2          | 9.5 ± 0.8         |
| MHC IIb        | 16.7 ± 5.8   | 20.0 ± 4.7         | 22.9 ± 4.0        |
| **Gastrocnemius** |            |                    |                   |
| MHC I          | 2.6 ± 0.4    | 6.2 ± 1.1          | 5.6 ± 0.6         |
| MHC IIa        | 6.1 ± 2.3    | 12.6 ± 1.1         | 15.8 ± 1.7        |
| MHC Ix         | 8.2 ± 2.2    | 21.3 ± 3.5         | 20.6 ± 1.9        |
| MHC IIb        | 83.1 ± 4.7   | 59.9 ± 4.6         | 58.2 ± 3.7        |
| **Plantaris**  |              |                    |                   |
| MHC I          | 2.4 ± 0.5    | 2.9 ± 0.6          | 5.1 ± 0.9         |
| MHC Ia         | 11.1 ± 0.7   | 23.3 ± 1.5         | 26.9 ± 2.9        |
| MHC Ix         | 15.5 ± 3.6   | 40.2 ± 5.3         | 37.3 ± 4.2        |
| MHC IIb        | 71.0 ± 3.8   | 32.5 ± 6.6         | 30.7 ± 6.0        |

* p < 0.05.
* p < 0.01.
* p < 0.001.

**FIG. 4.** Enzyme activities in muscle. Enzymatic activities are expressed in international units. Values are given as mean ± S.E. Control, n = 4; transgenics (hemizygotes), n = 6; three MCK-UCP1–13 and three MCK-UCP1–20.

**FIG. 5.** NMR spectra of phosphorylated intermediates in muscle. NTP, nucleotide triphosphate.
phosphorylate the same amount of ADP into ATP. After inhibition of UCP1 by GDP, the ADP cycle occurred as with the control mitochondria.

In the stationary state, the backflow of protons across the inner membrane compensates for the proton pumping by the respiratory chain. If we assume a constant stoichiometry between proton pumping by the respiratory chain and oxygen consumption, then the backflow of protons across the inner membrane is linearly related to the respiratory rate. According to Ohm’s law the proton flux is determined by both the membrane potential and membrane conductance. The curves showing the resulting membrane potentials for the decreasing state 4 respiratory rates generated after gradual inhibition of succinate dehydrogenase by malonic acid could be used to calculate the conductance of the inner membrane to protons at variable membrane potentials. This curve is modified in mitochondria from transgenics (Fig. 7). Addition of albumin (fatty acid chelator) diminishes the conductance, as does GDP. Finally, in the presence of both inhibitors (GDP + albumin) the conductance of the mitochondrial inner membrane of transgenics is unchanged in comparison to control. Therefore the protonophoric activity of the UCP1 can be fully inhibited whatever the value of the membrane potential. Neither GDP nor albumin alone could lead to full inhibition of UCP1 in mitochondria, both are requested. This calls to mind the observation made a long time ago with brown adipose tissue mitochondria where UCP1 is naturally present (24). If the conductance of the membrane was constant for all potential values, the curves would be straight lines. This is not the case, at low membrane potential values the conductance remains relatively low, whereas at higher potentials the conductance increases abruptly. When UCP1 is absent or inactive, the respiratory rate (conductance) increases 6–10-fold between 130 mV, the value of potential in state 3 (Fig. 6B), and the maximal value attained in state 4 (>170 mV). Although less marked, this “non-Ohmicity” of the curves is still apparent in the presence of UCP1, and the difference in proton leakage at the potential observed in state 3 (130 mV) and the maximal value in state 4 remains considerable as soon as UCP1 is partially inhibited.

Mitochondrial Oxidation of L-α-Glycerophosphate—The comparison of mitochondria from type IIb and type I fibers (25) showed that the glycerophosphate dehydrogenase is present at a 10 times higher level in mitochondria from type IIb fibers. The relative oxidation of succinate and of L-α-glycerophosphate by muscle mitochondria from wild-type and transgenic mice is shown in Fig. 8. ADP and the uncoupler CCCP were added to increase the respiratory rate and thus to evidence the respiratory control. The conditions used led to complete inhibition of the UCP1 and no difference was found in the presence of succinate. At the opposite, in the presence of L-α-glycerophosphate mitochondria from transgenics failed to show an increase in respiratory rate after addition of ADP or CCCP indicating a poor capacity of these mitochondria to use L-α-glycerophosphate as a substrate. Under the same conditions, the membrane potential of individual mitochondria was evaluated by rhodamine 123 staining and flow cytometry (Fig. 9). A large majority of the particles showed energization upon addition of substrate (true mitochondria), but a minority remained at the fluorescence values observed in the absence of membrane potential (non-mitochondrial particles or damaged mitochondria). In the presence of succinate, energizing particles (mitochondria) from transgenic muscles reached the same value of membrane potential as the controls. With L-α-glycerophosphate the fluorescence of the whole population was lower than with succinate. Moreover, no mitochondria were able to reach the high potential values attained in the presence of succinate. L-α-Glycerophosphate led to an almost identical energization of mitochondria from transgenic mice, which is not contradictory to the experiment shown in Fig. 8, because a very modest respiratory rate is able to support energization of mitochondria, whereas response to ADP and uncoupler required a significant increase in the respiratory rate. In fact the distribution obtained with mitochondria from transgenic mice showed a slightly higher frequency in the lower fluorescence values, which reflects the reduced ability of these mitochondria to utilize L-α-glycerophosphate.

**DISCUSSION**

Two recently published reports have described how a moderate expression of UCP1 (13) or an enhancement of the expression of UCP3 (12) in the muscle of transgenic mice results in a resistance to diabetes and obesity. This is well in line with the idea that expression of a bona fide uncoupling protein such as UCP1 or overexpression of its close homologue (UCP3) found in muscle is able to increase energy expenditure by mitochondrial uncoupling. Therefore these experiments illustrate how the recruitment of uncoupling proteins could help in the treatment of metabolic disorders like overweight and obesity in man. Our model of transgenic mice produced a slightly different phenotype probably because we obtained higher expression levels of UCP1 in muscle. Actually mice with similar properties have probably been obtained (13), but little characterization of the effects of the UCP1 overexpression on muscle contractile and metabolic phenotypes were provided, nor was the activity of the uncoupling protein in muscle mitochondria examined.

Our transgenic mice showed a significant reduction in body weight with a normal chow diet. It seems that we have attained a maximal effect of the UCP1 because increasing the copy number of the transgene by mating the two transgenic lines together did not decrease body weight further (Fig. 2). Moreover, in subsequent experiments no significant difference was observed when a distinction was made between hemizygote and homozygote mice for the transgene (Tables II–IV). Similarly, no difference in the phenotypical alterations provided by the transgene was found between the two transgenic lines (Fig. 3, Table IV). The food intake of transgenic mice equalled that of controls but their reduced body weight made them hyperphagic when the food intake was expressed relative to body weight (Table I). The relationship between energy expenditure and body weight is shifted toward hypermetabolism in transgenic mice (Fig. 3), indicating that UCP1 is likely to be active as an uncoupler in muscle in vivo. This conclusion is strengthened by...
the observation that despite a normal total creatine content, the ratio between phosphocreatine and ATP at rest is decreased in transgenics. The relative contribution of muscles to the body weight is decreased, whereas it is unchanged or even increased for the other organs studied (Table I). The case of the adipose tissue is of special interest (Fig. 2): whereas no difference was found in the percent body fat between transgenics and most of the controls, it seems likely that the presence of UCP1 prevented the rise in body fat percentage observed in several of the control animals. The reduction in muscle mass was mainly observed in fast muscles and explained by a decreased fiber cross-sectional area of fast twitch fibers but likely also by a shift toward type IIx and IIa fibers, at the expense of type IIb.

The experiments shown were done with mitochondria from MCK-UCP1–13 males (hemizygotes) and their littermate controls. Similar results were obtained with mitochondria from the MCK-UCP1–20 transgenic line. A, effect of palmitic acid and GDP under state 4 conditions; top, control mitochondria; bottom, mitochondria from MCK-UCP1 mice. The values of the respiratory rate in nanomole of O₂/min/mg of protein are indicated below the oxygen electrode trace (Ox). Membrane potential values in millivolts are indicated in italics above the TPP⁺ electrode trace (mV). Additions: CAT, carboxyatractylate, 1 μM final; Palm., palmitic acid + 5 μM (increase of 1 unit in the ratio palmitic acid to albumin); GDP, GDP + 0.5 mM; CCCP, CCCP + 150 mM. B, response to ADP of muscle mitochondria. ADP, +150 nmol of ADP. The values of respiratory control ratios are indicated. The rest of the legend is as above.
this latter type being known as thicker (26). As expected, this fast to slow transition in fiber type composition was paralleled by concomitant decreases in the activity of creatine kinase and lactate dehydrogenase, two mainly glycolytic enzymes (Fig. 4). It was also seen in isolated mitochondria because muscle mitochondria from transgenic mice had a reduced ability to oxidize α-glycerophosphate (Figs. 8 and 9). Finally, our model of transgenesis indicates that a high amount of functional UCP1 in heart mitochondria seems to have no influence on the heart under normal conditions. A first explanation would be that endogenous concentrations of nucleotides keep UCP1 completely inhibited in the heart. A second explanation would be that regular contraction, hence a high ATP demand, would render UCP1 without influence on cardiac muscle metabolism, and on differentiation. This second explanation is consistent with the observations made in skeletal muscles: the soleus, a slow twitch postural muscle, predominantly composed of slow oxidative fibers, recruited during quiet standing and over-ground locomotion (27), is protected from the effects of UCP1 in vivo by concomitant decreases in the activity of creatine kinase and lactate dehydrogenase, two mainly glycolytic enzymes (Fig. 4). In contrast, the plantaris and gastrocnemius showed a significant decrease in their mass, glycolytic capacity, and type IIb MHC isoform content. The mechanism by which the presence of UCP1 may have different effects according to the contractile activity of different muscles could be easily explained by the dependence of the proton leak through UCP1 on the membrane potential value (Fig. 7), compared with the values of membrane potential shown in Fig. 6.B: 130 mV when phosphorylation occurred (state 3), and 170 mV after conversion of all the ADP into ATP (state 4) when a maximal value of the ATP/ADP ratio was reached. As we said before, UCP1 is very likely to be active as an uncoupler in the muscle of transgenic mice. On the other hand it is very likely that the endogenous concentrations of nucleotides maintain it under partial inhibition. One may therefore conclude that the state of UCP1 in vivo ranges between the extreme situations of no inhibition (black circles) and complete inhibition (black squares), shown in Fig. 7. Accordingly in state 3, with a membrane potential of 130 mV, the fraction of the respiratory rate diverted from ATP synthesis can be deduced from the ordinate in Fig. 7. It appears that it is a minor or negligible fraction in comparison with the value of the respiratory rate in state 3, which exceeds 100 nmol of O₂/min/mg of protein (Fig. 6B). However, when mitochondria are in state 4 the rise in membrane potential dramatically enhances the influence of UCP1, for example, in Fig. 7 at 170 mV the proton leak because of the incompletely inhibited UCP1 (white circles) almost doubled the respiratory rate in comparison with the situation of complete inhibition in muscle mitochondria. In heart mitochondria the influence was even more dramatic because the incompletely inhibited UCP1 prevented the membrane potential reaching the value of 170 mV, something also observed under the conditions used for the traces shown in Fig. 6. Therefore in muscles undergoing sustained or repetitive contractions, where mitochondria operate in state 4, UCP1 would induce a marginal loss of metabolic energy, whereas in muscles at rest where mitochondria are expected to operate in a state close to state 4, UCP1 would induce a significant increase in mitochondrial respiration and a decreased membrane potential. In this respect, “from the point of view of the mitochondrial respiratory chain,” the presence of UCP1 mimics a moderate but continuous ATP production.

The hypothesis that UCP1 has virtually no effect when ATP production is intense is not in contradiction with the results of the NMR experiment, because the latter was done at rest. Consequently, muscle mitochondria are expected to be mainly in state 4, and the UCP1 effect would be the strongest. The
creatinine kinase reaction is assumed to be near equilibrium (28) and the PCR/ATP ratio negatively correlates with cytosolic free ADP (29). The observed decrease in PCR/ATP ratio thus indicates an increase in cytosolic ADP in transgenic mice. In terms of inorganic phosphate, any change would be an increase in transgenic mice. This increase in ADP therefore reflects a decrease in phosphorylation potential. According to Mitchell’s theory the latter would be the consequence of an impaired &Delta;μH⁺ across the inner membrane and the decrease in the PCR/ATP ratio is a direct consequence of the UCP1 protonophoric activity. An alternative could be that type IIb fibers, which are less abundant in transgenics, have the highest PCR/ATP ratios to be reached. However, it is still expected that these PCR/ATP ratios equilibrate with the maximal mitochondrial potential of mitochondria, otherwise ATP would be used by mitochondria. Consequently, this would predict that control mice possess more mitochondria, thereby sustaining a higher membrane potential in the presence of substrate. This is poorly supported by our experimental data with flow cytometry that allows the detection of subpopulations. First l-α-glycerophosphate failed to produce in any mitochondria a polarization as high as succinate, and second the upper limits of membrane potential (fluorescence) seemed almost identical in controls and transgenics (Fig. 7). This therefore suggests that the decreased PCR/ATP ratio directly reflects UCP1 uncoupling activity rather than its indirect consequences on muscular fiber phenotype. However, no significant change in the activity of citrate synthase, a mitochondrial enzyme, was associated with the decreased expression of the fastest myosin isoform in fast muscles. It has been hypothesized that the imbalance between energy requirement and energy supply may explain the responses of muscle to endurance training. However, the fast to slow conversion observed in transgenic mice differs from that in skeletal muscle after endurance training, which is mainly determined by an increase in oxidative capacity. This suggests either that distinctive pathways control the expression of genes of the oxidative metabolism, or that there is a gradation of the effects according to the extent of mitochondrial recruitment. This latter hypothesis may be explained because in these transgenic mice the increase in mitochondrial respiratory activity because of UCP1 in state 4 is expected to be marginal in comparison with the full respiratory capacity of mitochondria, and the decrease in membrane potential modest with regard to the decrease induced by oxidative phosphorylation in state 3. This study gives an example where modifications of mitochondrial characteristics, regardless of the ATP production rate, influence gene expression.

Acknowledgments—We thank Marie France Chapey for technical assistance, and Laurence Bernard, Danièle Chamereau, and Edwige Declerq for dedicated care to animals.

REFERENCES

1. Nicholls, D. G., and Locke, R. M. (1984) Physiol. Rev. 64, 1–64
2. Klingenberg, M., Echtay, K. S., Bienengraeber, M., Winkler, E., and Huang, S. G. (1999) Int. J. Obes. Relat. Metab. Disord. 23, Suppl. 6, S24–S29
3. Ernestbäck, S., Jacobson, A., Simpson, E. M., Guerra, C., Yamashita, H., Harper, M. E., and Kokaz, L. P. (1997) Nature 387, 90–94
4. Ross, O., Hagen, T., and Lowell, B. B. (2000) Diabetes 49, 143–156
5. Billonvaud, V., Coplan, E., Remmer, C., and Riequier, D. (2001) Biochim. Biophys. Acta 1504, 107–119
6. Riequier, D., and Bouillaud, F. (2000) Biochem. J. 345, 161–179
7. Rial, E., Gonzalez-Barroso, M., Fleury, C., Iturrizaga, S., Sanchis, D., Jimenez-Jimenez, J., Riequier, D., Goubert, M., and Bouillaud, F. (1999) EMBO J. 18, 5827–5833
8. Echtay, K. S., Winkler, E., Frischmuth, K., and Klingenberg, M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1416–1421
9. Vidal-Puig, A. J., Grujie, D., Zhang, C. Y., Hagen, T., Boss, O., Ido, Y., Szczepanek, A., Wade, J., Motta, V., Cortright, R., Musio, D. M., and Lowell, B. B. (2000) J. Biol. Chem. 275, 16538–16566
10. Gong, D. W., Menendez, S., Gervilova, O., Leon, L. R., Marcus-Samuels, B., Chou, C. J., Everett, C., Kokaz, L. P., Li, C., Deng, C., Harper, M. E., and Reitman, M. L. (2000) J. Biol. Chem. 275, 16251–16257
11. Arsenijevic, D., Osuna, H., Perpeux, C., Rainhaull, S., Manning, B. S., Mireux, B., Coplan, E., Goubert, M. C., Goubert, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S., and Riequier, D. (2000) Nat. Genet. 26, 435–439
12. Clapham, J. C., Arch, J. R., Chapman, H., Haynes, A., Lister, C., Moore, G. B., Pierry, V., Carter, S. A., Lehner, I., Smith, S. A., Beeley, J. L., Godden, R. J., Herrity, N., Skelih, M., Changani, K. K., Hockings, P. D., Reid, D. G., Squires, B. M., Hatcher, J., Trail, B., Latcham, J., Rastan, S., Harper, A. J., Cadenas, S., Buckingham, J. A., Brand, M. D., and Abuin, A. (2000) Nature 406, 415–418
13. Li, B., Nolte, L. A., Ju, S. S., Han, D. H., Coleman, T., Holloszy, J. O., and Semenekov, C. F. (2000) Nat. Med. 6, 1115–1120
14. Jaynes, J. B., Chamberlain, J. S., Buskin, J. N., Johnson, J. E., and Hauschka, S. D. (1986) Mol. Cell. Biol. 6, 2855–2864
15. Sigmund, K., Hellberg, N., Savendahl, L., Johansson, M. S., Berglind, T., Rosarud, I., and Ohlsson, C. (2001) J. Nutr. 131, 2963–2966
16. Klaus, S., Munzberg, H., Truloff, C., and Heldmaier, G. (1998) J. Physiol. 523, R287–R293
17. Weir, J. R. (1949) J. Physiol. 109, 1–9
18. Locke, R. M., Rial, E., Scott, I. D., and Nicholls, D. G. (1982) Eur. J. Biochem. 129, 373–380
19. Tanigadzic, R. J., and Roy, R. R. (1993) J. Appl. Physiol. 75, 2337–2340
20. Larsson, N. G., Garman, J. D., Oldfors, A., Barsh, G. S., and Clayton, D. A. (1996) Nature Genet. 13, 296–302
21. Gordon, J. W., Rungu, A. A., Inagaki, H., and Hood, D. A. (2001) J. Appl. Physiol. 90, 389–396
22. McDermott, J. C., Cardoso, M. C., Yu, Y. T., Andres, V., Leifer, D., Krain, D., Lipton, S. A., and Nadal-Ginard, B. (1993) Mol. Cell. Biol. 13, 2564–2577
23. Leifer, D., Krainc, D., Yu, Y. T., McDermott, J., Breithart, R. E., Heng, J., Neve, R. L., Kosofsky, B., Nadal-Ginard, B., and Lipton, S. A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1546–1550
24. Nicholls, D. G., and Lindberg, O. (1973) Eur. J. Biochem. 37, 523–530
25. Jackman, M. R., and Willis, W. T. (1996) Am. J. Physiol. 270, C673–C678
26. Sieck, G. C., Zhang, W. Z., Prakash, Y. S., Daoud, M. M., and Watchco, J. F. (1995) J. Appl. Physiol. 79, 1629–1639
27. Booth, P. W., and Thomason, D. B. (1991) J. Physiol. 417, 541–585
28. Veech, R. L., Lawson, J. W., Cornell, N. W., and Krebs, H. A. (1979) J. Biol. Chem. 254, 6538–6547
29. Lawson, J. W., and Veech, R. L. (1979) J. Biol. Chem. 254, 6528–6537
30. Ren, J. M., Ohira, Y., Holloszy, J. O., Hamalainen, N., Traub, I., and Pette, D. (1995) Pflugers Arch. 430, 389–393
31. Pette, D. (1998) Acta Physiol. Scand. 162, 367–376
32. Chin, E. R., Olson, E. N., Richardson, J. A., Yang, Q., Humphries, C., Shelton, J. M., Wu, H., Zhu, W., Bassel-Duby, R., and Williams, R. S. (1998) Genes Dev. 12, 2499–2509
33. Talmadge, R. J. (2000) Muscle Nerve 23, 661–679
34. Danielson, P. E., Forss-Petter, S., Brow, M. A., Calavetta, L., Douglass, J., Milner, R. J., and Sutcliffe, J. G. (1988) DNA 7, 261–267
High Level of Uncoupling Protein 1 Expression in Muscle of Transgenic Mice Selectively Affects Muscles at Rest and Decreases Their IIb Fiber Content

Elodie Couplan, Chantal Gelly, Marc Goubern, Christophe Fleury, Bruno Quesson, Mathieu Silberberg, Eric Thiaudière, Philippe Mateo, Michel Lonchampt, Nigel Levens, Catherine de Montrion, Silvia Ortmann, Susanne Klaus, Maria-del-Mar Gonzalez-Barroso, Anne-Marie Cassard-Doulcier, Daniel Ricquier, A. Xavier Bigard, Philippe Diolez and Frédéric Bouillaud

J. Biol. Chem. 2002, 277:43079-43088.
doi: 10.1074/jbc.M206726200 originally published online September 6, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M206726200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 13 of which can be accessed free at http://www.jbc.org/content/277/45/43079.full.html#ref-list-1