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Induction of Uncoupling Protein 3 Gene Expression in Skeletal Muscle of Preterm Newborns

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ABSTRACT

Prematurity is associated with delayed postnatal activation of mitochondrial oxidative phosphorylation and impaired switch from glycolytic to oxidative metabolism. Fatty acids (FA), which represent a major energy substrate in mature muscle cells, are engaged in the postnatal activation of genes of energy metabolism and lipid oxidation. To understand the mechanism activating mitochondria in human newborns, expression of the genes for mitochondrial uncoupling proteins (UCP) was characterized in autopsy samples of skeletal (n = 28) and cardiac (n = 13) muscles of preterm neonates, who mostly died during the first postnatal month, and two aborted fetuses. Transcripts levels for UCP2, UCP3, and also for genes engaged in the transport of FA between cytoplasm and mitochondria were measured using real-time reverse transcriptase PCR. In accordance with studies in mice, our results document postnatal induction of UCP3 gene expression in skeletal muscle, involvement of nutritional FA in the induction, and a role of UCP3 in mitochondrial FA oxidation. They suggest impaired postnatal activation of UCP3 gene in neonates delivered before approximately 26 wk of gestation. Mean levels of the UCP3 transcript in skeletal muscle were by two orders of magnitude higher than in the heart. In contrast to UCP3, the UCP2 gene was active in fetuses, and its expression was not affected by nutrition. Our results support a role of UCP3 in postnatal activation of lipid oxidation in skeletal muscle and suggest the involvement of UCP3 in the delayed activation of mitochondrial energy conversion in very immature preterm neonates. **(*Pediatr Res* 53: 691–697, 2003)**

Abbreviations

CPT-1, carnitine palmitoyltransferase I isoform β
EF-1α, elongation factor 1α
FA, fatty acids
MTE-1, mitochondrial thioesterase 1
PPAR, peroxisome proliferator-activated receptor
UCP, uncoupling protein

Postnatal switch of glycolytic to oxidative metabolism is of crucial importance for all mammalian neonates. Several studies (1–3) demonstrate the recruitment of energy conversion and ATP synthesis in mitochondria during the early postnatal period as well as insufficient maturation of this mechanism in premature newborns (4, 5). The recruitment of mitochondrial oxidative phosphorylation in the tissues of the newborns reflects changes in the hormonal status and also a shift from glucose to lipids as the major energy source during the early postnatal period (6). However, nutritional FA serve not only as the source of energy but also as modifiers of the gene expression in many tissues (7). The effects of FA on gene expression in the newborns are of great significance with respect to the postnatal maturation of energy metabolism and mitochondrial functions. To understand the mechanisms underlying the adaptation of premature newborns to the extrauterine life, genes engaged in the control of energy metabolism must be identified. The role of the developmental status of the organism and of other possible external factors, such as nutrition, on the expression of the genes must be clarified.

UCP represent an important example of developmentally regulated genes participating in the control of mitochondrial energy conversion. They belong to a family of mitochondrial inner membrane transporters and are implicated in the regulation of the proton leak across the membrane. Thus, all UCP may lower the efficiency of mitochondrial ATP production;

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however, the functional roles of different UCP in cellular metabolism as well as the expression of the genes for UCP are mostly tissue specific (8). UCP1 is a characteristic marker of brown fat, where it has a profound thermogenic role (9). However, minute amounts of UCP1 have recently been found also in smooth muscle cells (10). UCP2 transcript is present in fetal tissues of rodents (11–13), and its level in both skeletal (12) and cardiac (14–16) muscle increases during the first postnatal weeks. In adult rodents, the UCP2 protein was detected in white adipose tissue, spleen, lung, and stomach (8, 17, 18), and it was implicated in the regulation of reactive oxygen species production (19, 20). UCP3 is present only in skeletal muscle, heart, and brown fat (21–25). It is linked specifically to lipid metabolism (26–31), and indirect evidence suggests that this protein contributes to the control of energy expenditure in humans [for refs. see (23)]. Recent studies indicate that expression of UCP3 in both skeletal (12, 13) and heart (14, 16) muscle of rodents is negligible in fetuses and is switched on after birth. They also show that UCP3 gene expression is induced by FA (26) and that the postnatal induction of UCP3 gene in skeletal muscle of mice depends on the initiation of lipid intake during suckling (13). Therefore, UCP3 is an important candidate for the control of mitochondrial functions and the postnatal augmentation of lipid metabolism in muscles.

No data concerning the expression of the genes for UCP in developing muscles in humans have been published so far. Nothing is known about the functional significance of the UCP3 gene induction in human newborns. Therefore, the present research was designed to characterize the expression of the UCP3 gene in both skeletal and cardiac muscle during perinatal development in humans. To clarify possible links of uncoupling proteins to lipid metabolism in the developing muscles, the expression of the genes engaged in the transport of FA between mitochondria and cytoplasm, CPT-1 (32), and MTE-1 (29, 31) was analyzed. UCP2 gene expression was also characterized. Premature neonates, who died at different postnatal ages, and also aborted fetuses were studied.

METHODS

Human material. Samples of skeletal muscle (*musculus quadriceps femoris*) were obtained from 26 human newborns (gestational age at birth: median, 25 wk, range, 22–37 wk; birth weight: median 695 g, range, 380–3210 g) during autopsy 2–3 h after death. Most of them were extremely low birth weight (<1000 g) newborns (20 cases; 77% of the newborns involved; Table 1). There were also six newborns with a birth weight >1000 g (and gestational age >28 wk). Two fetuses aborted at 23 wk of gestation were also included (cases Ab1 and Ab2; Table 1). A majority of the newborns died during the first postnatal month, the maximum length of survival was 140 d (Table 1). Samples of left atrial and ventricular myocardium were also collected from 13 newborns. Nutritional and other clinical data were recorded (Table 1). Infants born from mothers who suffered from endocrinological disorders or with drug abuse were not eligible for the study. For isolation of total RNA and characterization of gene expression (see below), samples were frozen and stored in liquid nitrogen, or they were preserved using RNAlater (Ambion, Austin, TX, U.S.A.) and stored at −70°C. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, and it was *a priori* approved by the Committees of Medical Ethics at all the collaborating institutions. Informed consent was obtained from the parents.

RNA analysis. Total RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA, U.S.A.). RNA (300 ng) was treated with RNase-free DNAse, and cDNA was obtained by reverse transcription with polyT primer using M-MLV reverse transcriptase (Invitrogen). Starting from 1/200 of cDNA obtained by the reverse transcription, real-time quantitative PCR (LightCycler instrument, Roche Molecular Biochemicals, Mannheim, Germany) was performed using QuantiTect SYBR Green PCR kit (QIAGEN, Valencia, CA, U.S.A.) with specific primers (Table 2). The PCR cycling profile consisted of an initial 15-min activation at 95°C, followed by 40 cycles of annealing (20 s, 94°C), extension (15–20 s, 60–62°C), and denaturation (20 s, 72°C), with melting curve analysis for the final step. Values for each transcript represent means of at least three measurements. To correct for intersample variations in both the amount of RNA and the yield of the reverse transcription, concentrations of UCP2, UCP3, CPT-1, and MTE-1 transcripts were normalized using either elongation factor EF-1α or β-actin as housekeeping/maintenance genes (see "Results" and Ref. 33).

Statistics. Spearman correlation coefficient was used to evaluate the relationship between the gene expressions. Statistical significance of differences between groups was evaluated using the Mann-Whitney *U* test. Differences among skeletal muscle, cardiac atrium, and cardiac ventricle were assessed by nonparametric Friedman test for one-way ANOVA model with *post hoc* tests using Wilcoxon matched-pairs test with Holm's adjustment of the significance level of individual comparisons between groups. The level of significance of all tests was set at $p = 0.05$.

RESULTS

Quantification of gene expression. To measure the differences in gene expression, housekeeping/maintenance genes are used as internal standards for the normalization of the estimated transcript levels. The genes used for the normalization should turn on early in fetal life and stay on, and they should be equally expressed in different tissues (33). To optimize quantification of various transcripts in developing human muscles, we used two housekeeping/maintenance genes, EF-1α and β-actin (33). In both the skeletal muscle and the heart samples, positive correlations between β-actin and EF-1α transcript levels were found (Fig. 1). However, significant differences were detected in the levels of EF-1α and β-actin transcripts among the tissues. When 13 newborns in which gene expression in the cardiac samples was characterized were compared (Table 1), the levels of EF-1α in skeletal muscle, heart atrium, and heart ventricle were 6.4 ± 2.1, 14.5 ± 4.1, and 5.8 ± 1.5 arbitrary units, respectively. The corresponding values for β-actin were 14.6 ± 5.2, 68.8 ± 22.7, and 36.6 ± 9.2. Because the differences in the mean values were smaller

Table 1. *Cases examined and levels of UCP2 and UCP3 transcripts in skeletal muscle*

Case	Sex	Gestational age at birth (wk)	Birth weight (g)	Survival* (d)	Nutrition†			Clinical diagnosis and pathological anatomy characteristics	Transcript level‡	
					E	P	PL		UCP2	UCP3
D14§	F	25	730	5		+		G, IA, ICH, NEC, RDS	0.22	0.19
A31§	F	24	600	140	+			CLD, IA, M, RDS	1.15	0.48
A44§	F	27	800	90			+	G, HIE, NEC, P	4.00	10.03
A48§	F	27	635	7		+		DIC, ICH, PDA, PH, RDS, SGA	0.40	0.82
A50§	M	23	550	8	+		+	G, ICH, RDS, S, PDA	0.53	0.62
A61§	M	23	630	36		+		CLD, HY, NEC, PDA, RF, RDS, S	0.18	1.15
A63§	F	26	750	38			+	CLD, HY, ICH, NEC, PDA, PP, RDS, S	1.49	1.98
A64§	M	30	1240	**9 h**		+		HS, IA, ICH, SGA	3.19	7.55
A65§	F	22	450	9		+		HY, IA, S, SGA	0.20	0.03
A66§	F	31	1450	5		+		HIE, IA	0.23	0.07
A67§	F	24	635	39			+	G, ICH, NEC, PP, RDS, S	0.20	0.00
A69	F	25	850	75		+		PA, RDS, PNO, S, I CH, PVL, PDA, NEC, MOF	0.14	0.00
A70	F	35	1950	13			+	PE, RDS, PIE, HK, PHC, MOF	1.70	6.40
A71	M	24	680	2		+		PFC, P, CH	1.41	1.10
A72	M	24	680	27			+	IA, RDS, IVH, PHC, NEC	0.19	0.00
A73	M	25	435	69	+			G, H, RDS, MOF, P, ICH, IUT-D	0.25	0.04
A74	F	25	380	6			+	SGA, PE, RDS, PDA, S, P, ICH	0.55	0.53
A75	M	30	1295	10			+	RDS, M	0.33	11.18
A76	M	32	750	7			+	RDS, SGA, G, S, DS	0.96	2.74
A77	M	25	690	4		+		ICH, PVL,	0.61	0.11
A78	M	25	750	20			+	RDS, S, NEC, ICH, PHC, MOF	0.88	0.05
A79	M	25	650	16		+		G, S, RDS, NEC, PDA, MOF	0.27	0.00
A80	M	25	700	3			+	G, IUT-A, HK, RDS, ICH	0.32	0.07
A81	F	28	1050	**3 min**				Multiple malformations	2.86	0.37
A82	M	23	690	14			+	RDS, PH, PDA, NEC, S, M	0.14	0.16
A83	M	37	3210	**4 h**		+		LH, PIE, PNO, RF	0.31	0.04
Ab1§‖	F	23	450	**5 min**				Osteochondrodysplasia	0.23	0.00
Ab2‖	F	23	470	**3 min**				Chorioamnionitis	0.39	0.00

CHT, chromosomal translocation 45/14q21q; CLD, chronic lung disease; DIC, disseminated intravascular coagulation; G, gemini; HIE, hypoxic-ischemic encephalopathy; HK, hypertrophic cardiomyopathy; HS, hemorrhagic shock; HY, severe hypothermia (body temperature <31 °C) during the early postnatal period; IA, intrauterine asphyxia; ICH, intracranial hemorrhage; IUT-A, intrauterine transfusion acceptor; IUT-D, intrauterine transfusion donor; LH, lung hypoplasia; M, meningitis; DS, Down syndrome; MOF, multiorgan failure; NEC, necrotizing enterocolitis; P, pneumonia; PA, perinatal asphyxia; PDA, patent ductus arteriosus; PE, preeclampsia; PH, pulmonary hemorrhage; PHC, posthemorrhagic hydrocephalus; PIE, pulmonary emphysema; PNO, pneumothorax; PP, peripheral pancytopenia; RDS, respiratory distress syndrome; RF, respiratory failure; S, sepsis; SGA, small for gestational age; TGV, transposition of the great vessels.

* In days, except when indicated otherwise (in bold).

† Type of nutrition during last 48 h before death: E, enteral nutrition (human milk or preterm formula); P, parenteral nutrition without lipids; PL, parenteral nutrition containing lipids (*i.e.* lipid emulsions containing both long- and medium-chain FA; the Lipofundin MCT/LCT 20%, B. Braun Melsungen AG, Melsungen, Germany, or the Nutralipid MCT 20% Emulsion, ICN Czech Republic, Prague).

‡ Transcripts of UCP2 and UCP3 genes were quantified using real-time reverse transcriptase PCR and expressed relative to that of EF-1α (see "Methods"). Values are means ± SE. In all cases, the levels of CPT-1 and MTE-1 transcripts were also estimated (see Tables 3 and 4). In 13 newborns (A66, A67, A69, A70, A71, A72, A73, A74, A75, A76, A77, A81, and A82) the cardiac expression of UCP2, UCP3, CPT-1, and MTE-1 was also evaluated (see Fig. 1 and Table 4).

§ Cases examined in previous studies 35,36.

‖ Abortion.

Table 2. *Sequences of PCR primers*

Gene	Sense primer (5′-3′)	Antisense primer (5′-3′)	GeneBank accession no. for cDNA
EF-1α	GAACCATCCAGGCCAAATAAGC	CCACCGCAACTGTCTGTCTCATA	AY043301.1
β-actin	TGGTGGGCATGGGTCAGAAG	CCAGAGGCGTACAGGGATAGCAC	XM_037239.1
UCP2	CCTGCGGCTCGGACACATA	GGGGCACCTTTAATCAGCAACA	AF019409.1
UCP3	AGAACCATCGCCAGGGAGGAAGGA	CACCGGGGAGGCCACCACTGT	XM_006360.1
CPT-1*	GCACGCCAGGCCTTCTTTAGC	GATGACCGCCTGGCACTGTTTT	U66828.1
MTE-1	TGGGCAGCGGCATTTCAACTCC	TAATTCTTTCTCCCCTCAGCCCCATCC	NM_012332.1

* The primers detect all known isoforms of transcripts of CPT-1β gene. They correspond to exons 11 and 14.

with the EF-1α gene than with β-actin and the EF-1α gene showed only minimum changes in its expression during ontogeny (33), it was used for the normalization.

Gene expression in skeletal muscle. The main focus of the present study was on premature newborns who died as a result of various pathologic conditions associated with the immaturity. RNA was isolated from skeletal muscle for the quantification of UCP2, UCP3, CPT-1, and MTE-1 transcripts in all the newborns and fetuses involved in this study (Table 1). The expression data were analyzed to reveal the effects of gesta-

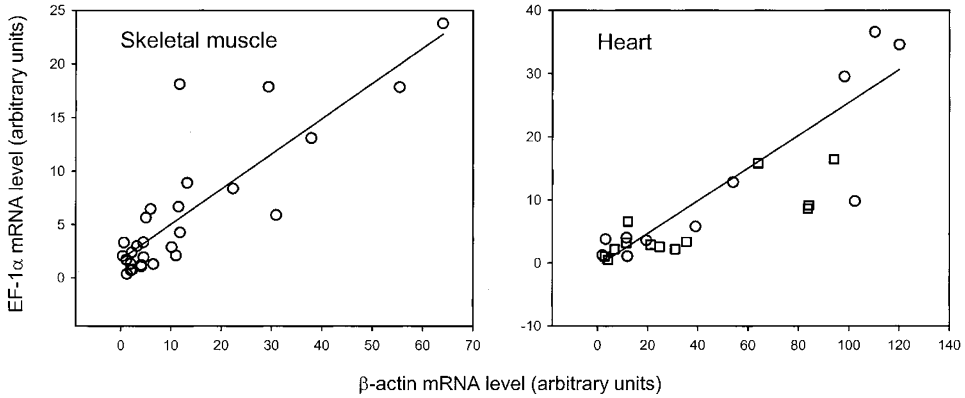

Figure 1. Correlations between concentrations of β-actin and EF-1α transcript in skeletal muscle and heart. The concentrations were estimated in RNA isolated from *musculus quadriceps femoris* of two fetuses and 26 newborns (*left*; see Table 1), as well as in heart atrium (*right*, *open circles*) and ventricle (*right*, *open squares*) of 13 newborns (see note to Table 1). Positive correlations between transcript levels were found in both skeletal muscle ($R = 0.78$; $p < 0.001$) and heart ($R = 0.87$; $p < 0.001$).

tional age, length of survival (postnatal age), and nutrition during the last 48 h before death. Concerning the nutrition, it was studied whether the character of nutrition (*i.e.* enteral nutrition, parenteral nutrition without any lipids, parenteral nutrition containing lipids, or absence of the nutritional stimulus after birth; Table 1) could influence gene expression.

The UCP2 transcript was found in all the newborns and aborted fetuses. In contrast, in none of the fetuses, or in about 30% of the newborns, the UCP3 transcript could be detected or its levels were negligible compared with the newborns exhibiting the highest levels of expression (Table 1). In both UCP2 and UCP3 (Fig. 2), inspection of the data suggests an effect of the gestational age at birth on the transcript levels, as newborns with lower gestational age (and fetuses) seemed to have lower levels. Moreover, in the case of UCP3 but not UCP2, the effect of the type of nutrition was also apparent, namely in newborns born at gestational age >25 wk (Fig. 2). Therefore, statistical analysis was performed to compare gene expression in newborns with lower and higher gestational age, with the borderline set empirically between 25 and 26 wk (Fig. 2). The tendency to increase the expression of UCP2 with increasing gestational age at birth was not unequivocally supported by the statistical analysis of differences in the expression of the two groups ($n = 10$–16) of the newborns ($p = 0.07$). However, UCP3 transcript levels in newborns with gestational age at birth above 25 wk were significantly higher than in the newborns delivered at earlier stages of the intrauterine development ($p = 0.02$), namely when the newborns on parenteral nutrition containing lipids were compared ($p < 0.001$, Fig. 2). No effect of the length of survival on the expression of either UCP2 or UCP3 gene could be found (Fig. 2). No statistically significant correlation between the gestational age at birth and the length of survival was observed (not shown; for the data see Table 1). It was also found that within the group of neonates with higher gestational age at birth, the UCP3 transcript levels were significantly higher in neonates receiving parenteral nutrition with lipids, compared with those receiving parenteral nutrition without lipids, or with all other neonates (Table 1 and Fig. 2). In fact, only one out of six newborns showing relatively high UCP3 transcript levels (*i.e.* $\geq$2 arbitrary units) did not receive any lipids in the nutrition (case A64; see Table 1 and Fig. 2). This effect of nutrition was not observed in neonates delivered before 26 wk of gestation (Table 1 and Fig. 2). Possible differences between the effect of enteral and parenteral nutrition (*i.e.* nutrition during the last 48 h before death) on UCP3 gene expression could not be evaluated, because all the neonates receiving enteral nutrition were born before 26 wk of gestation (Table 1; cases D14, A48, and A73). Expression of UCP2 was not significantly affected by the type of nutrition (Fig. 2). The same conclusions were made when β-actin (not shown) was used for the normalization of tran-

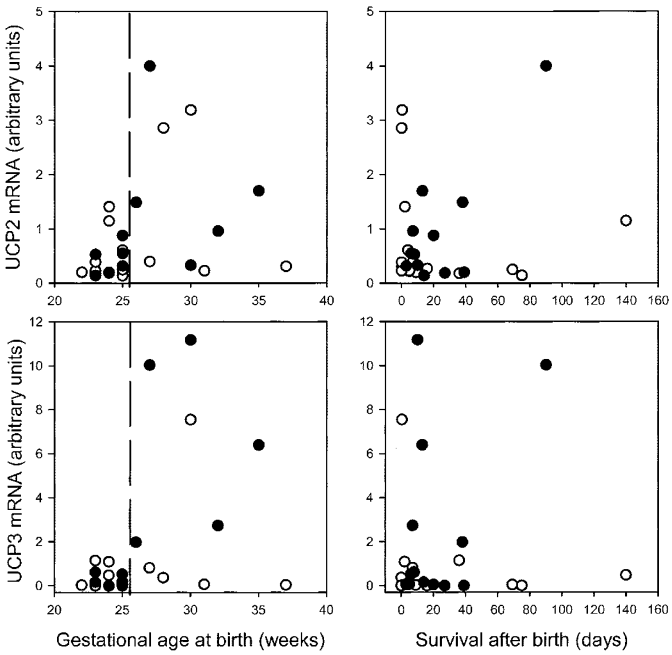

Figure 2. Expression of UCP2 and UCP3 genes normalized using EF-1α in human skeletal muscle during early postnatal development. The levels of the UCP2 and UCP3 transcripts normalized using EF-1α in skeletal muscle of 26 newborns and two fetuses (see Table 1) are plotted as a function of gestational age at birth and postnatal age. The type of nutrition during the last 48 h before death is also indicated. *Filled symbols*, parenteral nutrition containing lipids; *open symbols*, only enteral nutrition, or parenteral nutrition without lipids, or any nutrition, respectively (see Table 1). *Dashed line* separates age groups differing significantly in UCP3 gene expression (see "Results").

script levels instead of EF-1α (Table 1 and Fig. 2). The results indicate a postnatal induction of the UCP3 gene in skeletal muscle of human newborns and they suggest interplay between the effects of the gestational age at birth and nutrition in the control of the activity of the UCP3 gene.

In all the newborns and fetuses in our cohort (Table 1), the levels of the transcripts for CPT-1 and MTE-1 genes in skeletal muscle were also quantified. In all cases and with both genes, distinct levels of the transcripts were detected. In contrast to the expression of the gene for UCP3, no effect of the gestational age at birth or the postnatal age, or the type of nutrition could be observed (not shown). However, a positive correlation between the expression of the CPT-1 gene and that of all the other genes (*i.e.* UCP2, UCP3, and MTE-1) was found (Table 3). A positive correlation between UCP2 and UCP3 gene expression was also observed (Table 3). No correlation could be detected between the expression of MTE-1 and either of the genes for UCP.

Gene expression in the heart. Analysis of gene expression in cardiac muscle was performed in 13 newborns from our cohort (see note to Table 1) and the data were compared with the gene expression in the skeletal muscle in these newborns (Table 4). The transcript levels for UCP3 and MTE-1 were similar in atrium and ventricle, whereas the levels of UCP2 and CPT-1 were higher in the ventricle (about 2- and 4-fold, respectively) than in the atrium. The differences in transcript levels between heart and skeletal muscles were not greater than 3-fold, except for UCP3. The mean values of UCP3 transcript levels were much lower (about 60-fold) in the heart compared with skeletal muscle (Table 4), as also indicated by a comparison between the levels in skeletal and cardiac muscle of the individual newborns. Thus, among 13 newborns characterized for the cardiac expression, the newborns A70 and A76 showed relatively high levels of UCP3 transcript in skeletal muscle (*i.e.* 6.40 and 2.74 arbitrary units, respectively; Table 1), whereas the levels of the UCP3 transcript in their hearts were <0.004 arbitrary units. In fact, the highest level of UCP3 transcript measured in heart was 0.13 arbitrary units (Fig. 3), *i.e.* much less than the levels in skeletal muscle (Fig. 2 and Table 4). In contrast to skeletal muscle (Fig. 2), no effect of either the gestational age at birth, or the nutrition, on the expression of UCP3 in the heart could be detected (Fig. 3).

Table 3. *Correlations between the levels of transcripts for UCP2, UCP3, CPT-1, and MTE-1 genes in skeletal muscle*

	UCP2	UCP3	CPT-1
UCP3			
R	0.65		
P	†		
CPT-1			
R	0.39	0.39	
P	*	*	
MTE-1			
R	−0.15	−0.05	0.40
P	NS	NS	*

Spearman rank order correlation test was used to perform pairwise comparison of transcript levels in skeletal muscle of human fetuses and neonates (n = 27–28; see Table 1). NS, difference is not statistically significant ($p > 0.05$). * p = 0.01–0.05; † $p < 0.01$.

DISCUSSION

This study reflects our continuous effort to comprehend the role of mitochondria and particularly UCP in postnatal maturation of tissues in human neonates. It is based on a unique collection of autopsy materials from premature neonates, mostly extremely low birth weight newborns (34–36). The present research is focused on mitochondrial UCP in developing human muscles. Our results demonstrate that gene expression could be characterized in autopsy samples of human newborn muscles using real-time quantitative PCR and that EF-1α is a reliable housekeeping/maintenance gene for the normalization of the transcript levels. In accordance with studies in mice, showing the activation of UCP3 gene in skeletal muscle by lipid intake during suckling (12, 13), a statistically significant association between UCP3 transcript level in skeletal muscle and presence of lipids in parenteral nutrition could be observed in neonates born after 25 wk of gestation (Fig. 2). Therefore, our data support the idea that circulating lipids are required for the postnatal activation of UCP3 gene expression in human skeletal muscle. They stress the importance of the lipid composition of nutrition for postnatal development (see Refs. 37–39).

The induction of the UCP3 gene may depend on the activation of its promoter (23) by FA *via* transcription factor(s), the peroxisome proliferator-activated receptor α (PPAR α) and/or PPAR δ (12, 40). However, other mechanisms may also be involved. Recent studies showed stimulation of the activity of both UCP2 and UCP3 genes in skeletal muscle by AMP-activated protein kinase, which reflects the energy status of the cell (41–43). Moreover, UCP3 gene promoter activity depends on MyoD, the master regulator of differentiation program of muscle cells, and MyoD maybe responsible for preferential expression of UCP3 gene in differentiated muscle cells (44). It remains to be clarified whether, besides the lipid intake (and

Table 4. *Levels of various transcripts in skeletal muscle, cardiac atrium, and ventricle*

	Transcript level (arbitrary units)			
Tissue	UCP2	UCP3	CPT-1	MTE-1
Skeletal muscle	0.73 ± 0.23	1.746 ± 0.932	0.54 ± 0.11	0.67 ± 0.15
Atrium	0.23 ± 0.06*	0.024 ± 0.011*	0.47 ± 0.08	1.04 ± 0.20*
Ventricle	0.49 ± 0.06†	0.036 ± 0.009*	1.70 ± 0.18*†	1.20 ± 0.24*

Newborns in which gene expression in cardiac samples was characterized were compared (n = 13; see legend to Table 1). Different transcripts were quantified using real-time reverse transcriptase PCR and are expressed relative to that of EF-1α (see "Methods"). Values are means ± SE.

* Significantly different from skeletal muscle.

† Significantly different from atrium.

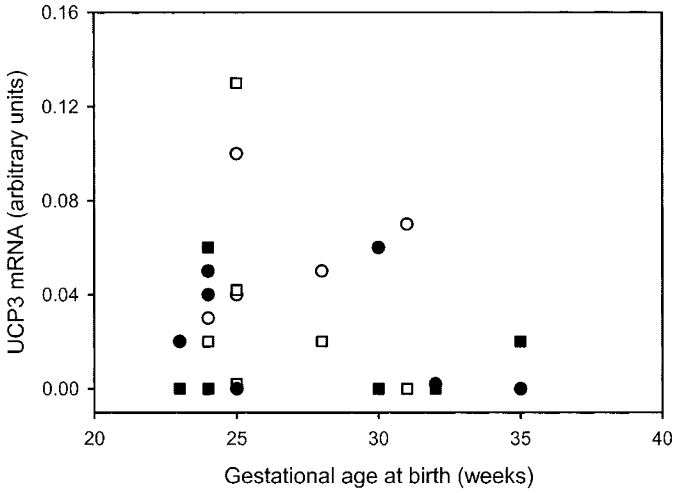

Figure 3. Expression of UCP3 gene normalized using EF-1α in human heart as a function of gestational age at birth. Levels of UCP3 transcript normalized using EF-1α in cardiac atrium (*squares*) and ventricle (*circles*) are plotted as a function of gestational age at birth. The type of nutrition during the last 48 h is indicated as in Figure 2 by *open* and *filled symbols*, respectively. Data from 13 newborns (see note to Table 1).

PPAR), the AMP-activated protein kinase or MyoD also contribute to the large variability in UCP3 gene expression among the neonates born after 25 wk of gestation.

In skeletal muscles of all the newborns delivered before 26 wk of gestation, relatively low UCP3 transcript levels were found. That UCP3 gene expression in skeletal muscle seems to depend on the gestational age at birth rather than on the postnatal (and postconceptional) age is striking. It is possible that the postnatal induction of the UCP3 gene is programmed during intrauterine development, and that appropriate conditions for the induction are not met by current clinical treatment of the very immature preterm newborns. Further studies on the influence of intrauterine factors on the postnatal development are clearly required.

Our results document substantial differences between activities of UCP2 and UCP3 genes in skeletal muscle during human perinatal development. The UCP2 transcript was detected in skeletal muscle of fetuses at 23 wk of gestation, whereas the UCP3 gene was silent during fetal life and became activated only after birth (see the expression of UCP2 and UCP3 genes in fetuses; Table 1, cases Ab1 and Ab2). Differential control of the expression of UCP2 and UCP3 genes was observed previously also in adult rodents (45, 46). Also, in contrast to UCP3, expression of UCP2 gene is mainly controlled translationally. This causes dissociation between the UCP2 transcript and protein level in most tissues, including muscle, where the content of the protein may be relatively low (18). Because UCP2 may decrease the formation of reactive oxygen species in mitochondria (19, 20), it may protect muscle cells against oxidative damage. UCP3 may also have a similar role (47). This role of UCP2 and UCP3 could be of special importance in premature neonates that are at high risk of the damage (48).

The postnatal induction of UCP3 gene is associated with maturation of the mechanism of oxidative phosphorylation, the major ATP supplier in mammalian tissues (1–4). Several pieces of evidence strongly suggest involvement of UCP3 in lipid metabolism, namely in facilitating β-oxidation of FA in mitochondrial matrix (26–28, 31). It was also hypothesized that UCP3 served as a carrier exporting FA accumulated inside mitochondria to the cytoplasm. According to this hypothesis, positive correlation between UCP3 and MTE-1 transcript levels was expected (29, 31). However, no such correlation was observed in the skeletal muscle of human newborns in this study (Table 3). On the contrary, the positive correlation found between UCP3 and CPT-1 transcript levels in the muscles supports the stimulatory effect of UCP3 on lipid oxidation, because CPT-1 is involved in the transport of long-chain FA from cytoplasm to mitochondria (32). In agreement with the notion that lipid oxidation is required for normal glucose metabolism and insulin sensitivity of muscle cells (49), it may be speculated that induction of UCP3 in skeletal muscle of the newborn is required not only for the increase of lipid metabolism but also for insulin sensitivity and glucose uptake (50, 51).

Similarly, as in adult rodents (21, 22, 24) and humans (25), in human newborns UCP3 transcript levels were much lower in the heart compared with skeletal muscle. In contrast to human adults (15, 52, 53), UCP2 gene expression was similar in both types of muscle. Moreover, in the heart but not in skeletal muscle, similar levels of UCP3 transcript were detected in neonates born at various stages of gestation, and nutritional lipids seemed to be without any major effect on the cardiac UCP3 gene expression. Therefore, our data indicate substantial differences in the control of UCP3 gene expression between skeletal and heart muscles of human newborns. Further studies are required to quantify the UCP3 gene expression at the protein level using antibodies. However, reliable antibodies that would be specific for human UCP3 are not commercially available (54).

In conclusion, this study documents specific patterns of expression of genes engaged in the control of energy metabolism in skeletal muscle and heart, the postnatal induction of UCP3 gene expression in skeletal muscle, and the role of UCP3 in lipid oxidation in human newborns. The data are in accordance with the role of dietary lipids in the postnatal induction of UCP3 gene in skeletal muscle of human newborns, and indicate involvement of UCP3 in delayed activation of mitochondrial energy conversion in very immature preterm neonates. These observations are important for understanding the development of muscle energy metabolism in human neonates, and for developing new strategies for treatment of preterm newborns.

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