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Original article

Mitochondrial and peroxisomal fatty acid oxidation capacities increase in the skeletal muscles of young pigs during early postnatal development but are not affected by cold stress

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Abstract — In pigs, the optimal utilization of energy substrates within muscle fibers is a prerequisite of the utmost importance for successful adaptation to extra-uterine life. In the present work we demonstrate that fatty acid (FA) oxidative capacities increased within the first five days of life in piglet skeletal muscle. Mitochondrial FA oxidation capacities increased more in the *rhomboideus* oxidative than in the *longissimus lumborum* glycolytic muscle (+114% vs. +62%, $P < 0.001$). The apparent rate of fatty acid degradation by peroxisomes represents 30 to 40% of total FA oxidation capacities and increased by about 170% ($P < 0.001$) with age in both muscles. The postnatal enhancement of skeletal muscle oxidative capacities was further supported by a rise in acid-soluble and long-chain acylcarnitine tissue levels (+67%, $P < 0.01$), and plasma levels of albumin (+160%, $P < 0.001$). Cold stress had no effect on mitochondrial and peroxisomal FA oxidation but greatly enhanced (+61%, $P < 0.05$) the circulating levels of non-esterified fatty acids at five days of life.

oxidation / skeletal muscle / piglet / cold / mitochondria / peroxisomes

1. INTRODUCTION

Nutritional and physiological adaptations are required at birth to ensure optimal survival of the neonates. New-born pigs are particularly at risk because they are naturally exposed to cold immediately after birth, poorly insulated and contain very little adipose tissue of any type at birth. They rely almost exclusively on shivering

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thermogenesis [1] and the optimal utilization of energy substrates within muscle fibers is a prerequisite of the utmost importance for successful adaptation to extra-uterine life. In this context, the change from carbohydrate fuel in utero to a high fat and low carbohydrate colostrum after birth requires an increased capacity for fatty acid oxidation. Whole-body investigations have clearly demonstrated that lipid utilization increases progressively after birth. Indeed, the respiratory quotient decreases postnatally [2] and the data of Chiang et al. [3] and Herpin et al. [4] showed that fatty acid oxidation in the fed newborn pig contributes 10.6% to the thermoneutral maintenance energy required during the first 12 h of life, and 41.7% **for** the subsequent 12 h. A further 40% enhancement is observed in the cold.

At the organ level, i.e. the heart, the kidneys, and especially the liver, conclusive evidence exists on the postnatal enhancement of total and mitochondrial fatty acid oxidation capacities [5]. Regarding skeletal muscle, the key thermogenic tissue of the piglet, direct demonstration of the postnatal enhancement of fatty acid oxidation is missing and the effects of muscle fiber type and cold stress are poorly documented. However, we have already shown that within the first five days of life, the in vivo hindquarter blood flow [6], mitochondrial protein mass and oxidative potential of skeletal muscles [7] are increasing. Furthermore, the inhibition of CPT I activity by malonyl-CoA was progressively relieved [8], which should favor fatty acid oxidation in the skeletal muscle. Therefore, the present work was set up to confirm this increase in oxidation capacities by measuring the mitochondrial and peroxisomal oxidation capacities in whole muscle homogenates prepared without any protease treatment [9]. The measurements were performed at birth and at five-days of life on two muscles: a slow-oxidative one (*rhomboideus*, RH) and a fast-glycolytic one (*longissimus lumborum*, LL). The effect of cold stress was also tested at both ages. Peroxisomal β-oxidation is an alternate pathway of fatty acid catabolism present in many tissues of mammals including pigs [5, 10]. In addition, it may play a role in thermogenesis [11] because the first oxidation step catalyzed by fatty acyl-CoA oxidase is not coupled to ATP production and the energy is released as heat. Changes in tissue (carnitine) and circulating (albumin, non-esterified fatty acids, carnitine) levels of substrates or proteins involved in fatty acid metabolism were also followed to refine the interpretation of the data.

2. MATERIALS AND METHODS

2.1. Animals

Twenty-four Piétrain × Large White crossbred piglets from six litters from the INRA (Institut National de la Recherche Agronomique) herd were used in this experiment. Parturition was induced with an intramuscular injection of a prostaglandin analog on d 113 of gestation, to ensure farrowing on d 114. At birth, four piglets of average body weight (1.5 kg) were selected in each litter for the experiment. Two piglets were removed from the sow and maintained for a 4-h period in a respiratory chamber either in thermoneutral (TN, 34 ºC, $n = 6$) or in cold (C, 24 ºC, $n = 6$) conditions for heat production measurements. The two other piglets were maintained with the sow under normal farrowing house conditions for five days. At this age, heat production was measured in the respiratory chamber either in thermoneutral (TN, 30 ºC, $n = 6$) or in cold (C, 15 ºC) conditions during a 4-h period. These temperatures were chosen to take into account the downward shift of the lower critical temperature with age [12] and to achieve a similar increase in heat production at both ages [6]. An oral dose of fresh sow colostrum (30 $g \cdot kg^{-1}$ BW) was given to the newborn piglets before being put into the chamber to ensure that, at both ages,

measurements were made on fed piglets. After the measurement of whole-body energy metabolism, all the piglets were anesthetized by halothane inhalation and then killed by exsanguination. LL and RH muscles were immediately removed and either used for immediate determination of the fatty acid oxidation potential on fresh homogenates or frozen in liquid nitrogen and stored at –80 °C for subsequent biochemical measurements. Blood was taken on heparin at killing, centrifuged at 7500 × *g* for 5 min and the plasma was stored at –20 °C for further analysis.

2.2. Whole-body oxygen consumption

Oxygen consumption, heat production and the respiratory quotient (RQ) were measured by indirect calorimetry as previously described [12]. Rectal temperature was recorded before killing.

2.3. Fatty acid oxidation in skeletal muscle homogenates

Oxidation rates were measured in muscle homogenates as described by Veerkamp and Van Moerkerk [13], using oleate in its acid form as the substrate [8]. Whole muscle homogenates (5% weight/volume) were prepared in 0.25 M sucrose, 2 mM Na_2-EDTA and 10 mM Tris-HCl (pH 7.4) by hand homogenization using a glass-glass homogenizer. Two pestles with different diameters were used (intervening space 0.075 mm and then 0.050 mm). Oleate oxidation was performed in a total volume of 0.5 mL containing 100 µL of muscle homogenate in 25 mM Sucrose, 75 mM Tris-HCl (pH 7.4), 10 mM K_2HPO_4, 5 mM $MgCl_2$ and 1 mM EDTA. This buffer was supplemented with 1 mM NAD+, 5 mM ATP, 25 µM cytochrome-c, 0.1 mM coenzyme A, 0.5 mM L-malate and 0.5 mM L-carnitine. Peroxisomal oleate oxidation was determined in the presence of inhibitors of mitochondrial oxidation, i.e., antimycin A (75.6 µM) and rotenone (10 µM) or in the presence of malonyl-CoA (150 µM), which is an inhibitor of CPT I. All assays were made in triplicate. The flasks were preincubated for 5 min at 37 °C before addition of 100 µL of 600 µM [1-^{14}C]oleate bound to albumin in a 5:1 molar ratio (final concentration: 120 µM). The specific activity usually averaged 1.6 µCi·µmol^{-1}. Incubation was carried out for 30 min at 37 °C with agitation and stopped by 0.2 mL of 3 M perchloric acid. The released $^{14}CO_2$ was trapped in 0.3 mL ethanolamine/ethylene glycol (1:2 v/v) and was measured by liquid scintillation counting in 5 mL of Ultima Gold (Packard, Groningen, The Netherlands). After 90 min at 4 °C, the acid incubation mixture was centrifuged for 5 min at 10 000 × *g* and 450 µL containing ^{14}C-labeled perchloric acid-soluble products was assayed for radioactivity by liquid scintillation. Oleate oxidation rates were calculated from the sum of $^{14}CO_2$ and ^{14}C-labeled perchloric acid soluble products and were expressed in nmol of oleate per min per g tissue wet weight.

2.4. Activity of cytochrome-c oxidase

Cytochrome-c oxidase (COX) activity was used here to calculate mitochondrial oxidation/COX activity ratios in order to appreciate the contribution of mitochondrial proliferation to the observed changes in the mitochondrial oxidation capacity.

Homogenates (5% w/v) from the muscles were prepared in a 0.1 M sodium phosphate buffer at pH 7.0. Mitochondria contained in tissue homogenates were broken up with 2% final v/v Triton X-100. The solution was centrifuged for 10 min at 800 × *g* and the supernatant was filtered and used for cytochrome-c oxidase activity. The cytochrome-c oxidase activity was measured according to the method of Errede et al. [14]. Supernatant (5 µL) was added to 1 mL of 56 µM reduced horse heart cytochrome-c (Roche Diagnostics) in a potassium

phosphate buffer (35 mM KH_2PO_4, 1 mM EDTA, pH 7.4). The rate of oxidation of cytochrome-c was monitored for 1 min at 550 nm against a reference sample consisting of a 56 µM cytochrome-c solution totally oxidised by potassium ferricyanide. Cytochrome-c oxidase activity was expressed in µmol of substrate per min and per g of wet tissue.

2.5. Tissue concentrations and plasma levels of carnitine

Tissue extracts for the determination of acid-soluble carnitine (free carnitine + carnitine linked to short-chain fatty acids) and long-chain acylcarnitine were prepared as described by Kerner et al. [15]. Briefly, the sonicated homogenates were mixed with 0.45 N $HClO_4$ and centrifuged at 1500 × *g* for 10 min at 4 °C. Acid-soluble carnitine was recovered in the supernatant, while long-chain acylcarnitine stayed in the pellet. Measurement of acid-soluble carnitine was performed with the radiochemical method of Cederblad and Lindstedt [16] using 21 µM [1-^{14}C]acetyl-CoA (specific radioactivity, 1.02 µCi·µmol^{-1}) as the substrate. The reaction was initiated by adding 2 units of carnitine acetyl-transferase. After incubation at room temperature for 30 min, 150-µL samples were applied to an ion exchange column (AG2-x8, 100-200 mesh, BioRAD) to separate [1-^{14}C] acetyl-CoA in excess from the [1-^{14}C] acetyl-carnitine formed. Eluate and washing (500 µL) were transferred in scintillation vials containing 10 mL of scintillation liquid and were counted. The long-chain acylcarnitine concentration was determined as described above but after alkaline hydrolysis of the pellet sample using Tris-base 1 N and KOH 0.4 N. Similarly, to determine plasma levels of total carnitine, we started with an alkaline hydrolysis of 100 µL of plasma before using the radiochemical method described above.

2.6. Plasma levels of non-esterified fatty acids (NEFA) and albumin

NEFA were measured on 10 µL of plasma with an enzymatic method using a commercial kit (NEFA C-test, Wako Chemicals, Dardilly, France) and an automatic analyzer (Cobas Mira automate, Roche, Switzerland). Plasma albumin was determined spectrophotometrically using bromcresol green (BCG) at pH 4.2 with a commercial kit (Albumin-kit, BioMérieux, Marcy-l'Etoile, France).

2.7. Statistical analysis

The effects of age and cold stress on oxygen consumption, RQ, rectal temperature, plasma levels of total carnitine, NEFA and albumin, and the interaction between both factors were tested by ANOVA (2 × 2 factorial design) using the GLM procedure of SAS [17]. For total mitochondrial and peroxisomal oxidation, as well as tissue concentration of carnitine, the effects tested included age, cold stress, animal nested within group (i.e. age and cold stress), muscle type and interaction between those factors. The effects of age and cold stress were tested against animals within groups. The residual square mean was used as the error term for other effects. Individual means were compared by Duncan analysis when interactive effects were significant. All the results are expressed as means associated to the pooled standard errors.

3. RESULTS

As expected, the energy metabolism of the piglets was affected by both age ($P < 0.01$) and cold stress ($P < 0.05$) (Tab. I). Oxygen consumption increased by 68% between birth and five days of life in thermoneutral conditions and was enhanced by 94 and 61% in the cold in newborn and five-day old piglets, respectively. RQ decreased with age ($P < 0.001$) and increased

Table I. Energy metabolism of new-born and 5-d old piglets exposed to thermal neutrality or to the cold[1].

Age	New-born		5 days		Statistics[2]	
Temperature	TN	C	TN	C	Pooled SE	Significance
O_2 consumption (mLO_2·(min·kg body weight)$^{-1}$)						
	11.1	21.6	18.6	29.9	2.37	A*** C***
Respiratory quotient						
	0.87	0.91	0.78	0.80	0.027	A*** C*
Rectal temperature (°C)						
	38.4	37.2	38.9	38.4	0.60	A** C**

[1] Values are means, $n = 6$. Piglets were exposed to thermal neutrality (TN, 34 °C at birth, 30 °C at 5 days) or to cold (C, 24 °C at birth, 15 °C at 5 days) for a 4-h period.
[2] Statistics: A, effect of age; C, effect of cold; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

slightly but significantly in the cold ($P < 0.05$). However, the comparison of the individual means indicated that this increase was not significant at five-days of life ($P > 0.05$). Rectal temperature increased slightly with age ($P < 0.01$) but the piglets were not able to maintain homeothermia in the cold ($P < 0.05$).

Fatty acid oxidation capacities are presented in Table II. In both muscles, total oxidation capacities increased with age ($P < 0.001$) but this increase was more

Table II. Total, mitochondrial and peroxisomal oxidation capacities of [1-^{14}C]-oleate in *longissimus lumborum* (LL) and *rhomboideus* (RH) muscle homogenates from new-born and 5-d old piglets exposed to thermal neutrality or to the cold[1].

Age	New-born		5 days		Statistics[2]	
Temperature	TN	C	TN	C	Pooled SE	Significance
Total oxidation (nmol carboxyl-C·(min·g muscle)$^{-1}$)						
LL muscle	25.6^{a}	32.1^{a}	54.4^{b}	55.9^{b}	6.97	A*** M***
RH muscle	31.0^{a}	32.4^{a}	75.5^{c}	72.6^{c}		A × M***
Peroxisomal β-oxidation (nmol carboxyl-C·(min·g muscle)$^{-1}$)						
LL muscle	8.40^{a}	6.51^{a}	21.1^{b}	19.7^{b}	2.80	A*** M***
RH muscle	12.7^{c}	11.6^{c}	34.4^{d}	29.8^{d}		
Mitochondrial oxidation (nmol carboxyl-C·(min·g muscle)$^{-1}$)						
LL muscle	17.2^{a}	25.5^{a}	33.4^{b}	36.2^{b}	6.60	A*** A × M*
RH muscle	18.4^{a}	20.8^{a}	41.0^{bT}	42.8^{bT}		
Mito β-ox / COX activity (nmol carboxyl-C·(μmol cyt c oxid)$^{-1}$)						
LL muscle	5.30	5.93	4.04	4.47	1.61	M***
RH muscle	2.32	2.09	1.32	2.20		

[1] Values are means, $n = 6$. Piglets were exposed to thermal neutrality (TN, 34 °C at birth, 30 °C at 5 days) or to cold (C, 24 °C at birth, 15 °C at 5 days) for a 4-h period before the measurements.
[2] Statistics: A, effect of age; M, effect of muscle; A × M, interaction between both effects; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. There was no effect of cold exposure.
[a,b,c,d] Individual means with different superscript letters are significantly different ($P < 0.05$).
[T] Difference between LL and RH muscle at 5 days tended to be significant ($P < 0.10$).

important in RH than in the LL muscle (134 vs. 94%, $P < 0.001$). It follows that the total oxidation capacities did not differ between the muscles at birth but were 34% higher in the RH than in the LL muscle at five-days of life ($P < 0.05$). Similarly, mitochondrial oxidation did not differ between the muscles at birth and increased with age (+114% in RH and +62% in LL, $P < 0.001$). An interaction between age and muscle type was observed ($P < 0.05$), indicating that the age-related rise in mitochondrial oxidation was more pronounced in the RH than in the LL muscle. Indeed, the oxidation capacities tended to be higher ($P < 0.1$) in the former at five days. When mitochondrial oxidation was expressed relative to COX activity the effect of the muscle type was still significant ($P < 0.001$) but there was no effect of age. Peroxisomal β-oxidation capacities were higher in RH than in LL from birth ($P < 0.001$). They increased by about 170% with age in both muscles ($P < 0.001$). Finally, it is noticeable that cold stress had no significant effect on fatty acid oxidation capacities.

The contribution of peroxisomal oxidation did not change with age and after cold stress and was higher in RH than in the LL muscles (42 vs. 32%, $P < 0.001$).

The ratio of CO_2 production to mitochondrial oxidation (Tab. III) was low and did not change with age in the LL muscle whereas an age-related increase was observed in RH ($P < 0.05$). This ratio was significantly reduced after a cold stress ($P < 0.05$).

As a regulator of CPT I activity, malonyl-CoA plays a pivotal role in the regulation of fatty acid oxidation. In our experimental conditions (Fig. 1), the inhibition of total oxidation by malonyl-CoA decreased with age in both muscles (–24% in LL, –18% in RH; $P < 0.01$). This inhibition was less pronounced in the RH muscle (29 vs. 67%, $P < 0.001$) and did not change in the cold.

Plasma levels of total carnitine did not change with age or cold stress (Tab. IV). On the contrary, NEFA levels increased by about 160% with age ($P < 0.001$), an additional increase being observed in the cold at five days (+60%, $P < 0.05$). Plasma albumin levels increased by 150% with age ($P < 0.001$) and were not affected by cold stress.

Finally, tissue levels of carnitine are presented in Table V. Total carnitine levels increased by about 70% with age in both muscles ($P < 0.01$). This postnatal rise was

Table III. The ratio of CO_2 production to mitochondrial oxidation of [1-^{14}C] oleate in *longissimus lumborum* (LL) and *rhomboideus* (RH) muscle homogenates from new-born and 5-d old piglets exposed to thermal neutrality or to the cold[1].

Age	New-born		5 days		Statistics[2]	
Temperature	TN	C	TN	C	Pooled SE	Significance
ratio of CO_2 production to mitochondrial oxidation (%)						
LL muscle	12.0^{a}	10.9^{a}	10.8^{a}	9.44^{a}	4.07	M** C*
RH muscle	13.9^{a}	10.4^{a}	20.1^{b}	13.7^{aT}		M × A*

[1] Values are means, $n = 6$. Piglets were exposed to thermal neutrality (TN, 34 °C at birth, 30 °C at 5 days) or to cold (C, 24 °C at birth, 15 °C at 5 days) for a 4-h period before the measurements.
[2] Statistics: A, effect of age; M, effect of muscle; C, effect of cold; M × A, interaction between both effects; **$P < 0.01$, *$P < 0.05$.
[a,b] Individual means with different superscript letters are significantly different ($P < 0.05$).
[T] Difference between LL and RH muscle at 5 days in the cold tended to be significant ($P < 0.10$).

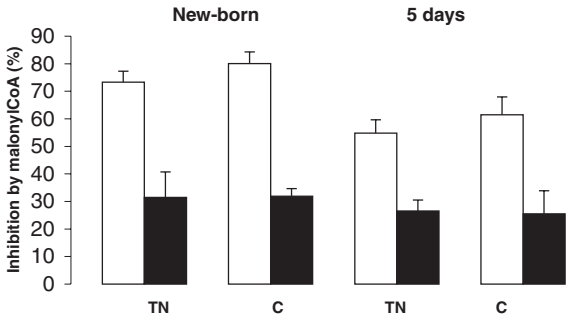

Figure 1. Inhibition of the total oxidation capacity of [1-^{14}C]-oleate by malonyl-CoA in *longissimus lumborum* (□, LL) and *rhomboideus* (■, RH) muscle homogenates from new-born and 5-d old piglets exposed to thermal neutrality (TN) or to the cold (C). The values are means + SE (n = 6). The effects of age ($P < 0.01$) and muscle type ($P < 0.001$) are significant, but there is no interaction and no effect of cold.

Table IV. Plasma levels of total carnitine, non-esterified fatty acid (NEFA) and albumin in new-born and 5-d old piglets exposed to thermal neutrality or to the cold[1].

Age	New-born		5 days		Statistics[2]	
Temperature	TN	C	TN	C	Pooled SE	Significance
Total carnitine (μmol·L^{-1})						
	21.9	24.9	26.9	29.1	7.50	NS
Non-esterified fatty acids (μmol·L^{-1})						
	56.0^{a}	57.0^{a}	148.1^{b}	238.9^{c}	57.50	A*** C^{T} A × C^{T}
Albumin (g·L^{-1})						
	11.1	10.9	28.2	27.6	3.75	A***

[1] Values are means, n = 6. Piglets were exposed to thermal neutrality (TN, 34 °C at birth, 30 °C at 5 days) or to cold (C, 24 °C at birth, 15 °C at 5 days) for a 4-h period before the measurements.
[a,b,c] Individual means with different superscript letters are significantly different ($P < 0.05$).
[2] Statistics: A, effect of age; C, effect of cold; A × C, interaction between both effects; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ^{T} $P < 0.06$.

explained by an increase in both long-chain acylcarnitine ($P < 0.01$) and acid-soluble carnitine ($P < 0.001$) concentrations. Concerning acid-soluble carnitine concentrations, an interactive effect is noticed between age and muscle type ($P < 0.05$) indicating that the postnatal rise was more marked in RH than in the LL muscle. Tissue levels of carnitine were not affected by cold stress.

4. DISCUSSION

The present data demonstrate that skeletal muscle mitochondrial and peroxisomal fatty acid oxidation capacities increase during early postnatal development in pigs. These changes are muscle-specific and are not affected by cold stress. These data complete the description of developmental changes in peroxisomal β-oxidation in the

Table V. Tissue levels of carnitine and acylcarnitine in *longissimus lumborum* (LL) and *rhomboideus* (RH) muscle homogenates from new-born and 5-d old piglets exposed to thermal neutrality or to the cold[1].

Age	New-born		5 days		Statistics[2]	
Temperature	TN	C	TN	C	Pooled SE	Significance
Acid-soluble carnitine (nmol/g wet wt)						
LL muscle	393[a]	511[a]	653[b]	713[b]	89.7	A***
RH muscle	391[a]	454[a]	747[bT]	814[bT]		M × A*
Long-chain acylcarnitines (nmol/g wet wt)						
LL muscle	69	73	84	112	30.8	A**
RH muscle	32	53	85	100		
Total carnitine (nmol/g wet wt)						
LL muscle	463	584	737	824	105.8	A**
RH muscle	423	507	832	914		

[1] Values are means, $n = 6$. Piglets were exposed to thermal neutrality (TN, 34 °C at birth, 30 °C at 5 days) or to cold (C, 24 °C at birth, 15 °C at 5 days) for a 4-h period before the measurements. Acid-soluble carnitine represents the sum of free carnitine and acid-soluble short-chain acylcarnitine.
[2] Statistics: A, effect of age; M, effect of cold; A × M, interaction between both effects; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.
[a,b] Individual means with different superscript letters are significantly different ($P < 0.05$).
[T] Difference between LL and RH muscle at 5 days tended to be significant ($P < 0.10$).

kidneys, liver and heart of young pigs [5], and our previous investigations on the in vivo metabolism [6], morphofunctional characteristics [18], mitochondrial metabolism [7] and CPT I activity [8] of skeletal muscle from young pigs.

4.1. Skeletal muscle fatty acid oxidation capacities increased postnatally

Whole-body oxygen consumption increases between birth and five-days, by which time adequate thermoregulation is achieved. As shown previously [12], this is accompanied by a major rise in NEFA plasma levels, and a decline in the respiratory quotient reflecting the increased involvement of lipids as an energy source. This metabolic switch is likely to occur at the muscular level because, in these conditions, skeletal muscle is known to contribute 34–40% to whole-body oxygen consumption [6]. Indeed, we observed a marked increase in total fatty acid oxidation capacities expressed by g tissue wet weight between birth and five-days of life. Our data thus confirmed that the overall capacity of the newborn pig to oxidize fatty acids is low at birth [3, 19] and increases postnatally in the skeletal muscle [20]. The present data also showed that this increase is more marked in the RH muscle, a slow-twitch oxidative muscle in mature pigs. Interestingly, this rise was supported by an increase in both mitochondrial and peroxisomal oxidation rates and a lowering of the inhibitory effect of malonyl-CoA.

Regarding mitochondrial oxidation, this result was consistent with the postnatal proliferation of muscle mitochondria [7, 18], the postnatal enhancement of CPT I activity in subsarcolemmal mitochondria, and the fact that the inhibition of CPT I activity by malonyl-CoA is progressively relieved in intermyofibrillar mitochondria [8].

Furthermore, no effect of age was evidenced when the results were expressed in relation to COX activity, which confirmed that mitochondrial biogenesis is the primary factor involved in this postnatal enhancement of the mitochondrial oxidation capacity. Similarly, it is very likely that the expression of the data per mg of muscle proteins in the homogenate would have completely masked this effect of age because muscle protein contents are known to increase between birth and 5 days of age (data not shown). However, the expression of the data per g tissue wet weight more accurately reflects the in vivo changes [20].

We simultaneously, reported an increase in the peroxisomal β-oxidation rate between birth and five days of age and calculated that the apparent rate of fatty acid degradation by peroxisomes contributed 32–42% to total oxidation capacities of skeletal muscles at both ages. Yu et al. [5] found a peroxisome contribution of 37–51% in the liver, 28–41% in the kidney and 26–31% in the heart of pigs. Similarly, the relative contribution of peroxisomes was quite similar among tissues in humans (19–33% [13]) and bovines (22–49%; [21]) whereas large variations were noticed in rat tissues (< 10% [22], up to > 30% [13]), especially within the same study (14% in skeletal muscles up to 51% in the liver [21]). However, peroxisome contribution could also vary according to the fatty acid chain length [23]. Therefore, knowing the wide age range and nutritional conditions used by these different authors, it is difficult to conclude on the significance of these variations. Whatever it might be, our results confirm that peroxisomes contribute significantly to the in vitro fatty acid oxidation capacity and to its postnatal enhancement in pig muscles. We must also keep in mind that the apparent rate of fatty acid degradation by peroxisomes is proportionally exaggerated by our in vitro approach probably due to the large dilution of the cytoplasmic pool by homogenization of muscles which could delay the transfer of shortened fatty acids from peroxisomes to the mitochondria. Little is known about the proliferation of peroxisomes in the skeletal muscle of the neonatal swine but it is likely that peroxisomal mass and/or enzyme activities change postnatally. Indeed, in the pig liver, peroxisomal numbers increase during the first 28 d of life [24] and the activity of fatty acyl-CoA oxidase, the rate-limiting enzyme for peroxisomal β-oxidation, increases within 24 h post-partum [25]. As proposed by Yu et al. [5], this relatively high peroxisomal β-oxidation rate may act as a compensatory mechanism to the limited mitochondrial ability to oxidize the milk fatty acids. This mechanism may also operate in skeletal muscle. However, considering the incompleteness of peroxisomal oxidation [26] and the subsequent mitochondrial degradation of peroxisomal oxidation products, it is likely that peroxisomal proliferation will be of limited help to the piglet without a concomitant increase in mitochondrial mass. Alternatively, the initiation of fatty acid β-oxidation at the peroxisomal level should be very useful, considering the limited development of the skeletal muscle capillary bed at birth (low capillary-to-fiber ratio) [18] and thereby, the low capacity for transport and diffusion of oxygen to the mitochondria at this age.

Peroxisomal and mitochondrial oxidation capacities were higher in RH than in LL muscle, the difference between both muscles increasing with age. These changes parallel the well-known metabolic differentiation of skeletal muscles in pigs during the early postnatal life [7, 27]. The contribution of peroxisomal oxidation to total oxidation was higher in RH, i.e. the most oxidative muscle, which further confirms that peroxisomal and mitochondrial oxidation pathways are probably coordinated mechanisms as suggested by various authors [5, 26]. In our experiment, RH and LL muscles also differed regarding malonyl-CoA sensitivity, with a much lower inhibition of oleate oxidation in the RH. This nonsuppressible component of CPT I activity is probably not

related to mitochondrial damage and exposure of CPT II because it is unlikely that (i) mitochondrial damage occurred in a manner that differed systematically between red and white muscles, and (ii) mitochondrial damage allowed CPT I independent acyl-CoA entry into the mitochondrial matrix without concomitant disruption of the β-oxidative pathways. Although surprising, this result was in agreement with a recent study showing a greater resistance of palmitate oxidation to malonyl-CoA inhibition in red than in white muscle homogenates of rats [28]. This suggests that red muscle expressed a malonyl-CoA-resistant CPT I isoform or sub-population. Indeed, Kim et al [28] have shown that two CPT-1β splice variants were more abundant in red than in white muscles which may be related to fiber type-specific expression and/or post-translational modulations.

Skeletal muscle fatty acid oxidation has also been probably triggered off postnatally by the rise in plasma albumin and tissue carnitine levels. Plasma albumin levels are low in the fetal pig [29] and at birth [30], and this postnatal rise should promote the NEFA transport capacity. Simultaneously, acid-soluble and long-chain acylcarnitine levels increased three-fold in both muscles within five days. This result is in agreement with the 20% increase reported by Kerner et al. [15] in the *biceps femoris* muscle. Interestingly, we demonstrate that the postnatal rise in acid-soluble carnitine was more marked in RH than in LL muscle, in agreement with the increasing difference in the fatty acid oxidation capacity between those two muscles after birth.

4.2. Cold stress had no effect on skeletal muscle fatty acid oxidation capacities of the piglets

At birth, the oxidation of fatty acids in skeletal muscle was very limited and did not change during cold stress. The oxidation capacity is low and limited amounts of fatty acids are available at the tissue [18] and plasma (present results) levels. Therefore, the respiratory quotient was high and increased in the cold, reflecting the preferential utilization of glycogen stores as an energy source.

At five days of life, the metabolic response of the piglet to cold had changed considerably. The respiratory quotient was much lower and did not rise in the cold which suggested a concomitant increase in both fat and carbohydrate utilization. The cold-induced enhancement of NEFA plasma levels indicates that adequate mobilization of fat stores occurred. In similar cold conditions, we have also shown that, at this age, fatty acid uptake is stimulated in the skeletal muscle [6] thereby increasing fatty acid availability within the muscle cell. This mechanism was probably responsible for the enhancement of fatty acid utilization because very limited changes in fatty acid oxidation were noticed. The total as well as mitochondrial, peroxisomal, and malonyl-CoA-dependent oxidation capacities were not affected by cold stress, which suggests that this potential is probably sufficient to cover the piglet's energy needs. Alternatively, inhibition by malonyl-CoA was probably already maximal at thermoneutrality because it was measured on tissues from the fed piglet, and therefore the present conditions could hardly reveal a difference in the cold. The contribution of peroxisomal β-oxidation did not increase after cold stress which gives little credibility to the existence of a significant thermogenic role of this metabolic pathway under such conditions. Although thermogenesis might also be enhanced by slight physiological uncoupling of the mitochondria, evidence is still missing for the existence of such mechanisms in the newborn pig. Surprisingly enough, the ratio of CO_2 production to mitochondrial oxidation decreased in the cold. This suggests that the capacity of the citric acid cycle

relative to that of the β-oxidation pathway was lower in the cold. In other words, this may indicate a decreased channeling of mitochondrial acetyl-CoA into the citric acid cycle and thereby an increased activity of other pathways, such as more acetyl-CoA being converted into acetyl-carnitine. This possibility requires further investigations considering the slight – although not significant ($P = 0.12$) – increase in acid-soluble carnitine in both muscles of cold-exposed piglets.

In conclusion, the ability of piglet skeletal muscles to use fatty acids as an energy source increases rapidly after birth. This is supported by a rise in both mitochondrial and peroxisomal oxidation capacities. The ability to mobilize body lipids increases simultaneously and is probably the main event involved in the enhancement of lipid utilization in the cold at five days of life.

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