Expression of Lung Uncoupling Protein-2 mRNA Is Modulated Developmentally and by Caloric Intake

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Lung expresses a high concentration of uncoupling protein-2 (UCP-2) mRNA, but neither its pulmonary regulation nor function is known. We measured lung UCP-2 mRNA expression in two animal models: in neonatal rats when both the metabolic rate, as measured by oxygen consumption, and levels of serum free fatty acids (FFAs) increase and in adult mice during decreased food intake, when levels of serum FFAs increase but the metabolic rate decreases. In rat lung, the concentration of UCP-2 mRNA was low and unchanged during late gestation, increased approximately twofold within 6 hrs after birth, and, compared with late gestation, remained approximately threefold higher from day 1 to adulthood. The early postnatal rise in the lung UCP-2 mRNA concentration was partially blocked by an antithyroid drug and was increased by treatment with triiodothyronine. Unlike lung, heart UCP-2 mRNA levels were lower during adulthood than at day 15. In adult mice, lung UCP-2 mRNA concentrations increased approximately fivefold within 12 hrs of 67% calorie restriction (CR), remained elevated during 2 weeks of CR, fell to control levels within 24 hrs of refeeding (CR-RF), and positively correlated with serum FFA concentrations. Heart UCP-2 expression during CR and CR-RF was similar to that of lung; liver UCP-2 mRNA levels were slightly lower during CR and returned to control levels during CR-RF. These data suggest that the regulation of UCP-2 is at least partly tissue-specific and that, in the adult mouse, lung UCP-2 is regulated not by oxygen consumption but by FFAs. Moreover, lung UCP-2 mRNA levels in mice fed *ad libitum* was increased by the intraperitoneal administration of Intralipid, a 20% fat emulsion. On the basis of these data in adult mice, together with the findings of others that levels of FFAs increase by 2 hrs after birth, we propose lung UCP-2 is regulated by FFA. Exp Biol Med 229:479–485, 2004

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Uncoupling protein-2 (UCP-2) belongs to the uncoupling protein family, which is composed of mitochondrial inner membrane proteins with homology to UCP-1 (1). UCP-1 is found exclusively in brown adipose tissue and was termed an "uncoupling" protein because it conducts protons through the mitochondrial membrane down an electrochemical gradient without generating adenosine triphosphate (ATP), thereby uncoupling oxygen consumption from adenosine diphosphate phosphorylation (1–3). The function of UCP-1 is to dissipate energy stored in the proton electrochemical gradient as heat instead of ATP production (1–3). Four mammalian UCP-1 homologs (UCP-2 to UCP-4 and brain mitochondrial carrier protein-1) and two plant UCP homologs have been described (1). All have predicted mitochondrial transmembrane domains and UCP-specific sequences in the first, second, and fourth alpha helices (4). Whereas the function of UCP-1 is thermogenesis, the function and regulation of the UCP homologs are varied and, in many cases, still uncertain.

Whereas lung appears to express only UCP-2, UCP-2 mRNA is also expressed in a variety of organs, with a relatively larger amount in spleen, heart, lung, white adipose tissue, stomach, and testis and a lesser amount in brain, kidney, liver, and muscle (4–6). The UCP-2 amino acid sequence has high homology across species: rat UCP-2 is 99% and 95% identical to mouse and human UCP-2, respectively (7). The strong conservation of the sequence across species and its widespread expression among organs indicate that UCP-2 is a physiologically important protein. In addition to the uncoupling of respiration by proton transport and thermoregulation, UCP-2 has been proposed to play a role in lipid metabolism, insulin resistance, glucose utilization, the regulation of reactive oxygen species, and macrophage-mediated immunity (1, 8, 9).

A number of factors have been proposed to regulate the expression of UCP-2, including metabolic rate and free fatty

acids (FFAs). The goal of the present study was to examine the expression and regulation of lung UCP-2 mRNA in animal models that are physiologically relevant and during which the lung undergoes important alterations in lung structure, energy expenditure, and lipid metabolism (9–13). First, we measured lung UCP-2 mRNA expression during perinatal development, when FFAs and the metabolic rate, as measured by oxygen consumption, increase (13–15). The lungs of both rats and mice undergo morphological maturation during the first 2 postnatal weeks by septation of the saccules present at birth; the result is an increase in the number of alveoli and an increased surface area for gas exchange (16, 17). We chose to use rats for the perinatal study because of their relatively larger body weight and number of pups per litter. However, in neonates, FFAs are mainly derived from the dam's milk and are not easily experimentally altered. Therefore, for these studies, we chose to use adult animals in which the metabolic rate, FFAs, and number of alveoli can be experimentally manipulated by changes in food intake (12, 18–20). In addition, the use of two species increases the general importance of the study.

Materials and Methods

Animal Studies. Timed-pregnant Sprague Dawley rats were obtained from Taconic Farms, maintained in the Animal Care Facility of Georgetown University Medical Center on a 12:12-hr light:dark cycle, and allowed intake of food (Rodent Chow 5001; PMI Nutrition, Brentwood, MO) and water *ad libitum*. For developmental studies, pregnant rats were delivered by hysterotomy at gestational days 18–22 or allowed to deliver naturally at gestational day 22. Rats were designated to be 1 day old on the day of birth. At all ages tested, we used pups from a minimum of two separate litters. In addition, for the postnatal longitudinal studies, the litters were maintained at 10 pups per litter by 24 hrs after birth, to eliminate variances due to litter size. This was achieved by randomly mixing pups from three to six litters born on the same day, to minimize the effect of litter differences. In some experiments, immediately after birth, pups were injected subcutaneously with triiodothyronine (T3; Sigma Chemical Co., St. Louis, MO) at a dose of 0.1 μg/g body weight; other animals were treated with the same volume of diluent (0.075 *M* NaCl and 10 m*M* NaOH). Animals were killed 6 and 24 hrs after injection. Other rats were killed immediately after birth (zero time) without the administration of T3. Propylthiouracil (PTU) crosses the placenta and mammary gland (21) and blocks the synthesis of T3 and thyroxine (T4) in the thyroid gland and the conversion of T4 to T3 in peripheral tissues (22). Therefore, we used PTU to test the effect of blocking thyroid hormone. PTU (0.01% in tap water; Sigma) was made fresh every other day and was provided as drinking water for pregnant rat dams. PTU treatment was started at gestational day 12 and continued until the end of the experiment. We have previously shown (23) that this concentration of PTU in the tap water of dams affects the neonatal lung, decreasing lung volume, surface area, and surface-to-volume ratio. Control animals received regular tap water without PTU. Adult male Sprague-Dawley rats (Taconic Farms, Germantown, NY) at a body weight of ~250 gm (10–12 weeks of age) were used for the studies in adult rats.

For studies of calorie restriction (CR), we followed our protocol described elsewhere (19, 20). Adult male C57BL/6J mice (weight, ~20–25 g at age ~8 weeks) were purchased from Jackson Laboratories (Bar Harbor, ME) and placed in separate cages on their arrival at Georgetown University. The individual daily food (Rodent Chow 5001; PMI Nutrition) of each mouse was recorded over a period of at least 5 days. At the start of the experiment, animals in the CR group were fed one-third their normal food intake with *ad libitum* access to water. Food was given daily at 0900 hr. Animals in the group with CR followed by refeeding (CR-RF) were given food *ad libitum* after CR. In some experiments, mice fed *ad libitum* were injected at 0900 hr intraperitoneally with water or with 2 ml of Intralipid (Baxter, Deerfield, IL), a 20% fat emulsion that contains 20% soybean oil, 2.3% lecithin, and 4.5% glycerin; these mice were then killed 6 hrs after the injection.

All animal procedures adhered to the National Research Council's *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committee of Georgetown University. Animals were anesthetized by the intraperitoneal injection of xylazine (12 mg/kg) and ketamine (90 mg/kg), and they were killed by cutting the abdominal aorta. Lungs and other tissues were removed, frozen in liquid nitrogen, and stored at –70°C. In some experiments, after anesthesia, blood was obtained from the aorta and serum was collected to measure T3 using the I^{125} RIA Coat-A-Count Total T3 kit (Diagnostic Products Corp., Los Angeles, CA). In other experiments, blood was used to measure the level of FFAs in serum using the half-micro kit (Roche, Indianapolis, IN).

Generation of Murine UCP-2 cDNA. A mouse UCP-2 plasmid was generated using polymerase chain reaction (PCR). The PCR primers used for cloning mouse UCP-2 were sense, 5′-AGG AAG TCA GAA TCA TGG TTG GT-3′, and antisense, 5′-AGT ATC TTT GAT GAG GTC ATA GGT-3′. A cDNA strand was reverse transcribed (RT) from mouse lung total RNA using the Superscript kit (Gibco BRL, Gaithersburg, MD). The PCR products were cloned into the pCRII vector using the TA Cloning Kit (Invitrogen, Carlsbad, CA). The ligation product was transformed into INV'F-competent cells that were then plated on X-gal ampicillin plates for bacterial selection on the basis of interruption of the β–galactosidase gene. The insertion of the correct length fragment (603 bp) was confirmed by *Eco*RI restriction-enzyme digest. The sequence was verified using the Sanger dideoxy method with the Sequenase version 2.0 Kit (USB, Cleveland, OH).

Construction of Cytochrome c Oxidase, Subunit IV (Cox IV) cDNA. To measure mRNA expression of a second mitochondrial protein, Cox IV cDNA was generated using a procedure analogous to the construction of the mouse UCP-2 probe described above. The RT-PCR primers for Cox IV were sense, 5'-TCTTA TGRRG ATCGG CGTGA-3', and antisense, 5'-CTCA TTGGTG CCCTT GTTCA-3'. The Cox IV fragment was generated by RT-PCR using rat lung total RNA and cloned into pCRII/TA vector. The sequence of the 189-bp Cox IV insert was 95% homologous with the mouse Cox IV mRNA sequence and was used to measure both rat and mouse COX IV mRNA.

Measurement of UCP-2 and Cox IV mRNA. Total RNA was isolated from tissue using Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH). RNA was quantitated by absorbance at 260 nm. UCP-2 and Cox IV mRNA concentrations were measured by a ribonuclease protection assay (RPA) using the RPA II Kit (Ambion, Austin, TX). Antisense strand riboprobes were transcribed *in vitro* (Riboprobe Kit; Promega, Madison, WI) from a rat UCP-2 cDNA plasmid kindly provided by Dr. Wai-Yee Chan (Georgetown University) and from the rat Cox IV and mouse UCP-2 cDNA plasmids described above. For use as an internal standard, an 18s rRNA antisense probe was transcribed from an 18s RNA template (Ambion) using the MEGAshortscript T7 Kit (Promega). Ribonuclease-protected hybrids were resolved on a 6% nondenaturing polyacrylamide gel; the gel was dried under vacuum and exposed to Hyperfilm (Amersham, Piscataway, NJ) to visualize the bands. RNA was quantified using laser densitometry and analyzed using ImageQuant software (both from Molecular Dynamics, Sunnyvale, CA). Concentrations of UCP-2 and Cox IV mRNAs were expressed as densitometry units (DU) relative to the concentration of 18s rRNA in the same sample.

Statistical Analysis. Statistical calculations were done using StatMost Statistical Analysis and Graphics (version 2.5; DataMost Corp., Salt Lake City, UT). The values for individual samples were averaged per experimental group, and the SEM was calculated. The significance of the difference between two groups was obtained by an unpaired, two-tailed Student's *t* test. The significance of the difference between more than two groups was determined by ANOVA with Duncan's multiple range test extension. Regression analysis was used to calculate the correlation coefficient between UCP-2 mRNA expression and serum levels of FFA.

RESULTS

Rat Lung UCP-2 mRNA Levels Are Markedly Increased at Birth. The UCP-2 mRNA concentration was low and unchanged during late gestation; between gestational day (gd) 21 and postnatal day 1, levels of UCP-2 mRNA increased approximately threefold (Fig. 1). UCP-2 mRNA expression was higher in all postnatal lungs compared with each fetal time point measured ($P < 0.05$). UCP-2 mRNA expression in adult rat lung was slightly but significantly higher compared with expression on postnatal day 1. In a separate set of experiments, the UCP-2 mRNA concentration increased significantly within 7 hrs after birth and, by 24 hrs, UCP-2 mRNA levels had increased approximately fourfold compared with gd 21 and gd 22 (Table 1). There was no difference in UCP-2 mRNA concentrations between sexes at any time point measured; moreover, the induction of UCP-2 after birth was similar in both sexes (Table 1). On the basis of these findings, we used both male and female rat pups in further studies.

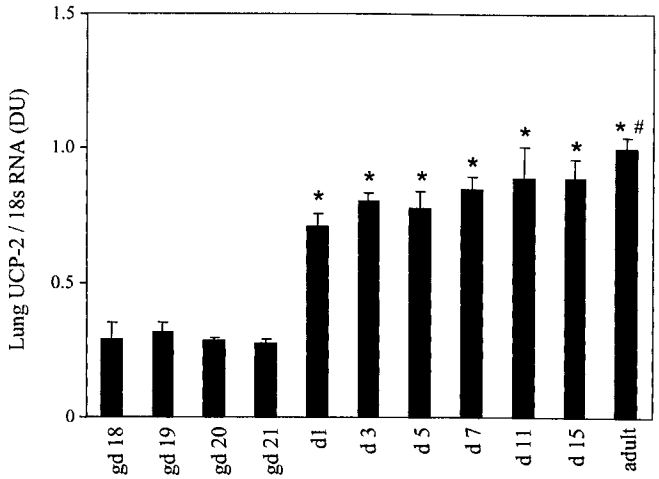

Figure 1. Rat lung UCP-2 mRNA expression during perinatal development. The RNA concentration was measured by RPA; data were quantified by densitometry and normalized to 18s rRNA. UCP-2 mRNA data are presented as relative DU mean ± SE. *n* = three lung samples at each age tested. *$P < 0.05$ compared with all time points examined in fetal lungs. #$P < 0.05$ compared with day 1.

Developmental Pattern of Rat UCP-2 mRNA Expression Differs Between Lung and Heart. To examine the specificity of our findings in lung, the expression of UCP-2 in rat heart was measured at different developmental ages. We found that the expression of UCP-2 mRNA in heart was low and unchanged during late gestation, was slowly upregulated after birth, and reached

Table 1. Comparison of Perinatal Rat Lung UCP-2 mRNA Concentrations, by Sex

Age	Female	Male	*P*
Gestation day 21	0.80 ± 0.10	0.91 ± 0.10	>0.05
Gestation day 22	1.12 ± 0.06	1.06 ± 0.11	>0.05
7 hrs after birth	3.52 ± 0.09[a]	3.58 ± 0.22[a]	>0.05
24 hrs after birth	4.58 ± 0.30[a]	4.05 ± 0.09[a]	>0.05
Postnatal day 1	3.05 ± 0.40[a]	2.65 ± 0.36[a]	>0.05
Postnatal day 7	3.68 ± 0.32[a]	3.77 ± 0.54[a]	>0.05

UCP-2 mRNA/18s rRNA DU. The data are given as mean ± SE of five separate lungs at each time point tested. *P* values are for female *vs.* male.

[a] $P < 0.05$ compared with gd 21 or 22.

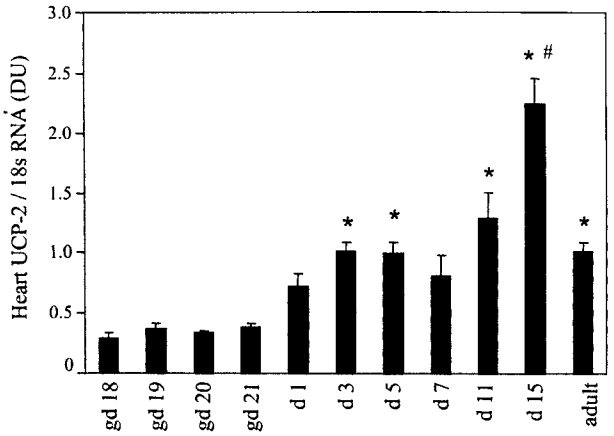

Figure 2. Rat heart UCP-2 mRNA expression during perinatal development. RNA concentration was measured as described in the legend of Figure 1. Data are given as mean ± SE of three separate heart samples at each age tested. *$P < 0.05$ compared with all time points examined in fetal hearts. #$P < 0.05$ compared with all other time points.

a high concentration at postnatal day 15 (Fig. 2). Unlike lung, the level of UCP-2 mRNA expression in heart was lower at adulthood than at postnatal day 15.

Rat Lung UCP-2 mRNA Expression Is Upregulated by Thyroid Hormone. At birth, we administered T3 to rat pups at a dose of 0.1 μg/g body weight. T3 and T4 are the two major forms of circulating thyroid hormones, but T3 is considered to be the physiological functional form of the hormone. Six hours after the T3 injection, all animals had serum thyroid hormone concentrations >600 ng/dl, a level markedly higher than in diluent-treated control rat pups, whose serum T3 levels were barely detectable (data not shown). Compared with untreated pups at birth, both the diluent-injected and T3-injected pups had a significantly higher expression of lung UCP-2 mRNA 6 hrs after birth (Fig. 3A). Moreover, UCP-2 mRNA concentrations were significantly greater at 6 hrs after birth in the lungs of T3-treated pups than in diluent-injected control pups (Fig. 3A). Given from gd 12 in the dam's drinking water, PTU, an antithyroid drug (21, 22), resulted in a lower concentration of lung UCP-2 mRNA in pups 6 and 24 hrs after birth compared with age-matched pups from dams given only tap water (Fig. 3B).

Mouse Lung UCP-2 mRNA Expression Is Modulated by Calorie Restriction and Refeeding and Correlates Positively with the Serum Concentration of FFA. Lung UCP-2 mRNA expression was increased 5-fold within 12 hrs of CR, remained elevated during 14 days of CR, and fell to control concentrations within 24 hrs of refeeding after either 72 hrs or 14 days of CR (Fig. 4A). The concentration of serum FFA was measured in the same mice in which lung UCP-2 mRNA levels were determined during CR and CR-RF (Fig. 4B). The concentrations of UCP-2 mRNA and FFA exhibited a positive correlation ($r = 0.64$; $P < 0.01$). In a shorter time-course study, mice were fasted (no food given) for 1, 3, and

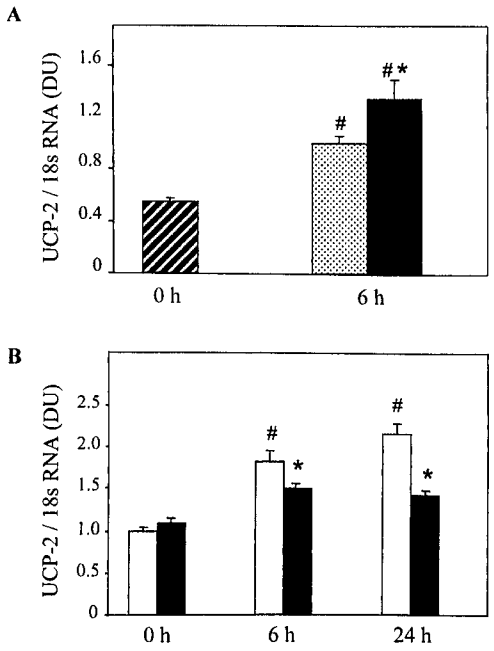

Figure 3. Regulation of perinatal rat lung UCP-2 mRNA expression by thyroid hormone. RNA concentration was measured as described in the legend of Figure 1. (A) At birth, rat pups were treated with T3 (0.1 μg/g body weight); age-matched control animals were given the same volume of diluent; zero-time control rats were untreated. Lungs were isolated at zero time and at 6 hrs after treatment with T3. Data are given as mean ± SE of five separate lungs at each time point tested. *$P < 0.05$ in the lungs of T3-treated rats (solid bar) compared with age-matched diluent treated rats (stippled bar). # $P < 0.05$ compared with the zero-time untreated rats. (B) Treatment with 0.01% PTU in rat dams' drinking water was started at gd 12. Control rats were given normal tap water. RNA was isolated from rat pup lungs at 0, 6, and 24 hrs after birth. Data are given as mean ± SE of five separate lungs at each time point tested. *$P < 0.05$ in PTU-treated pups (solid bars) compared with age-matched controls (open bars). #$P < 0.05$ compared with zero-time controls

6 hrs; another group of mice was refed for 1, 3, and 6 hrs following 72 hrs of 67% CR. Lung UCP-2 expression increased significantly after 3 and 6 hrs of fasting, was diminished within 1 hr of refeeding, and was not significantly different than that of fed control mice after 6 hrs of refeeding (Fig. 5). In these animals, lung UCP-2 mRNA concentrations correlated with serum FFA levels ($r = 0.61$; $P < 0.01$). Thus, FFA and UCP-2 RNA concentrations were altered concurrently and very quickly in response to changes in food intake.

CR and CR-RF Do Not Alter Cox IV mRNA Expression. To ascertain whether increased lung UCP-2 expression during CR was part of a general upregulation of mRNAs coding for mitochondrial proteins, lung Cox IV mRNA expression was measured during CR. Cox IV, like UCP-2, is coded by nuclear DNA and is transported into mitochondria after being translated. In contrast to UCP-2, Cox IV mRNA concentrations did not change in lung during CR and CR-RF, which indicates that, among mitochondrial proteins, the regulation of UCP-2 mRNA was at least partly specific (Fig. 6A).

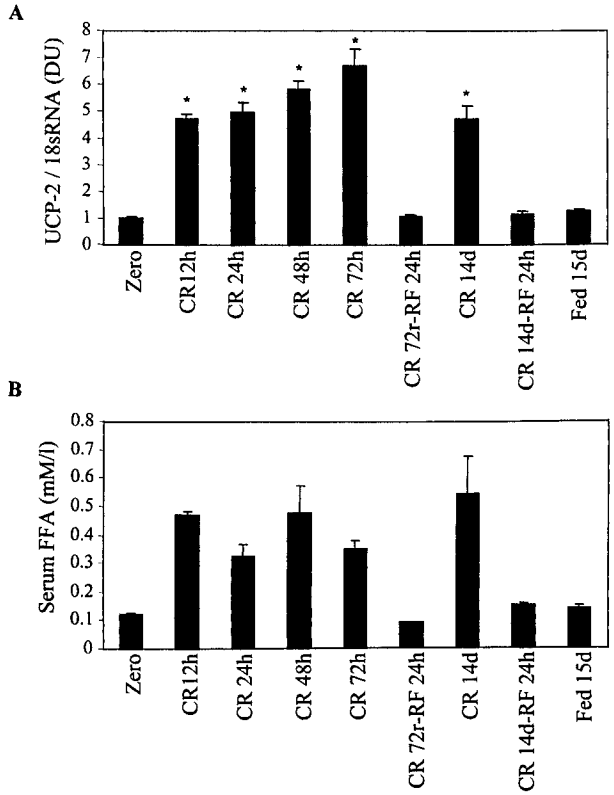

Figure 4. Time course of mouse lung UCP-2 mRNA expression during CR and CR-RF. (A) RNA concentrations were measured as described in the legend of Figure 1. UCP-2 mRNA data are given as mean ± SE of five separate mouse lungs at each time point tested. $^*P < 0.05$ compared with the time-zero group. (B) Blood samples were collected from the right ventricles of the mice used in panel A, and serum was obtained. The serum FFA level (in m*M*/l) was measured using the halfmicro kit (Roche). The concentrations of UCP-2 mRNA and FFA exhibited a positive Pearson correlation ($r = 0.64$; $P < 0.01$).

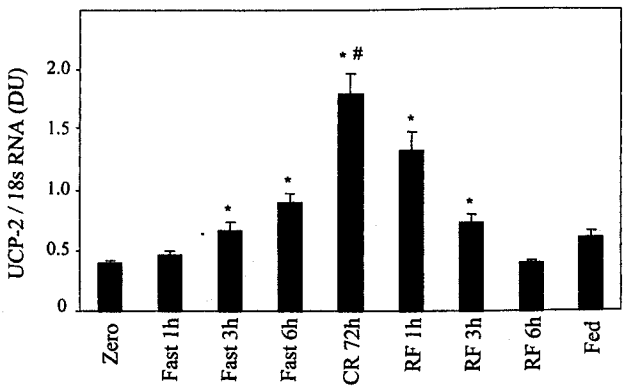

Figure 5. Lung UCP-2 mRNA expression during short-term fasting and short-term refeeding. The RNA concentration was measured as described in the legend of Figure 1. UCP-2 mRNA data are given as mean ± SE of five separate mouse lungs at each time point tested. $^*P < 0.05$ compared with the zero-time animals. $\#P < 0.05$ compared with all other groups.

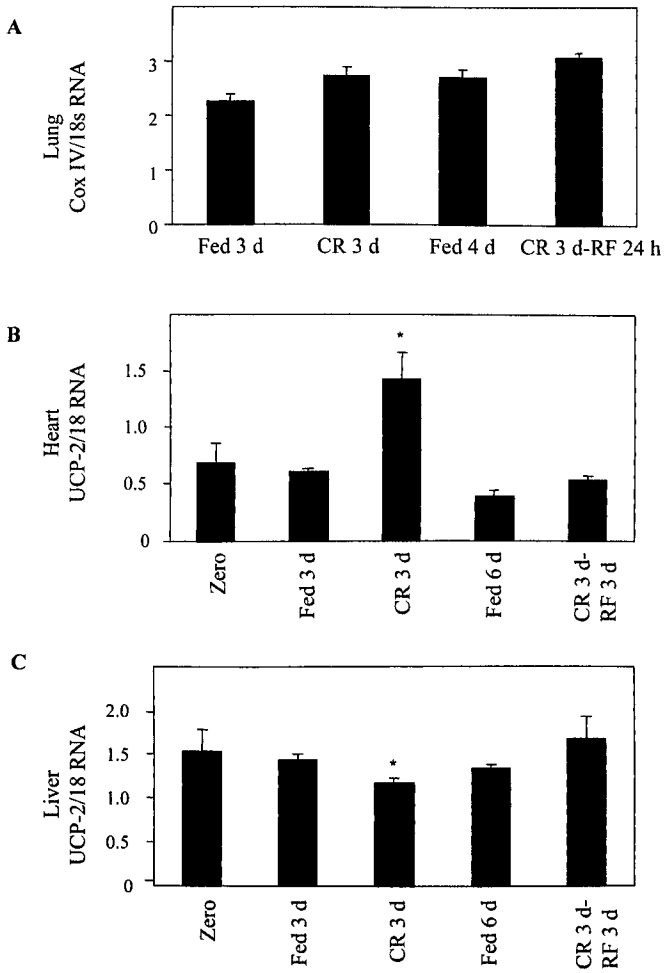

Figure 6. Mouse lung Cox IV mRNA and UCP-2 mRNA expression in mouse heart and liver during CR and CR-RF. RNA concentration was measured as described in the legend of Figure 1. (A) Lung Cox IV mRNA levels were measured in mice allowed food ad libitum (Fed), those on 67% CR for 3 days, or those on 67% CR for 3 days followed by 24 hrs of food ad libitum. Data are given as mean ± SE of five separate mouse lungs at each time point tested. $^*P > 0.05$ among all four groups. (B) Heart UCP-2 mRNA levels in in mice allowed food ad libitum, those on 67% CR for 3 days, and those on 67% CR for 3 days followed by 3 days of food ad libitum. Data are given as mean ± SE of five separate hearts at each time point tested. $^*P < 0.05$ compared with all other groups. (C) Liver UCP-2 mRNA levels in mice allowed food ad libitum, those on 67% CR for 3 days, and those on 67% CR for 3 days followed by 3 days of food ad libitum. Data are given as mean ± SE of five separate livers at each time point tested. $^*P < 0.05$ compared with all other groups.

Effect of CR and CR-RF on UCP2 mRNA Expression in Mouse Heart and Liver; Partial Tissue Specificity. To evaluate whether other organs responded similar to lung during CR and CR-RF, UCP-2 mRNA concentrations in heart and liver were measured. As in lung (Figs. 4 and 5), heart UCP-2 mRNA concentrationd increased after 3 days of CR and decreased to control levels after refeeding (Fig. 6B). Conversely, liver UCP-2 mRNA levels were modestly but significantly downregulated by CR and returned to control concentrations after refeeding (Fig.

6C). These data indicate that the regulation of UCP-2 mRNA expression by food intake was, in part, organ specific. The variation among tissues could also have arisen from differences in the type of substrate preferentially utilized by various organs.

Lung UCP-2 mRNA Is Increased by Exogenous Administration of Lipid. To further investigate the relationship between UCP-2 mRNA expression and lipid, the lung UCP-2 mRNA concentration was measured in mice fed *ad libitum*, 6 hrs after intraperitoneal injection with 2 ml of a 20% fat emulsion (Intralipid) or water. The lung UCP-2 mRNA concentration increased 78% in animals injected with Intralipid, compared with animals injected with water (5.5 ± 0.5 *vs.* 3.1 ± 0.5; $n = 6$ in each group; $P = 0.022$). These data indicate that increased fatty acids upregulate lung UCP-2 expression.

DISCUSSION

Although lung UCP-2 mRNA is present at a high concentration, little is known about the regulation of UCP-2 expression in the lung. On the basis of its role in other systems, we reasoned that the UCP-2 mRNA concentration might be altered during the perinatal period because of the challenges the lung experiences at birth, including an increased metabolic rate and a requirement for protection against the relative hyperoxia of breathing air at birth. Our findings show rat lung UCP-2 mRNA expression was upregulated within 6 hrs after birth and that the high level of UCP-2 mRNA in lung was maintained throughout postnatal life into adulthood (Fig. 1). The developmental expression of lung UCP-2 differs from that in brown adipose tissue, where UCP-2 mRNA is only transiently upregulated after birth (6); in liver, where UCP-2 mRNA is expression downregulated during the postnatal period (24); or in heart, where UCP-2 mRNA levels increase slowly, peak at postnatal week 2, and then fall during adulthood (Fig. 2). The variations in the pattern of expression among tissues indicate that UCP-2 is developmentally regulated differently among organs and may perform distinct functions in different tissues or perform the same function but at different times in development.

The physiological changes at birth are complex and include an alteration in hormone levels. To begin to understand the mechanisms responsible for the sharp upregulation of UCP-2 within the first few hours after birth, we tested the effect of thyroid hormone, because (i) the plasma concentration of thyroid hormone increases rapidly after birth (25); (ii) the tissue to plasma ratio of T3 in rat lung reaches a peak within 6 hrs after birth, which indicates that lung tissue is capable of concentrating thyroid hormone during the early postnatal period (26), at the time when we found an increase in lung UCP-2 in otherwise untreated rats (Fig. 1); and (iii) a thyroid hormone response element is present in the promoter region of human UCP-2 (27), which allows for the possibility of the direct regulation of UCP-2 transcription by thyroid hormone. Our data showed that UCP-2 mRNA levels were increased by thyroid hormone (Fig. 3A), which agrees with other findings in heart, adipose tissue, and liver (28, 29). Further support for our hypothesis that thyroid hormone plays a physiological role in the regulation of lung UCP-2 expression was provided by data showing that PTU, an agent that produces a hypothyroid state (21, 22), prevented the increase in lung UCP-2 expression at 6 and 24 hrs after birth (Fig. 3B).

Whereas the neonatal model examined the regulation of UCP-2 mRNA levels during a state of increasing oxygen consumption (13), decreased calorie intake led to a decrease in metabolic rate (12). However, we did not find the inverse of the neonatal results; instead, CR and short-term fasting upregulated lung UCP-2 mRNA concentrations (Figs. 4 and 5). In concert with our data showing that UCP mRNA levels increased 5-fold by CR, Pecqueur *et al.* (30) showed that 24 hrs of fasting increased lung UCP-2 protein expression approximately 6-fold. This increase in protein is the same order of magnitude as our increase in mRNA after 24 hrs of CR, which suggests that, during food restriction, lung UCP-2 transcription and translation are concordant.

The rapid induction of lung UCP-2 mRNA by fasting and quick decline of UCP-2 mRNA on refeeding suggest that the factor(s) regulating UCP-2 expression responds swiftly to changes in food intake and that UCP-2 mRNA likely has a short half-life. Serum FFA regulates UCP-2 expression in other systems (18, 31–33) and was a reasonable candidate as a potential regulator of lung UCP-2 expression, because CR caused a rapid increase in FFA (16). In the CR-RF experiments, we found a positive correlation between serum FFA levels and lung UCP-2 mRNA expression (Fig. 4). Moreover, we found that the FFA treatment of fed mice increased lung UCP-2 concentration. On the basis of these data, together with the findings of others that FFA levels increase by 2 hrs after birth (15) and a 6-fold increase in FFA levels by 16 hrs after birth (14), we propose that FFA regulates lung UCP-2 gene expression in both adult and neonatal animals.

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