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Dietary coconut oil affects more lipoprotein lipase activity than the mitochondria oxidative capacities in muscles of preruminant calves

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The presence of coconut oil in a milk replacer stimulates the growth rate of calves, suggesting a better oxidation of fatty acid in muscles. Because dietary fatty acid composition influences carnitine palmitoyltransferase I (CPT I) activity in rat muscles, this study was designed to examine the effects of a milk replacer containing either tallow (TA) or coconut oil (CO) on fatty acid utilization and oxidation and on the characteristics of intermyofibrillar (IM) and subsarcolemmal (SS) mitochondria in the heart and skeletal muscles of preruminant calves. Feeding CO did not affect palmitate oxidation rate by whole homogenates, but induced higher palmitate oxidation by IM mitochondria (+37%, $P < 0.05$*). CPT I activity did not significantly differ between the two groups of calves. Heart and* longissimus thoracis *muscle of calves fed CO had higher lipoprotein lipase activity (+27% and 58%, respectively;* $P < 0.05$*) but showed no differences in fatty acid binding protein content or activity of oxidative enzymes. Whatever the muscle and the diet, IM mitochondria had higher respiration rates and enzyme activities than those of SS mitochondria (*$P < 0.05$*). Furthermore, CPT I activity of the heart was 28-fold less sensitive to malonyl-coenzyme A inhibition in IM mitochondria than in SS mitochondria. In conclusion, dietary CO marginally affected the activity of the two mitochondrial populations and the oxidative activity of muscles in the preruminant calf. In addition, this study showed that differences between IM and SS mitochondria in the heart and muscles were higher in calves than in other species studied so far.* (J. Nutr. Biochem. 11:231–238, 2000)

Keywords: preruminant calves; coconut oil; fatty acid oxidation; mitochondria; cardiac and skeletal muscles

Introduction

From birth to weaning, preruminant calves received a liquid milk diet that contains a high amount of triglycerides generally from animal sources (beef tallow or lard). However, alternate sources of triglycerides of vegetable origin may be of particular interest either (1) for the health of consumers by reducing the risk of the bovine spongiform encephalopathy from any animal product (e.g., beef tallow) or (2) for a better growth performance[1] and improved meat organoleptic qualities.[2] It was recently shown that lauric acid (C12:0) from a coconut oil (CO)-rich milk replacer (42% of total fatty acids) would be preferentially transported as chylomicrons and very low density lipoprotein (VLDL) by the lymphatic pathway rather than by the portal vein.[3] Therefore, a more important contribution of dietary lauric acid in energy supply to muscle and a higher oxidation rate of this fatty acid by the muscle tissue could explain the increased protein retention previously observed in this animal.[4]

The metabolism of fatty acids and their subsequent mitochondrial oxidation depends on the coordinated induction of enzyme activities involved in fatty acid metabolism at both the extramitochondrial level (i.e., fatty acid uptake,

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free fatty acid trafficking within the muscle fiber) and intramitochondrial level (i.e., flux of fatty acid through the β-oxidation)[5] and some of these steps have been shown to be regulated by dietary fats. For instance, the expression of lipoprotein lipase (LPL) and of fatty acid binding protein (FABP) involved in fatty acid uptake and fatty acid transport within the cell, respectively, may be regulated by the nature of dietary fats.[6,7] Furthermore, fatty acid oxidation takes place in two populations of mitochondria, which have been isolated in the heart and muscles of humans,[8] rats,[9,10] and pigs[11]: the subsarcolemmal (SS) mitochondria, localized just beneath the sarcolemma, and the intermyofibrillar (IM) mitochondria, inserted into the myofibrillar network. In bovine muscles, the biochemical characteristics of either IM or SS mitochondria have never been studied. The factors that regulate some of the enzyme activities in IM and SS mitochondria might subsequently affect fatty acid oxidation. For instance, both the level and the nature of dietary fats regulate carnitine palmitoyltransferase I activity (CPT I; EC 2.3.1.21), a rate-limiting step of mitochondrial fatty acid oxidation in the liver and muscles of rats.[12] In addition, fatty acid composition of dietary fats is known to change the lipid composition of mitochondrial membranes.[13,14] Supplementation with dietary CO, a natural source of triglycerides providing saturated medium-chain fatty acids, produced damage to the hepatic mitochondria of chicks.[15]

The oxidation rate of medium-chain (C12:0) or long-chain (C16:0, C18:1) fatty acids provided by CO or tallow (TA), respectively, has been recently measured in our group in bovine tissues.[3,16] We have used as substrates the major fatty acids, which vary between CO and TA (C12:0, C16:0, C18:1), for the determination of oxidation rates of each fatty acid by an in vitro approach. Significant differences were observed in total oxidation rates.[16] Therefore, the objectives of this work were to investigate the effects of dietary CO on (1) the expression and activity of some enzymes or binding proteins involved in fatty acid utilization (such as LPL activity and FABP expression) and oxidation (such as the CPT I activity) and (2) the biochemical characteristics of the IM and SS mitochondria in muscles. Thus, two groups of preruminant calves fed a conventional milk replacer providing either saturated and unsaturated long-chain fatty acids (TA) or saturated medium-chain fatty acids (CO) were compared.

Materials and methods

Materials

[1-^{14}C]Palmitic acid (2.11 GBq/mmol), [methyl-^{3}H]carnitine (2.92 TBq/mmol), and [^{3}H]triolein (1.85–2.96 GBq/mmol) were obtained from Amersham International (Amersham, Bucks, UK). Nagarse (E.C. 3.4.21.14) from *Bacillus subtilis* (9.0 units/mg), adenosine triphosphate (ATP), adenosine diphospate (ADP), NAD^+, and cytochrome *c* were supplied by Boehringer-Mannheim (Meylan, France). Acetyl-coenzyme A (CoA), malonyl-CoA, palmitoyl-CoA, fatty acid-free bovine serum albumin (BSA) L-carnitine, palmitic acid, oxaloacetate, L-malate, puruvate, and CoA were purchased from Sigma Chemical Co. (St. Louis, MO USA). Polystyrene 96-well flat-bottom microtiter enzyme-linked immunosorbent assay (ELISA) plates were obtained from Polylabo (Strasbourg, France) and horseradish peroxydase-conjugated swine-anti rabbit immunoglobulin G (IgG) from Dako A/S (Glostrup, Denmark). Others reagents were from Merck (Darmstadt, Germany).

Table 1 Composition of the experimental milk diets

	Tallow	Coconut oil
Ingredient* (*g/kg of dry matter*)		
Spray-dried skim milk powder	656	656
Whey	60	60
Tallow	224	—
Coconut oil	—	224
Maize starch	50	50
Vitamin and mineral mixture†	10	10
Metabolizable energy value (kJ/g diet)	19.7	20.0
Fatty acid composition (*g/100 g of total fatty acids*)		
8:0	0.1	0.6
10:0	0.1	4.0
12:0	2.9	42.4
14:0	4.2	17.9
16:0	22.3	12.8
16:1 (n-7)	2.4	0.5
18:0	19.2	5.0
18:1 (n-9)	37.8	12.1
18:2 (n-6)	2.4	3.0
18:3 (n-3)	0.4	0.3
20:0	0.3	0.1
20:1	0.4	0.2
Others‡	7.5	1.1
Saturated fatty acids	53.8	83.0
Monounsaturated fatty acids	42.0	13.0
Polyunsaturated fatty acids	4.3	4.0
S:U ratio	1.2	4.9

*All ingredients were purchased from Bridel Retiers SA, 35 230 Bourg Barré, France.
†Contained (per kilogram of mixture): 0.15 g $MgSO_4$, 0.30 g MgO, 8 mg $FeSO_4$, 10 mg $CuSO_4$, 80 mg $ZnSO_4$, 48 mg $MnSO_4$, 0.6 mg $CoSO_4$, 0.18 mg $CaCl_2$, 0.15 mg Na_2SeO_3, 25,000 IU retinol, 5,000 UI cholecalciferol, 50 mg tocopherol, 5 mg thiamine, 10 mg riboflavine, 25 mg panthothenic acid, 40 mg niacin, 0.08 mg cyanocobalamin, 4 mg pyridoxine, 2.5 mg menadione, 100 mg ascorbic acid, 0.1 mg biotin, 1.2 mg folic acid, 0.6 g methionine, 0.8 g lysine chloride, 0.8 g choline, 0.05 g virginiamycin, and 3 g sorbitol (Celtic Langlois, St. Jacques de la Lande, France).
‡Others: branched-chain fatty acids and minor fatty acids (<0.1%).
S:U–saturated:unsaturated fatty acids.

Animals and experimental design

Two groups of five 2-week-old preruminant crossbred Holstein-Friesian male calves were used to study the effects of dietary medium-chain fatty acids on fatty acid oxidation capacity of muscles. Calves were fed for 19 days with a conventional milk replacer containing either TA or CO (*Table 1*) as previously described.[16] Metabolizable energy intake did not significantly differ between the two groups of calves.[16] At slaughter (5 weeks of age), the mean body weight of calves was 68 kg. Calves were anesthetized by isoflurane inhalation and sacrificed by exsanguination. Samples of the heart, *rectus abdominis* (RA; oxidoglycolytic muscle), and *longissimus thoracis* (LT; glycolytic muscle) were quickly trimmed of visible fat and connective tissue. Then they were cut into pieces, which were divided into two parts as soon as they were obtained. One part was frozen less than 10 min post slaughter in liquid nitrogen and stored at −80°C for subsequent analyses. The second part was rapidly excised with scissors and immediately cooled in ice-cold buffer and homoge-

nized to measure fatty acid oxidation rate[16,17] and to isolate IM and SS mitochondria.

Some of the biochemical characteristics of IM and SS mitochondria were studied in the muscles taken from the 10 5-week-old preruminant crossbred Holstein-Friesian male calves and also from 6 15-month-old male Charolais bulls. Bulls were fed a mixed diet according to a feeding pattern designed to allow an average daily weight gain of 1 kg as previously described.[17] Bulls were sacrificed by stunning (captive-bolt pistol) and exsanguination. Samples of heart, RA, and LT were immediately removed for subsequent isolation of IM and SS mitochondria and determination of mitochondrial enzyme activities.

LPL activity

Activity of LPL (EC 3.1.1.34), expressed in nmol fatty acid liberated per (min g tissue) was measured at 25°C in muscle homogenates with rat serum as activator and Intralipid® (Pharmacia and Upjohn, Stockholm, Sweden) as substrate into which [^{3}H]triolein had been incorporated by sonification.[18]

FABP content of tissues

Quantitative measurement of the heart type FABP (H-FABP) concentration in cytosols was performed by ELISA.[19] Cytosolic proteins of tissues, prepared as previously described,[19] were diluted to the concentration of 1 mg/L in buffer B (50 mmol/L sodium carbonate, pH 9.6) and 50 μL aliquots were coated onto the wells of microtiter plates by overnight incubation at 4°C. Amounts of 0 to 2 ng of human H-FABP were also coated in buffer B in the same way for calibration. In our assay procedure, the ELISA coefficient for human H-FABP in comparison to bovine H-FABP was 3.54. Consequently, values determined in our samples were multiplied by 3.54. The wells were washed as previously described.[19] Then, 100 μL of 1,000 X diluted in buffer C (5.4 mmol/L sodium phosphate, 1.3 mmol/L potassium phosphate, 150 mmol/L NaCl, pH 7.4, 0.5 g/L Tween-20) antisera against H-FABP were added and the plates incubated for 1 hr at 20°C. The wells were washed as described above and 100 μL of 500 X diluted in buffer C horseradish peroxidase-conjugated swine-anti rabbit IgG (P 0217; DAKO, Glostrup, Denmark) were added. After 1-hr incubation at 20°C, the wells were washed again. The bound peroxidase was assessed with 100 μL of a freshly prepared solution of 2.2 mmol/L *o*-phenylene with incubation in the dark for 30 min at 20°C; the reaction was stopped by addition of 50 μL 12.5% H_2SO_4. The product of the peroxidase reaction was determined at 492 nm using an ELISA plate reader (Labsystems Multiscan, Helsinki, Finland). The amount of immunoreactive protein (μg FABP/g tissue wet weight) was determined from calibration curves calculated from linear regression analysis.

In vitro palmitate oxidation

Palmitate oxidation rates of homogenates were determined by the procedure previously described.[17,20] All assays were made in triplicate using [1-^{14}C]palmitate as substrate (specific activity 55–63 kBq/μmol) with or without addition of mitochondrial inhibitors of the respiratory chain [i.e., antimycin A and rotenone (73 μmol/L and 10 μmol/L final concentrations, respectively)]. Mitochondrial oxidation rates designed as the antimycin-rotenone sensitive oxidation rate (A/R sensitive oxidation) represented total oxidation rate (mitochondrial and peroxisomal) minus peroxisomal oxidation rate measured in the presence of mitochondrial inhibitors. Palmitate oxidation rates also were assayed with freshly isolated IM and SS mitochondria. Briefly, palmitate oxidation was carried out for 15 min at 37°C in a total volume of 0.5 mL containing 200 μg of mitochondrial protein and 100 μL of 600 μmol/L [1-^{14}C]-palmitate bound to albumin in a 5:1 ratio.[21] Palmitate oxidation rates were calculated from the sum of $^{14}CO_2$ and ^{14}C-labeled perchloric acid-soluble products and were expressed in nmoles of palmitate oxidized/(min · g tissue wet weight or mg mitochondrial protein).

Isolation of muscle mitochondria and oxygen consumption

Muscle SS and IM mitochondria were isolated separately by a modification of the procedure previously described.[11] All isolation procedures were carried out at 4°C. Muscle samples were homogenized in buffer A [100 mmol/L sucrose, 180 mmol/L KCl, 10 mmol/L ethylenediamine-tetraacetic acid (EDTA), 5 mmol/L $MgCl_2$, 1 mmol/L ATP, and 50 mmol/L Tris-HCl, pH 7.4] using a Teflon-pestle-glass Potter-Elvehjem homogenizer, and mitochondria were obtained by differential centrifugations and suspended in storage buffer B (250 mmol/L sucrose, 2 mmol/L EDTA, and 20 mmol/L Tris-HCl, pH 7.4). Use of Nagarse (8.5 U/mg; Boehringer Mannheim, Heidelberg, Germany) was necessary to digest myofibrils in the pellet (3 mg/g of heart for 2 min and 4 mg/g of skeletal muscle for 3 min) and to liberate IM mitochondria from this pellet. Aliquots were taken after the first centrifugation step from the supernatant (HS) used for SS isolation and from the resuspended pellet (HI) used for IM isolation to calculate protein content on base of citrate synthase (CS) activity. Then SS and IM mitochondria were isolated and washed as previously described.[11] Final centrifugations were performed at 9,600 × g and at 11,000 × g for 10 min to pellet mitochondria from heart and from skeletal muscle, respectively. The final mitochondrial protein concentrations were determined,[22] with BSA as standard. Isolated muscle mitochondria were then diluted to 20 g protein/L with buffer B and kept on ice. Yields of IM or SS mitochondria protein ranged from 0.91 to 1.71 mg protein/g tissue for skeletal muscles and from 2.81 to 5.03 mg for heart, yields of IM protein being 2-fold higher ($P < 0.01$) than those of SS protein in the heart and LT ($P < 0.01$). The mitochondrial contents for SS and IM mitochondria calculated using the ratio of total CS activity of the tissue fraction HS or HI [units/(min · g of tissue)] to the specific activity of CS [units/(min · mg of mitochondrial protein)] in the SS and IM fractions were 48, 23, and 17 mg protein/g for heart, RA, and LT, respectively.

Respiration rate of isolated mitochondria was measured polarographically at 25°C using a Clark O_2 electrode (oxygen Hansatech Ltd, London, UK). State 4 and state 3 of respiration were initiated by the addition of 10 μL of malate plus 10 μL of pyruvate (final concentration of each 5 mm/L) and, 2 or 3 min later, by the addition of 10 μL ADP 100 nmol (final concentration 100 μmol/L).[23] The values of respiratory control ratio (RCR)[24] were high in IM mitochondria, ranging from 3.5 to 8, and much higher than those found in SS mitochondria (1.5 to 3.5). The values of phosphorus-oxygen ratio (ADP/O)[24] were 2.7 in heart and skeletal muscle IM mitochondria and in heart SS mitochondria (results not shown).

Mitochondrial enzyme activities

Activity of CS (EC 4.1.3.7) and of cytochrome *c* oxidase (COX; EC 1.9.3.1) were measured in both sonicated homogenates and in isolated mitochondria as previously described.[16,17] Specific and total activities of CS and COX are expressed in μmol of coenzyme A liberated/(min · mg mitochondria protein or g tissue) and in μmol of cytochrome c oxidized/(min · mg mitochondria protein or g tissue), respectively. Activity of isocitrate dehydrogenase (ICDH; EC 1.9.9.42) was determined in tissue homogenates as previously described.[18] Activity was expressed in μmoles of $NADPH,H^+$ liberated/(min · g tissue).

Activity of CPT I from freshly isolated mitochondria was

Table 2 Influence of the fatty acid composition of the milk diet on lipoprotein lipase (LPL), isocitrate dehydrogenase (ICDH), citrate synthase (CS), and cytochrome *c* oxidase activities (COX), fatty acid binding protein (FABP) content, and mitochondrial palmitate oxidation rates in homogenates of the heart and skeletal muscles of preruminant calves*†

	Heart		*Rectus abdominis*		*Longissimus thoracis*		Significant effect‡
	CO	TA	CO	TA	CO	TA	
LPL [*nmol of fatty acid released/(min · g tissue)*]	2,777 ± 198^{A}	2,192 ± 123$^{a,\|}$	159 ± 15.9^{B}	156 ± 23.6^{b}	169 ± 15.8^{B}	107 ± 19.2$^{b,\|}$	D$^{\|}$, M** M × D††
FABP [*μg/g tissue*]	574 ± 53.4^{A}	525 ± 54.9^{a}	231 ± 13.8^{B}	193 ± 18.1^{b}	186 ± 20.6^{B}	179 ± 33.6^{b}	M**
ICDH [*μmol of NADPH,H^{+} liberated/(min · g tissue)*]	16.4 ± 0.52^{A}	17.4 ± 2.14^{a}	2.49 ± 0.19^{B}	2.35 ± 0.07^{b}	2.83 ± 0.27^{B}	2.80 ± 0.21^{b}	M**
CS [*μmol of CoASH liberated/(min · g tissue)*]	39.0 ± 7.92^{A}	28.2 ± 5.14^{a}	4.90 ± 0.69^{B}	4.71 ± 0.98^{b}	7.38 ± 1.17^{B}	4.80 ± 0.61^{b}	M**
COX [*μmol of cytochrome c oxidized/(min · g tissue)*]	215 ± 8.12^{A}	231 ± 39.4^{a}	25.0 ± 2.99^{B}	24.4 ± 6.26^{b}	23.1 ± 3.42^{B}	19.9 ± 6.61^{b}	M**
AR-sensitive oxidation§ [*nmol of palmitate oxidized/(min · g tissue)*]	145 ± 12.4^{A}	132 ± 16.1^{a}	12.4 ± 2.58^{B}	22.1 ± 4.09^{b}	17.6 ± 4.30^{B}	18.0 ± 3.53^{b}	M**

*Data are means ±SEM (n = 5–7).
†Calves were fed for 19 days a milk diet containing either coconut oil (CO) or tallow (TA).
‡Statistics: M–significant effect of muscle; D–significant effect of diet group. M × D–significant effect of the interaction between muscle and group.
$^{\|}P < 0.05$, $^{**}P < 0.001$, $^{††}P < 0.01$.
§AR-sensitive oxidation rate means antimycin-rotenone sensitive oxidation rate. It represents difference between oxidation rates measured in whole tissue homogenates without addition of mitochondrial inhibitors (i.e. peroxisomal and mitochondrial oxidation rates) and with addition of mitochondrial inhibitors (peroxisomal oxidation rate).
^{A,B,a,b}Means with different superscripts in the same row are significantly different between tissues for the CO and TA groups, respectively ($P < 0.05$), by the Student's t-test with an adjustment of the P-values by the Bonferroni correction for multi-comparisons.

assayed at 30°C as the formation of [^{3}H]palmitoyl-L-carnitine from [methyl-^{3}H]carnitine and palmitoyl-CoA.[25,26] Sensitivity of CPT I to malonyl-CoA inhibition was estimated by measuring the concentration of malonyl-CoA required for 50% inhibition of the enzyme activity (IC_{50}). Mitochondria (0.5–1 mg protein) were preincubated 3 min alone or in the presence of malonyl-CoA (0.005–150 μmol/L) in 0.45 mL reaction medium [75 mmol/L KCl, 50 mmol/L mannitol, 25 mmol/L N-[2-hydroxyethyl]piperazine-N^{1}[2ethanesulfonic acid] (HEPES), 2 mmol/L KCN, 0.2 mmol/L EGTA, 1 mmol/L dithiothreitol, 10 g/L fat-free BSA, 80 μmol/L palmitoyl-CoA, pH 7.3, and then incubated 15 min with 1 mmol/L L-carnitine and 37 kBq [methyl-^{3}H]carnitine. The reaction was stopped by addition of 1 mL of butanol and 1 mL of saturated $(NH_4)_2SO_4$ and the product ([^{3}H]palmitoyl-L-carnitine) was extracted and counted as previously described.[27] CPT I activity was expressed as nmol palmitoyl [^{3}H]-carnitine produced/(min · mg mitochondrial protein). IC_{50} was expressed in μmol/L. Maximum inhibition with 150 μmol/L of malonyl-CoA was 95.8 ± 2.97% and 74.9 ± 7.69% for IM and SS mitochondria, respectively, isolated from the heart.

Malonyl-CoA concentration of heart

Malonyl-CoA concentration was determined by reversed-phase high performance liquid chromatagraphy (HPLC) as described previously.[27] Briefly, 1 g of frozen tissue was powdered under liquid nitrogen and placed into a centrifugation tube. Two milliliters of 1.4 mol/L perchloric acid containing 200 mmol/L of isobutyryl-CoA as internal standard were added to the powder. The mixture was homogenized for 2 min on ice and centrifuged at 12,000 × g and 4°C for 10 min. The pH of the resulting supernatant was adjusted to approximately 3 with 356 mmol/L KOH, centrifuged at 1,000 × g and 4°C for 10 min and frozen at −80°C until further analysis. Finally, the pH of the samples was adjusted to approximately 6 just prior to the identification of the CoA esters by HPLC.

Statistical analysis

Analysis of variance of the data was made using the general linear models procedure of SAS.[28] For oxidation rates, enzyme activities, and FABP contents, the effects tested included diet (D), group of animals (TA or CO), animal (A) tested within treatment group, muscle (M), and interaction between group and muscle (D × M). The group factors were tested against animals within groups. The residual mean square was used as the error term for other effects. Results were expressed as means and standard errors of the means. Comparisons for specific activities of mitochondrial enzymes (CS, COX, and CPT) between tissues within one type of mitochondria, and comparisons between tissues within a diet group (CO or TA), were analyzed by the Student's t-test with an adjustment of the P-values by the Bonferroni correction used for multicomparison. In addition, comparisons between groups (TA or CO) were made using the Student's t-test for unpaired data.

Results

Effect of the nature of dietary fat on lipid muscle metabolism

LPL activity. LPL activity differed significantly between heart and skeletal muscle ($P < 0.001$) and between the CO and TA groups of calves ($P < 0.05$; *Table 2*). Indeed, whatever the dietary fat, LPL exhibited a 17-fold higher activity in the heart than in RA and LT ($P < 0.001$). In addition, LPL activity was 27% and 58% higher in the heart and in LT of CO than of TA calves, respectively ($P < 0.05$).

FABP content. Tissue FABP content was 2.8-fold higher in the heart than in both RA and LT ($P < 0.001$; *Table 2*). By contrast, substitution of TA for CO in the milk diet did not

Table 3 Influence of the fatty acid composition of the milk diet on palmitate oxidation rates, citrate synthase (CS), and cytochrome c oxidase (COX) activities in isolated subsarcolemmal (SS) and intermyofibrillar (IM) mitochondria from the heart and from two skeletal muscles of preruminant calves*†

	Heart		*Rectus abdominis*		*Longissimus thoracis*		Significant effect‡
	CO	TA	CO	TA	CO	TA	
Oxidation rate [*nmol of palmitate oxidized/(min · mg mitochondrial protein)*]							
SS mitochondria	7.56 ± 1.50^{a}	6.50 ± 0.80^{A}	$1.68 \pm 0.16^{a,b}$	2.09 ± 0.38^{B}	2.14 ± 0.75^{b}	1.90 ± 0.42^{B}	M§
IM mitochondria	1.55 ± 0.33^{a}	0.95 ± 0.10^{A}	0.58 ± 0.08^{a}	0.50 ± 0.08^{AB}	0.87 ± 0.25^{a}	0.44 ± 0.08^{B}	M‖, D**
CS [*μmol of CoASH liberated/(min · mg mitochondrial protein)*]							
SS mitochondria	0.62 ± 0.11^{a}	0.85 ± 0.23^{A}	0.19 ± 0.02^{a}	0.27 ± 0.09^{A}	0.19 ± 0.04^{a}	0.25 ± 0.09^{A}	M§
IM mitochondria	1.09 ± 0.19^{a}	0.95 ± 0.23^{A}	0.43 ± 0.12^{a}	0.56 ± 0.15^{A}	0.53 ± 0.15^{a}	0.77 ± 0.18^{A}	M§
COX [*μmol of cytochrome c oxidized/(min · mg mitochondrial protein)*]							
SS mitochondria	13.7 ± 5.52^{a}	5.44 ± 1.08^{A}	1.43 ± 0.22^{a}	1.54 ± 0.32^{B}	2.43 ± 0.77^{a}	1.36 ± 0.22^{B}	M‖
IM mitochondria	29.7 ± 4.30^{a}	$18.7 \pm 1.11^{A,\|}$	12.9 ± 4.05^{b}	10.5 ± 1.64^{B}	11.2 ± 3.41^{b}	12.2 ± 2.04^{B}	M§, M × D§

*Data are means ±SEM ($n = 5$).
†Calves were fed for 19 days a milk diet containing either coconut oil (CO) or tallow (TA).
‡Statistics: M–significant effect of muscle; D–significant effect of diet group. M × D–significant effect of the interaction between muscle and group.
§$P < 0.001$, ‖$P < 0.01$, **$P < 0.05$.
A,B,a,bMeans with different superscripts in the same row are significantly different between tissues for the CO and TA groups, respectively ($P < 0.05$), by the Student's *t*-test with an adjustment of the *P*-values by the Bonferroni correction for multi-comparisons. ‖$P < 0.10$, significant difference between the two diet groups.

affect the FABP content in either heart or skeletal muscles (*Table 2*).

Oxidation rates of palmitate. Oxidation rate of palmitate was measured in both IM and SS mitochondria and in whole tissue homogenates. The antimycin-rotenone sensitive palmitate oxidation rate (i.e., mitochondrial oxidation rate) was 7.9-fold higher in the heart than in skeletal muscles ($P < 0.001$, *Table 2*). In the same way, the oxidation rate of palmitate was 3.6- and 2.1-fold higher in SS and IM mitochondria isolated from the heart than from skeletal muscles, respectively ($P < 0.01$, *Table 3*). Palmitate oxidation rates were also 6.0- and 3.4-fold higher in SS than in IM mitochondria of the heart and skeletal muscles (RA and LT), respectively. The diet did not affect the rate of palmitate oxidation except in IM mitochondria since palmitate oxidation rate was 37% higher in muscles from calves fed the CO diet ($P < 0.05$; *Table 3*).

CS, ICDH, COX, and CPT I activities. Activity of CS, ICDH, and COX in tissue homogenates differed between heart and skeletal muscles but not between the CO and TA groups of calves (*Table 2*).

Similarly, specific activity of CS and COX in both IM and SS mitochondria from the three muscles did not significantly differ between CO and TA calves (*Table 3*). However, COX specific activity tended to be higher in IM mitochondria from the heart of the CO calves (1.5-fold, $P < 0.10$; *Table 3*). In the same way, the specific activity of CPT I was not affected by the dietary fatty acids, whatever the tissue and the mitochondrial populations considered (*Table 4*). Moreover, the sensitivity of CPT I for malonyl-CoA (IC_{50}) of IM and SS mitochondria as well as the malonyl-COA concentration did not differ significantly in the hearts between the CO and TA groups of calves (*Table 4*). However, IC_{50} of CPT I for malonyl-CoA was 28-fold higher for IM than for SS mitochondria from the heart, indicating a higher sensitivity of CPT I to malonyl-CoA in SS than in IM mitochondria (*Table 4*).

Mitochondrial enzyme activities in SS and IM mitochondria

CS specific activity was 1.5- to 2-fold higher ($P < 0.05$) in IM mitochondria than in SS mitochondria in the three studied muscles from calves and bulls (*Table 5*). IM mitochondria in these muscles were also characterized by much higher COX and CPT I activities (from 3.5- to 7.8-fold and from 2- to 10.8-fold, respectively, $P < 0.001$) than SS mitochondria (*Table 5*).

Enzyme activities of the two mitochondrial populations differed significantly between the heart and the skeletal muscles (*Table 5*). CS, COX, and CPT I activity in both IM and SS mitochondria were higher in the heart (from 2.0- to 4.0-fold for CS and COX and from 1.3- to 6.9-fold for CPT I activities, $P < 0.001$) than in RA and LT.

Mitochondrial enzyme activities did not differ significantly between the two diets in preruminant calves (*Tables 3 and 4*) and between bulls and calves (data not shown).

Discussion

Effects of dietary fatty acids on oxidative fatty acid metabolism in muscles

The higher LPL activity in the heart and LT of calves fed the CO diet may be explained by several direct or indirect factors. Among the hormones that modulate the LPL activity of peripheral tissues, insulin correlates positively with LPL activity in white adipose tissues and negatively with

Table 4 Influence of the fatty acid composition of the milk diet on specific carnitine palmitoyltransferase I (CPT I) activities in subsarcolemmal (SS) and intermyofibrillar (IM) mitochondria of the heart and two skeletal muscles of preruminant calves*†

	Heart		*Rectus abdominis*		*Longissimus thoracis*		Significant effect‡
	CO	TA	CO	TA	CO	TA	
CPT I activity [*nmol of palmitoylcarnitine formed/(min · mg mitochondrial protein)*]							
SS mitochondria	0.71 ± 0.22^{A}	0.80 ± 0.04^{a}	0.10 ± 0.04^{A}	0.12 ± 0.01^{b}	0.13 ± 0.06^{A}	0.08 ± 0.03^{b}	M§
IM mitochondria	1.46 ± 0.14^{A}	1.68 ± 0.06^{a}	1.08 ± 0.11^{B}	1.18 ± 0.10^{b}	1.09 ± 0.18^{A,B}	1.29 ± 0.10^{a,b}	M§
IC_{50}‡ (*μmol/L*)							
SS mitochondria	0.008 ± 0.002	0.009 ± 0.004	ND‖	ND‖	ND‖	ND‖	NS
IM mitochondria	0.14 ± 0.075	0.34 ± 0.16	ND‖	ND‖	ND‖	ND‖	NS
Malonyl CoA concentration (*nmol/g of tissue*)	1.94 ± 0.50	1.26 ± 0.29	ND‖	ND‖	ND‖	ND‖	NS

*Data are means ±SEM (n = 4–5).
†Calves were fed for up to 19 days a milk diet containing either coconut oil (CO) or tallow (TA).
‡Statistics: M–significant effect of muscle; D–significant effect of diet group. §$P < 0.001$.
§IC_{50} is the concentration of malonyl-CoA required for 50% inhibition of the CPT I activity.
‖IC_{50} values were not determined (ND) due to the limited amount of available mitochondria material. Consequently, malonyl-CoA concentrations were not determined as well.
A,B,a,bMeans with different superscripts letters in the same row are significantly different between tissues for the TA and the CO groups, respectively ($P < 0.05$), by the Bonferroni correction for multi-comparisons.
NS–nonsignificant.

that in skeletal and cardiac muscles.[29] In fact, the lower insulinemia observed in calves fed the CO diet[16] could explain, at least in part, the higher LPL activity of the heart and LT. The changes in insulinemia could also have regulated adipose tissue metabolism, thereby affecting fat partitioning between muscles and adipose tissues. In fact, preliminary results indicate that neither LPL activity nor lipogenic enzyme activities significantly differed between the two groups of animals in the perirenal fat depot (data not shown).

The higher LPL activity measured in both heart and LT of calves fed the CO diet also suggests an increase of fatty acid uptake by the muscle cells. However, this was not associated with higher levels of H-FABP in these tissues. As in the rat muscle,[30] the H-FABP content in bovine muscles were not affected by the type of dietary fatty acid. In the rat, regulation by the fatty acid composition of the diet of H-FABP content seemed to differ from that of the liver type FABP in some[7] but not all studies.[30]

Furthermore, the higher LPL activity and the lower triacylglycerol concentration observed in the heart of calves fed a diet containing CO[16] suggests a higher oxidation rate of medium-chain fatty acids. In fact, in our study, dietary medium-chain fatty acids marginally affected CPT I activity in both IM and SS mitochondria. Previous observations in the heart of rats fed a high-fat diet containing hydrogenated CO compared with rats fed a diet containing olive oil[12] showed a significant difference in total CPT I activity

Table 5 Specific activities of mitochondrial enzymes of subsarcolemmal (SS) and intermyofibrillar (IM) mitochondria of bovine heart and skeletal muscles*

	Heart		*Rectus abdominis*		*Longissimus thoracis*	
	SS	IM	SS	IM	SS	IM
CS [*μmol of CoASH liberated/(min · mg mitochondrial protein)*]	0.72 ± 0.11^{A}	1.06 ± 0.26^{a,†}	0.23 ± 0.04^{B}	0.46 ± 0.07^{b,†}	0.25 ± 0.04^{B}	0.51 ± 0.08^{b,†}
COX [*μmol of cytochrome c oxidized/(min · mg of mitochondrial protein)*]	6.69 ± 1.39^{A}	23.6 ± 2.67^{a,‡}	1.49 ± 0.19^{B}	11.6 ± 1.92^{b,‡}	1.84 ± 0.38^{B}	11.7 ± 1.77^{b,‡}
CPT I [*nmol of palmitoylcarnitine formed/(min · mg mitochondrial protein)*]	0.76 ± 0.11^{A}	1.57 ± 0.08^{a,‡}	0.11 ± 0.02^{B}	1.13 ± 0.07^{b,‡}	0.11 ± 0.03^{B,‡}	1.19 ± 0.10^{b,‡}

*Data are means ±SEM (n = 8–16 calves or bulls, no significant differences were observed between calves and bulls).
†$P < 0.01$, ‡$P < 0.001$, significant difference between IM and SS mitochondria for a given tissue.
A,B,a,b,Means with different superscript letters are significantly different between tissues and for SS and IM mitochondria, respectively ($P < 0.05$), by the Student's t-test with an adjustment of the P-values by the Bonferroni correction for multi-comparisons.
CS–citrate synthase. COX–cytochrome c oxidase. CPT I–carnitine palmitoyltransferase I.

between the two diet groups after 10 weeks of feeding. The duration of feeding in our study may have been too short to induce any modification of total CPT I activity in skeletal muscles of calves.

Our results also suggest that dietary CO marginally affects fatty acid oxidation by the two mitochondrial populations, whatever the metabolic parameters studied. Indeed, the slight increase in palmitate oxidation rates in IM mitochondria induced by the CO diet was not associated with any concomitant differences between the two groups of calves in CS, COX, and CPT I activities in IM mitochondria and in fatty acid oxidation rate in whole homogenates.[16] In addition, in SS mitochondria, neither palmitate oxidation rate nor CPT I activity differed between the two groups of animals. Finally, the concentration of malonyl-CoA, a potent regulator of CPT I activity, and thus of fat oxidation, did not differ significantly between the two groups of animals. Thus, this study and our previous work[16] demonstrated that the oxidative capacities of mitochondria in cardiac and skeletal muscles were marginally affected quantitatively or qualitatively by the presence of CO in the diet. The major nutritional effects induced by coconut oil are a higher LPL activity in cardiac and muscle tissues (this study) and a higher peroxisomal oxidation rate of laurate in the liver and in oxidative muscles.[16]

Properties of IM and SS mitochondria from bovine cardiac and skeletal muscles

Evidence of the existence of two biochemically distinct mitochondrial populations in the heart and skeletal muscles has been shown in a great number of animal species.[10,11,31,32] However, our study reported for the first time the functional characteristics of these two mitochondrial populations from cardiac and skeletal muscles in cattle, although an old study had previously described the functional properties of mitochondria in bovine muscles.[33] Yields of mitochondrial protein in our study were similar to those previously reported in piglet skeletal muscles.[11,34] Treatment of the myofibril pellet with nagarse did not alter the respiratory properties of the IM mitochondria.[35] On the contrary, SS mitochondria that were not exposed to nagarse were less well coupled than in previous studies in piglets[11] and exhibited much lower enzyme activities than observed in studies in rats.[31]

Thus, as previously shown in other species,[11,31,34] the IM mitochondria appeared to be a more biochemically active population than the SS mitochondria, and these differences do not depend on isolation procedure or specific treatment.[35] Differences in COX and CPT I activities between the IM and SS mitochondria in bovine skeletal muscles were greater than those in skeletal muscle from piglets[11,34] and rats.[31] In the bovine heart, IM CPT I was also 28-fold less sensitive to malonyl-CoA than CPT I of SS mitochondria. In fact, values of IC_{50}[12,36] and of COX activity[37] for isolated mitochondria of the rat heart were intermediate with our values for IM and SS bovine heart mitochondria.

CPT I activity expressed per milligram of mitochondrial protein was two- to eightfold lower in the bovine than in the rat heart[12,36] in agreement with a lower fatty acid oxidative capacity of the bovine heart.[17] Furthermore, the malonyl-CoA concentration in the heart was threefold lower in bovine than in the rat in the fed state.[36] This may be explained by a higher carnitine content of the ruminant muscle and heart, even in the preruminant state,[38] because carnitine binds acetyl-CoA and directs it to the acetylcarnitine pool rather than to its conversion into malonyl-CoA.

Another way to compare IM and SS mitochondria properties would be to calculate the ratio of the activities between an enzyme located in the inner mitochondrial membrane and an enzyme located in the mitochondrial matrix space, such as the COX:CS ratio. This ratio was much higher in IM (23.5) than in SS (7.7) mitochondria, which suggests that the development of the inner mitochondrial membrane and the number of cristae are higher in the IM population, leading to higher oxidative and ATP synthesis capacities.[39] Differences between IM and SS mitochondria are probably related to the different metabolic functions of these two mitochondrial populations; that is, to provide the energy required for the transport of nutrients from blood to within the muscle cells for SS mitochondria and to provide the energy required for contractile protein interaction and therefore, muscular contraction for IM mitochondria.[40]

The heart has a much higher oxidative capacity than skeletal muscles in the calf[17] due to higher specific activities of mitochondrial enzymes (CPT I, COX, CS) and higher contents of both IM and SS mitochondria. The 15- and 2.6-fold higher LPL activity and FABP content in the heart than in skeletal muscles are in agreement with observations in other species.[19,41] This confirms the relationship between the oxidative capacities and the FABP content of heart and muscles.[30] However, unlike in chick and rat skeletal muscles,[40,42] mitochondrial properties did not differ greatly between oxido-glycolytic (RA) and glycolytic (LT) bovine muscles.

In conclusion, we showed that dietary CO affects mainly LPL activity in the heart and skeletal muscles from calves and marginally the enzyme activities in both IM and SS mitochondria. This suggests that following their uptake by muscle cells, fatty acids may be directed to another subcellular compartment. This is in agreement with our previous results showing a significantly higher peroxisomal fatty acid oxidation rate in the heart of calves fed a milk replacer rich in CO.[16]

This study also showed that differences between IM and SS mitochondria properties were greater in bovine heart and muscles than in the corresponding tissues from others species suggesting species-specific differences in the biochemical characteristics of these two mitochondrial populations.

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