Effects of *trans* MUFA from dairy and industrial sources on muscle mitochondrial function and insulin sensitivity

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Abstract Epidemiological studies suggest that chronic consumption of *trans* MUFA may alter muscle insulin sensitivity. The major sources of dietary *trans* MUFA (dairy fat vs. industrially hydrogenated oils) have different isomeric profiles and thus probably different metabolic consequences. These effects may involve alterations in muscle mitochondrial oxidative capacity, which may in turn promote insulin resistance if fatty acid oxidation is reduced. We report that in Wistar rats, an 8 week diet enriched (4% of energy intake) in either dairy, industrial, or control MUFA did not alter insulin and glucose responses to an intraperitoneal glucose tolerance test (1g/kg). In C2C12 myotubes, vaccenic and elaidic acids did not modify insulin sensitivity compared with oleic acid. Furthermore, the ex vivo total, mitochondrial and peroxisomal oxidation rates of [1-^{14}C]oleic, vaccenic, and elaidic acids were similar in *soleus* and *tibialis anterior* rat muscle. Finally, an 8 week diet enriched in either dairy or industrial *trans* MUFA did not alter mitochondrial oxidative capacity in these two muscles compared with control MUFA but did induce a specific reduction in *soleus* mitochondrial ATP and superoxide anion production ($P < 0.01$ vs. control). In conclusion, dietary *trans* MUFA of dairy or industrial origin have similar effects and do not impair muscle mitochondrial capacity and insulin sensitivity.— Tardy, A-L., C. Giraudet, P. Rousset, J-P. Rigaudière, B. Laillet, S. Chalancon, J. Salles, O. Loreau, J-M. Chardigny, and B. Morio. **Effects of *trans* MUFA from dairy and industrial sources on muscle mitochondrial function and insulin sensitivity. *J. Lipid Res.* 2008.** 49: **1445–1455.**

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Plurimetabolic syndrome (syndrome X) affects 40% of men and 25% of women over the age of 55 in Europe (1). One of the features of this metabolic disorder is impaired insulin sensitivity 10 to 20 years before the development of type 2 diabetes, with a concomitant increased risk of cardiovascular disease (2). Several observational studies suggest that insulin resistance is primarily a lifestyle disorder (3), because overweight and low physical activity are the greatest risk factors for type 2 diabetes (4). Evidence is now emerging that the incidence of this metabolic disorder could also be affected by diet composition. High energy intake is a factor contributing to the development of insulin resistance by increasing body weight and fat deposition (5, 6), whereas several studies have shown that weight loss is very effective in reducing the prevalence of type 2 diabetes in overweight individuals (7, 8). Among all the macronutrients, dietary fatty acids have received particular attention as potential inductors of insulin resistance. Changes in the fatty acid composition of blood and muscle have been shown to correlate with insulin resistance in humans (9, 10). Furthermore, epidemiological studies have reported that mono- and polyunsaturated fat may reduce the risk of developing type 2 diabetes, whereas saturated fat has been reported to have a potentially adverse effect (11–13). These effects are independent of changes in body weight and composition. The KANWU (Kuopio, Aarhus, Naples, Wollong and Uppsala) interventional study confirms these effects, but only when total dietary fat consumption is lower than 37% of total energy intake (14).

The impact of dietary *trans* MUFA on insulin sensitivity is not fully understood. *Trans* MUFA are geometrical isomers of *cis* MUFA. Hydrogen atoms are on both sides of the fatty acid double bonds and therefore change their configuration and their chemical properties. The major sources of *trans* MUFA in food are partially hydro-

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Abbreviations: COX, cytochrome C oxidase; CS, citrate synthase; DF, dairy fat; IF, industrial fat; IPGTT, intraperitoneal glucose tolerance test; MSR, mitochondrial superoxide anion radical; ROS, reactive oxygen species.

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genated oil (industrial products) and ruminant fat (milk, dairy products, and meat). Elaidic acid (C18:1 *trans*-9) is the major *trans* MUFA formed during catalytic hydrogenation of oil, whereas the main *trans* fatty acid resulting from rumen biohydrogenation of polyunsaturated fatty acid is vaccenic acid (C18:1 *trans*-11) (15). Differences in the relative abundance in food of each of these *trans* MUFA isomers could probably lead to different metabolic effects. Epidemiological data suggest that chronic consumption of industrial *trans* MUFA could have adverse effects on insulin sensitivity, especially in obese women with low physical activity levels (11). However, intervention studies on this problem have been inconclusive. Short-term studies (4 to 5 weeks) have shown that dietary *trans* MUFA from both sources (industrial and ruminant) might not impair insulin sensitivity in healthy subjects (16–18), whereas a diet high in industrial *trans* MUFA induces an increase in postprandial insulinemia in obese and insulin-resistant subjects (19).

An additional aspect of the pathogenesis of type 2 diabetes is the impaired bioenergetic capacity of mitochondria. Mitochondria have been suggested to play a key role in the induction of insulin resistance in skeletal muscle (20). A significant number of mitochondrial dysfunctions have been observed in the skeletal muscle of type 2 diabetic patients. Briefly, studies have evidenced a deficit in subsarcolemmal mitochondria, which generate ATP for cellular processes (21), a reduction in key mitochondrial enzymes involved in mitochondrial fatty acid transport (carnitine palmitoyl transferase 1) (22) and oxidation [citrate synthase (CS), cytochrome C oxidase (COX), and hydroxyacyl-deshydrogenase] (20), and a change in ratio between mitochondrial oxidative capacity and glycolysis (20). Hence, it has been suggested that the accumulation of fatty acid metabolites within muscle cells resulting from reduced fatty acid oxidation by mitochondria could be responsible for inducing insulin resistance (23). However, insulin resistance itself can also induce alterations in muscle mitochondrial functions (24). Perfusion of insulin leads to a significant increase in mitochondrial mRNA synthesis, respiratory chain enzyme activity, and ATP production rates in the skeletal muscle of healthy subjects (25). Thus, an abnormal response to insulin, as observed among insulin-resistant patients, could be responsible for a reduction in muscle mitochondrial oxidative functions (24).

The primary endpoint of this study was to assess the effect of an 8 week diet enriched (4% of energy intake) in either dairy (vaccenic acid), industrial (elaidic acid), or control (oleic acid) MUFA on glucose tolerance and insulin sensitivity in Wistar rats. MUFA-induced alterations in skeletal muscle response to insulin were confirmed on C2C12 myotubes. The secondary endpoint of this study was to examine the oxidative capacity of mitochondrial *cis* and *trans* MUFA and the impact of the 8 week *cis* or *trans* MUFA-enriched (4% of energy intake) diet on skeletal muscle mitochondrial functions (i.e., respiration and ATP and superoxide anion production rates). These measurements were performed in the oxidative muscle *soleus* and on the glycolytic muscle *tibialis anterior*.

MATERIALS AND METHODS

General study designs

Animal experiments were performed in accordance with the French guidelines on the care and use of animals and were approved by the local ethical committee for animal experimentation (CREEA, Auvergne, CE10-06).

Study 1

Male Wistar rats (3 months old, ~400 g) (centre d'élevage R Janvier; Le Genest-Saint-Isle, France) were housed individually and maintained under standard conditions on a 12/12 light/dark cycle in a temperature-controlled room (20–22°C). After 1 week of acclimatization with standard laboratory chow, the animals were randomly divided into three groups: a control group (n = 14), an industrial hydrogenated oil group [industrial fat (IF), n = 13], and a dairy fat (DF) group (n = 10). The animals were then fed the experimental diets (UPAE; Jouy-en-Josas, France) for 8 weeks (**Table 1**). Food was distributed in individual ramekins, providing an average 96 kcal per day. The control group received dietary fat enriched in oleic acid (C18:1 n-9). The IF group received 4% of total energy intake as *trans* fat derived from hydrogenated oil, composed mainly of elaidic acid (*9t*, C18:1). The DF group received 4% of total energy intake as *trans* fat from dairy products, composed mainly of vaccenic acid (*11t* C18:1). The diets were enriched with *trans* fat of industrial and ruminant origin at 4% of total energy intake, a proportion that is the highest estimated content of *trans* fat intake in human diet (26). IFs and DFs were similar to those used in a previous study performed in healthy volunteers (27) who received either vaccenic acid-enriched milk fat or elaidic acid-enriched fat. Briefly, to obtain the vaccenic acid-enriched milk fat, Holstein Friesian cows at the Cornell University Teaching and Research Center were fed a corn-based total mixed ration supplemented with 2% soybean oil and 1% fish oil. Milk samples were analyzed daily for fatty acid composition, and the milk collected from the cows had a high fat content of vaccenic acid. The milk was then pasteurized and processed into anhydrous milk fat. The elaidic acid-enriched fat was obtained by mixing two commercially available partially hydrogenated vegetable oils obtained from Lipid Nutrition (Wormerveer, The Netherlands) and Fuji Oil Europe (Ghent, Belgium). These fats were a generous gift from Nestlé and Prof. Dale Bauman (Cornell University). For the purposes of this study, these fats were mixed with oleic sunflower, rapeseed, and sunflower oils (Lesieur® R and D; Coudekerque-Branche, France) to balance the animal diets, thereby producing the DF and the IF. Control fat was obtained by mixing palm stearin with oleic sunflower, rapeseed, and sunflower oils (Lesieur® R and D). Water was offered ad libitum. After anesthesia via an intraperitoneal injection of ketamin and valium, the animals were euthanized by decapitation. The *soleus* (oxidative muscle) and *tibialis anterior* (glycolytic muscle) were quickly removed and separated from fat and connective tissues. Intra-abdominal fat depots were estimated by summing weights of perirenal, periepididymal, and periomental fat pads.

Study 2

The experiment was performed using 3-month-old male Wistar rats (~400 g, n = 7). The animals were housed and maintained under standard conditions as described above. From weaning at 21 days through to euthanization, the rats were fed ad libitum on a standard laboratory chow diet (A04; Scientific Animal Food and Engineering, Augy, France). Water was also offered ad libitum. The animals were euthanized as described above. Oxidative (*soleus*) and glycolytic (*tibialis anterior*) muscles were quickly removed

TABLE 1. Composition of the experimental dairy *trans* (DF), industrial *trans* (IF) and *cis* (control) MUFA-enriched diets administered in study 2.

	DF	IF	Control
	Percentage of total energy intake		
Proteins	16.3	16.3	16.3
Carbohydrates	58.9	58.9	58.9
Fat	24.8	24.8	24.8
SFA			
4:0 to 12:0	1.67	0.29	0.00
14:0	1.40	0.23	0.00
15:0	0.14	0.00	0.00
16:0	4.55	5.62	4.94
17:0	0.09	0.02	0.00
18:0	1.24	2.96	0.70
19:0	0.00	0.03	0.00
20:0	3.97	0.13	0.06
22:0	0.00	0.05	0.09
Total SFA	*9.14*	*9.34*	*5.78*
MUFA			
16:1	0.30	0.02	0.03
18:1 n-9	6.85	7.67	14.21
Others *cis* 18:1	0.00	0.32	0.00
20:1	0.04	0.14	0.13
Total *cis* MUFA	*7.19*	*8.15*	*14.36*
trans 18:1			
4t, 18:1	0.01	0.00	0.00
5t, 18:1	0.02	0.00	0.00
6/8t, 18:1	0.15	0.59	0.00
9t, 18:1	0.16	1.85	0.00
10t, 18:1	0.68	0.84	0.00
11t, 18:1	2.77	0.43	0.00
12t, 18:1	0.25	0.19	0.00
13/14t, 18:1	0.00	0.12	0.00
15t, 18:1	0.00	0.01	0.00
Total *trans* 18:1	*4.05*	*4.05*	*0.00*
PUFA			
trans 18:2	0.00	0.20	0.01
9c, 11t 18:2	0.75	0.00	0.00
18:2 n-6	2.25	2.51	3.32
20:4 n-6	0.00	0.00	0.00
Others n-6	0.00	0.00	0.00
18:3 n-3	0.31	0.55	0.94
Others n-3	0.00	0.01	0.00
Total PUFA	*3.32*	*3.26*	*4.28*

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatt acid; SFA, saturated fatty acid. Diets were similarly enriched in fibers (cellulose: 40 g/kg dry diet), vitamins and minerals (60 g/kg dry diet).

and separated from fat and connective tissues. Muscle fat oxidative capacity was determined in vitro as described below.

Study 3

The effects of *cis* (oleic acid) and *trans* (vaccenic and elaidic acids) MUFA on insulin signaling were assessed on C2C12 myotubes as previously described by Chavez et al. (23). Saturated fatty acid (palmitic acid, C16:0) was used as a positive control.

Intraperitoneal glucose tolerance test (study 1)

Insulinemic and glycemic responses to an intraperitoneal glucose tolerance test (IPGTT) injection, (1 g glucose/kg body weight) were determined 5 days before euthanization as previously described (28). To minimize stress, the rats were handled by the same operator during weighing and the IPGTT. Blood samples were collected into heparinized tubes from the retro-orbital plexus immediately before glucose injection and at 15, 30, 60, and 120 min postinjection. After centrifugation at 3,000 *g*, plasma samples were stored at −80°C until insulin and glucose measurements. Glucose was measured using an automated analyzer (Konelab 20; Thermo Electron, Waltham, MA). The biochemicals were purchased from Randox (Crumlin, UK). Insulin was measured using an ELISA kit obtained from Biosource (Camarillo, CA). Insulin sensitivity was assessed by calculating areas under the curve (AUCs) for glucose and insulin (AUC_G and AUC_I, respectively).

Effect of fatty acids on insulin signaling in C2C12 myotubes (study 3)

Chemicals. DMEM, insulin, and palmitic, oleic, vaccenic, and elaidic acid were acquired from Sigma (St. Louis, MO). Fetal calf serum (FCS) and heat-inactivated horse serum were purchased from PAN-Biotech GmbH (Aidenbach, Germany). Antibiotics were supplied by Gibco-Invitrogen (Carlsbad, CA). Primary antibodies [rabbit polyclonal anti-phospho-Akt (Ser473) and anti-Akt] were obtained from Cell Signaling (Danvers, MA). Horseradish peroxidase-conjugated polyclonal swine anti-rabbit antibody was obtained from Dako (Glostrup, Denmark). Secondary antibodies were revealed using a kit acquired from Pierce (Rockford, IL). All other chemicals used were of the highest grade commercially available.

Cell cultures and free fatty acid experiments. Mouse C2C12 myoblasts (American Type Culture Collection; Manassas, VA) were grown in 100 mm culture dishes at 37°C in a 5% CO_2 atmo-

sphere in a medium composed of DMEM supplemented with 10% FCS and 1% antibiotics (5 U/ml penicillin, 50 μg/ml streptomycin). When cells reached 80–90% confluence, the medium was switched to a differentiation medium containing DMEM and 2% heat-inactivated horse serum. Myotubes were used for experiments 5 days following the cell differentiation. C2C12 were first incubated for 16 h in a medium containing DMEM, 1% FCS, and 2% free fatty acid BSA in the absence (control) or in the presence of either palmitic, oleic, vaccenic, or elaidic acid (0.75 mM). The myotubes were then washed with PBS and incubated with palmitic, oleic, vaccenic, or elaidic acid in serum-free DMEM for 2 h. Finally, the myotubes were incubated for 10 min with or without 100 nM of insulin and lysed at 4°C in a lysis buffer (HEPES, 50 mmol/l; NaCl, 150 mmol/l; EDTA, 10 mmol/l; NaPPi, 10 mmol/l; β-glycerophosphate, 25 mmol/l; NaF, 100 mmol/l; glycerol 10%; sodium orthovanadate, 2 mmol/l; Triton-X-100, 1%; protease inhibitors). Cell lysates were resolved by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with anti-Akt and anti-phospho-Akt antibodies. Proteins were then revealed using horseradish peroxidase-conjugated polyclonal swine anti-rabbit antibody. Detections were run on a Pierce ECL Western blotting substrate and BIOMAX Film (Kodak; Paris, France).

Muscle fatty acid oxidative capacity (study 2)

Chemicals. [1-^{14}C]oleic acid was purchased from Amersham International (Buckinghamshire, UK). [1-^{14}C]vaccenic acid and [1-^{14}C]elaidic acid were obtained by synthesis at the Commissariat à l'Energie Atomique (Gif-sur-Yvette, France). [1-^{14}C]vaccenic and elaidic acids were prepared using well-established procedures (29, 30). Briefly, the bromo precursors were synthesized from unlabeled fatty acids according to the procedure of Barton, Crich, and Motherwell (30). Reaction with K^{14}CN followed by alkaline hydrolysis gave the expected fatty acids. [1-^{14}C]oleic acid was 99.7% pure, and both [1-^{14}C]vaccenic acid and [1-^{14}C]elaidic acid were over 98% pure. Specific activities were found to be 7,451 dpm/nmol for oleic acid, 6,436 dpm/nmol for vaccenic acid, and 6,564 dpm/nmol for elaidic acid. ATP, NAD$^+$, fatty acid-free BSA, L-carnitine, L-malate, CoA, cytochrome C, oleic acid, vaccenic acid, and elaidic acid were obtained from Sigma. All other chemicals used were of the highest commercially available grade.

Soleus and tibialis anterior fatty acid oxidative capacities. Fatty acid oxidation rates were measured on muscle homogenates as previously described (4). Total and peroxisomal fatty acid oxidation were both assayed in duplicate on *soleus* and *tibialis anterior* muscle. Muscle biopsy samples (~150 mg) cut into small pieces were crushed in an ice-cold buffer (0.25 mmol/l sucrose, 2 mmol/l EDTA, and 10 mmol/l Tris-HCl, pH 7.4). Fatty acid oxidation was measured using sealed vials in a total volume of 0.5 ml containing 75 μl of muscle homogenate in a medium (25 mmol/l sucrose, 75 mmol/l Tris-HCl, 10 mmol/l KH_2PO_4, 5 mmol/l $MgCl_2$, and 1 mmol/l EDTA, pH 7.4) supplemented with 5 mmol/l ATP, 1 mmol/l NAD$^+$, 0.1 mmol/l CoA, 0.5 mmol/l L-carnitine, 0.5 mmol/l L-malate, and 25 μmol/l cytochrome C. Peroxisomal fatty acid oxidation was determined in the presence of mitochondrial oxidation inhibitors (rotenone and antimycin A) in a medium containing 5 mmol/l ATP, 1 mmol/l NAD$^+$, 0.1 mmol/l CoA, and 25 μmol/l cytochrome C. Blanks were produced by replacing the muscle homogenate with ice-cold buffer. After 5 min of preincubation at 37°C with shaking, the reaction was started by injecting 0.1 ml of 600 μmol/l of either [1-^{14}C]oleic acid, [1-^{14}C]vaccenic acid, or [1-^{14}C]elaidic acid. Incubation was carried out at 37°C for 30 min and stopped by the addition of 0.2 ml of 3 mol/l perchloric acid. After 90 min at 4°C, the acid incubation mixture was centrifuged for 5 min at 10,000 *g*, and the 0.5 ml supernatant containing ^{14}C-labeled perchloric acid-soluble products was assayed for radioactivity by liquid scintillation (Beckman Instruments; Fullerton, CA). Fatty acid oxidation rates were calculated from ^{14}C-labeled perchloric acid–soluble products and were expressed in nanomoles of fatty acids oxidized per minute per gram wet tissue weight. Mitochondrial oxidation rates were calculated by subtracting the peroxisomal rate from the total oxidation rate. $^{14}CO_2$ production was not determined, because it accounts for less than 1% of muscle fat oxidation rate when measured over a 30 min period (unpublished observation).

Analysis of *soleus* and *tibialis anterior* mitochondrial pellets (study 1)

Muscle isolated mitochondria preparation. About 250 mg of *soleus* and 300 mg of *tibialis anterior* muscle were kept at 4°C in a preservation solution (CaK_2EGTA, 1.9 mmol/l; K_2EGTA, 8.1 mmol/l; imidazole, 20 mmol/l; DTT, 0.5 mmol/l; $MgCl_2$, 9.5 mmol/l; MES, 53.3 mmol/l; taurine, 20 mmol/l; ATP, 2.5 mmol/l; phosphocreatine, 19 mmol/l) until mitochondrial isolation. Muscle mitochondria were isolated using a protocol adapted from Rassmussen, Andersen, and Rassmussen (31). The muscles were cut into small pieces and incubated for 2 min in a KCl-ATP ice-cold buffer (500 μl/100 mg muscle) composed of KCl, 100 mmol/l; Tris, 50 mmol/l; $MgSO_4$, 5 mmol/l; K_2EDTA, 2 mmol/l (KCl ice-cold buffer) supplemented with ATP (1 mmol/l); BSA, 0.2% (m/v); and subtilisin, 1.5 mg/ml. After incubation, the muscle pieces were rinsed in KCl-ATP ice-cold buffer (500 μl/100 mg muscle). After the buffer was removed, the samples were homogenized in KCl-ATP ice-cold buffer, the latter buffer (0.9 ml/100 mg muscle) using a polytron PT 1200 (Kinematica AG; Littau, Switzerland) and then centrifuged for 10 min at 1,000 *g*. The supernatant was removed and centrifuged for 10 min at 4,400 *g*. The pellet was suspended in KCl ice-cold buffer and centrifuged for 10 min at 7,000 *g*. The final pellet (mitochondria-enriched fraction) was suspended in a sucrose buffer (300 mmol/l) and stored at 4°C for further investigation of mitochondrial respiration and ATP and superoxide anion radical production. The mitochondrial pellets were stored at −80°C for further exploration of mitochondrial enzyme activity and the lipid composition of the mitochondrial membrane. Before each experiment, the protein content of the mitochondrial fraction was assayed using a Pierce proteins BCA kit. Purity of the mitochondrial pellets obtained by this method was previously assessed by Rooyackers et al. (32), showing that oligomycin inhibited ATPase activity by 92.5 ± 0.8% in the isolated mitochondria, indicating only a minor contamination of the mitochondrial pellet with nonmitochondrial fractions (32).

Measurement of mitochondrial respiration and ATP production. Mitochondria respiration rates in the *soleus* muscle and *tibialis anterior* muscle were measured at 25°C using an oxygraph system Oxytherm; Hansatech Instruments, Norfolk, UK). The respiration buffer contained CaK_2EGTA, 1.9 mmol/l; K_2EGTA, 8.1 mmol/l; imidazole, 20 mmol/l; DTT, 0.5 mmol/l; KH_2PO_4, 3 mmol/l; $MgCl_2$, 4 mmol/l; MES, 100 mmol/l; taurine, 20 mmol/l; EDTA (MgATPase inhibitor), 20 μmol/l; and 0.2% BSA. Respiration was assayed on 0.25 mg/ml of mitochondrial proteins using glutamate, 5 mmol/l; malate, 2.5 mmol/l; and succinate, 5 mmol/l (GMS) as substrates. State 2 was measured in the presence of respiratory substrates without ADP. State 3 was measured after the addition of 360 μmol/l of ADP (33). State 4 was assayed after addition of 60 μmol/l of atractyloside, which is a potent inhibitor of

the ATP/ADP carrier (adenine nucleotide translocator). Respiratory control rate was evaluated by dividing state 3 by state 4. ATP production was measured kinetically after adding ADP in the oxygraphic room. Ten microliters of the oxygraphic medium containing the ATP produced was removed and placed in 100 μl of DMSO and frozen at −20°C to stop the reaction. The synthesized ATP contained in DMSO was then assessed in a luminometer (Luminiskan Ascent, Thermo Labsystems, Waltham, WA) using an ATP reagent kit (Enliten® Luciferase/Luciferin Reagent, Promega, Madison, WI) (34, 35).

Measurements of mitochondrial superoxide anion radical production. The production of mitochondria-derived reactive oxygen species (ROS) was assessed using a procedure adapted from Li, Zhu, and Trush (36). The chemilumigenic probe used was lucigenin (bis-*N*-methylacridinium), which is specific to superoxide anion ($0_2^{\bullet-}$) production. Superoxide anion production was determined on isolated mitochondria of both *soleus* muscle and *tibialis anterior* muscle. The mitochondria (0.4 μg/μl) were placed in a microwell plate containing buffer (saccharose, 250 mmol/l; Tris Base, 10 mmol/l; and EGTA, 0.1 mmol/l) and substrates (glutamate, 5 mmol/l; malate, 2 mmol/l; succinate, 5 mmol/l) with or without rotenone (0.05 mmol/l). Lucigenin-derived chemiluminescence was inhibited by 2,2,2,6-tetramethylpiperidine-*N*-oxyl, 3 mmol/l. All experiments were performed in the dark at 37°C for 40 min using a luminometer (Luminiskan Ascent, Thermo Labsystems). Results were expressed as the area under the curve of lucigenin luminescence in 40 min arbitrary units/μg protein.

Measurement of mitochondrial muscle enzyme activities. Maximum activity of CS and COX was assessed spectrophotometrically on mitochondrial pellets from the *soleus* and *tibialis anterior* muscles, as described by Morio et al. (37).

Measurement of total lipid composition of soleus- *and* tibialis anterior-*derived mitochondrial membranes.* Total mitochondrial lipids were extracted as described by Folch, Lees, and Sloane Stanley (38). Fatty acid methyl esters were prepared via basic transesterification of sodium methoxide in methanol (Sigma-Aldrich, St. Louis, MO) followed by acid transesterification of boron trifluoride in methanol (14%, Sigma-Aldrich). The experiments were performed at room temperature to preserve and analyze the conjugated linoleic acid (CLA) content in our samples. Analytic gas chromatography analyses of lipids were performed using a gas chromatograph (Thermo Electron Corporation; Waltham, MA) equipped with a flame ionization detector. Helium was used as carrier gas at a constant flow rate of 400 kPa, and a split/splitless injector was used in splitless mode. The fatty acid methyl esters were analyzed using a CP-Select for FAME capillary column (100 m/0.25 mm internal diameter/0.25 mm film thickness) (Varian; Palo Alto, CA). The oven temperature program ran between 60°C and 220°C in three separate steps. The temperature of both injector and detector was 250°C.

Statistical analysis

Data are provided as means ± standard deviation. Statistical analyses were performed using Statview version 5.0 software (SAS Institute Inc., Cary, NC). One-way ANOVA was used to compare groups for the parameters of interest, and a Fisher's exact test was used as post hoc test. Owing to the small number of samples, the nonparametric Kruskall-Wallis test was used to compare lipid mitochondrial membrane composition of *soleus* and *tibialis anterior* muscles between the three groups. The Mann-Whitney test was used to compare differences between each group. Differences were considered significant at the 5% level.

RESULTS

Effects of *trans* fatty acids on insulin sensitivity

IPGTT (study 1). There were no adverse effects of DF and IF on insulin and glucose responses to IPGTT compared with control fat. The glucose and insulin AUCs are shown in **Fig. 1A, B**.

Quantification of Akt activation in C2C12 myotubes (study 3). As expected, palmitic acid inhibited insulin-stimulated Akt phosphorylation by 275% ($P < 0.001$ vs. control). In contrast, neither *cis* nor *trans* MUFA from both sources did impair insulin-stimulated Akt phosphorylation (P = NS vs. control). The results of Akt activation are presented in **Fig. 2A, B**.

Food intake and body composition (study 1). During the experimental diet period, energy intakes were the same in each group. There were no between-group differences (DF, IF, and control) in total body and tissue weight at euthanization (**Table 2**).

Muscle fatty acid oxidative capacity (study 2). Total, mitochon drial, and peroxisomal fatty acid oxidation rates were on average 48.0 ± 3.4% higher in *soleus* than in *tibialis anterior* muscle ($P < 0.0001$). Oxidation rates in *soleus* and *tibialis anterior* muscle were not statistically different between *cis* and *trans* MUFA (P = NS) (**Fig. 3A, B**). However, in *so-*

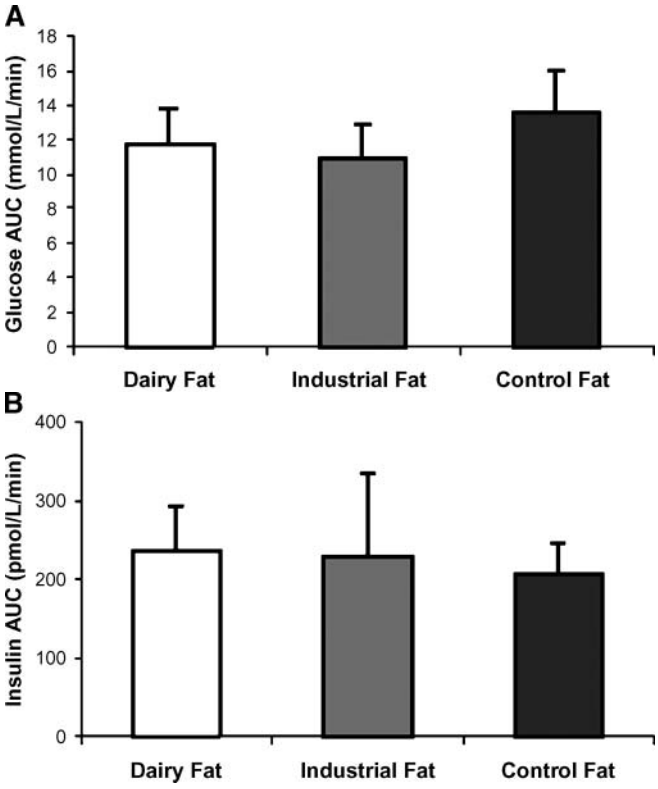

Fig. 1. Glucose (A) and insulin (B) areas under the curve in response to an intraperitoneal glucose injection in Wistar rats fed for 8 weeks on the dairy *trans*, industrial *trans*, or control *cis* MUFA-enriched diets. All values are given as means ± SD.

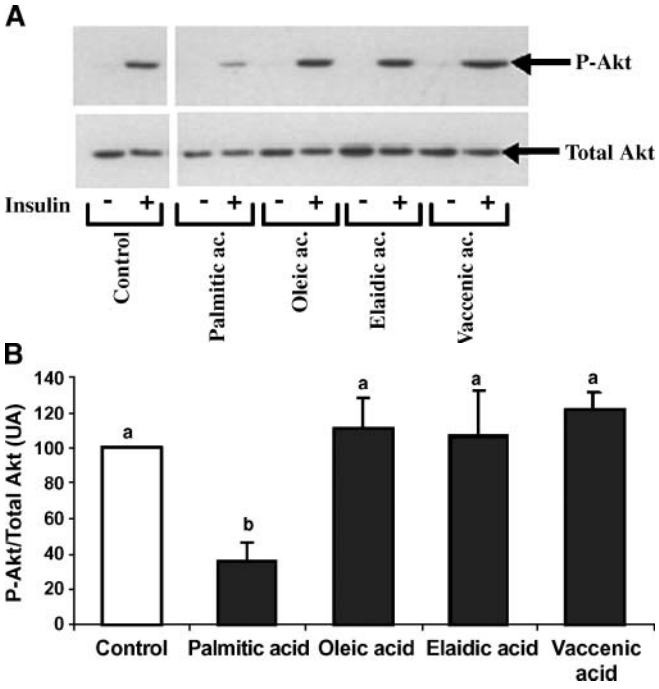

Fig. 2. Effects of palmitic, oleic, vaccenic, and elaidic acids on Akt phosphorylation and activation. Representative Western blot showing the level of phosphorylated and total Akt in C2C12 myotubes stimulated (+) or not (−) with insulin for 10 min (A). Histograms represent the ratio of phosphorylated Akt to total Akt content in insulin-stimulated conditions (B). Values are given as means ± SD. Means sharing the same superscript letter are not statistically different ($P > 0.05$). Data are representative of three to five independent experiments. Lowercase letters represent the differences between the groups.

leus, the mean total oxidative rates of vaccenic and elaidic acids were, respectively, 11.1% and 12.2% higher than for oleic acid (P = NS). In contrast, in *tibialis anterior*, all three MUFA demonstrated similar total oxidation rates. The proportion of total oxidation due to peroxisomal oxidation was on average similar for all three MUFA (13.5 ± 2.0% in *soleus* and 12.1 ± 3.8% in *tibialis anterior*).

Analysis of *soleus*- and *tibialis anterior*-derived mitochondrial pellets (study 2)

Isolated muscle mitochondria functions. In *soleus* muscle, the experimental diets did not alter state-2, state-3, or state-4 respiratory rates, and respiratory control ratio remained unchanged (**Table 3**). In contrast, ATP production and phosphate to oxygen ratio (P/O ratio) were higher in the control group than in the two *trans* MUFA groups ($P < 0.01$ vs. IF and DF). ATP production in the control group was 31.6% and 36.7% higher than in the DF and IF groups, respectively. Mitochondrial superoxide anion radical (MSR) production was on average 65% higher, with or without rotenone, in the control group than in the *trans* groups ($P < 0.05$) (**Table 4**). CS activity was higher in the control group than in the *trans* groups ($P < 0.05$), but there was no significant change in COX activity (Table 4).

In *tibialis anterior* muscle, state-2, state-3, and state-4 respiratory rates, ATP production, P/O ratio, and MSR production were similar in the three groups (Tables 3, 4). As in the *soleus*, CS activity was higher in the control group than in the *trans* groups ($P < 0.05$), but there was no significant change in COX activity (Table 4).

Fatty acid composition of mitochondrial membrane. To further investigate the role played by *trans* MUFA from both sources in the decreased mitochondrial ATP production in the oxidative muscle, we assessed the lipid composition of *soleus* and *tibialis anterior* mitochondrial membranes (**Table 5**). The lipid composition of *soleus*- and *tibialis anterior*-derived mitochondrial membranes was significantly different, except for total *trans* MUFA content, which remained similar between the two muscles (P = NS). The incorporation of oleic acid (C18:1 9 *cis*) and C22:5 n-3 was higher in *soleus* than in *tibialis anterior* mitochondrial membranes, irrespective of dietary source ($P < 0.05$), whereas the incorporation of other *cis* MUFA and docosahexaenoic acid (C22:6 n-3) was significantly lower ($P < 0.05$). Linoleic acid (C18:2 n-6) content was lower in the DF and IF groups and higher in the control group in the *soleus* compared with *tibialis anterior* mitochondrial membrane ($P < 0.05$). Arachidonic acid (C20:4 n-6) content was lower in the DF and control group in the *soleus*- compared with *tibialis anterior*-derived mitochondrial membrane ($P < 0.05$).

Trans MUFA incorporation in the *soleus* mitochondrial membrane reflected dietary lipid profile. No dietary effects were found in the mitochondrial membrane incorporation of palmitic acid (C16:0), total C16:1, linoleic acid (C18:2 n-6), arachidonic acid (C20:4 n-6), or C22:5 n-3 (P = NS). Stearic acid (C18:0) and oleic acid (C18:1 n-9) were lower, whereas other n-6 PUFA were higher in the two *trans* groups compared with the control group ($P < 0.05$). Finally,

TABLE 2. Effects of an 8-week supplementation with either dairy *trans* (DF), industrial *trans* (IF) or *cis* (control) MUFA on tissue and body weight

Group	Body	Liver	Intra-abdominal Adipose Tissue	*Soleus*	*Tibialis Anterior*
	g	*mg*	*mg*	*mg*	*mg*
DF	592 ± 20	19.5 ± 2.2	35.7 ± 5.1	244.4 ± 26.8	889.5 ± 49.6
IF	593 ± 33	18.0 ± 2.0	37.7 ± 4.8	232.8 ± 27.3	877.4 ± 53.1
Control	573 ± 25	18.5 ± 1.7	33.9 ± 5.5	242.6 ± 26.7	918.0 ± 70.7
ANOVA	NS	NS	NS	NS	NS

Values are given as means ± SD. NS = not statistically different ($P > 0.05$).

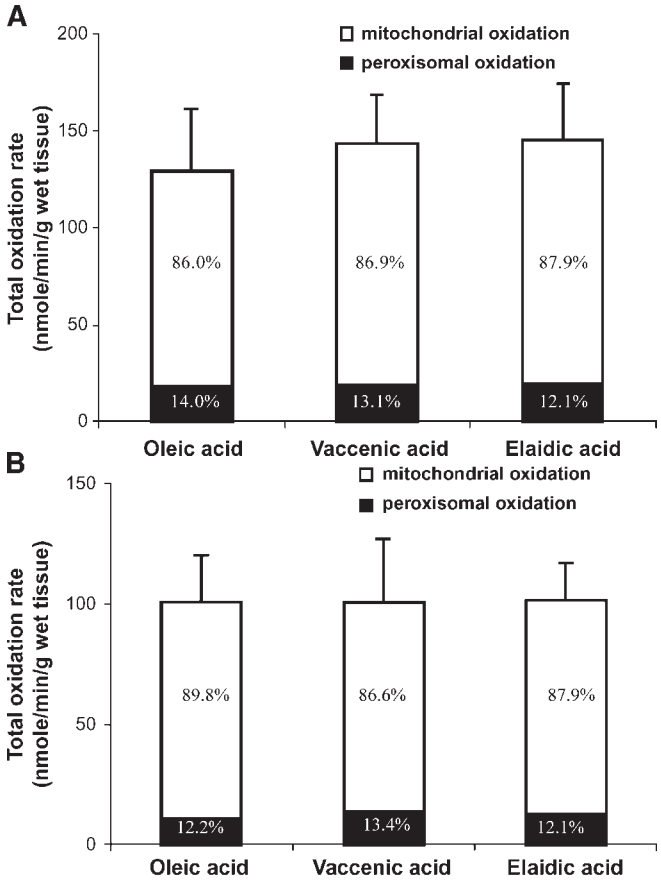

Fig. 3. Total, mitochondrial, and peroxisomal oxidation rates of oleic, vaccenic, and elaidic acids measured in homogenates of rat *soleus* (oxidative, A) and *tibialis anterior* (glycolytic, B) muscles. In-histogram data show the relative contributions of mitochondrial and peroxisomal oxidations to total oxidation. All values are means ± SD.

docosahexaenoic acid (C22:6 n-3) was lower in the DF group compared with the IF and control groups ($P < 0.05$).

Trans MUFA incorporation in the *tibialis anterior* mitochondrial membrane reflected dietary lipid profile. No dietary effects were found in the mitochondrial membrane incorporation of palmitic acid (C16:0), total C16:1, other *cis* MUFA, arachidonic acid (C20:4 n-6), or docosahexaenoic acid (C22:6 n-3) (P = NS). Oleic acid (C18:1 n-9) was higher, whereas linoleic acid (C18:2 n-6) and other n-6 PUFA were higher, in the two *trans* groups, compared with the control group ($P < 0.05$). Finally, stearic acid (C18:0) was lower in the DF group and C22:5 n-3 was lower in the IF group than in the other groups ($P < 0.05$).

DISCUSSION

The main purpose of our study was to evaluate the impact of *trans* MUFA-enriched diets on muscle insulin resistance. Our results suggest that *trans* MUFA of industrial source do not induce insulin resistance in healthy rats when given at 4% of energy intake. Our results in healthy rodents are in agreement with results reported for human healthy volunteers. In the Health Professional follow-up study, men's intake of *trans* MUFA was not associated with prevalence of diabetes after multivariate adjustment (39). Lovejoy et al. (40), in a population featuring a broad range of glucose tolerance, did not demonstrate any association between either self-reported *trans* MUFA intake or serum-lipid *trans* MUFA concentrations and insulin response to an oral glucose tolerance test. In nutritional interventional studies on healthy lean subjects (16, 17), *trans* MUFA of industrial origin did not have any adverse effect on insulin resistance. At variance with our results, *trans* MUFA of industrial origin induced an accumulation of intramyocellular triglycerides in the diaphragm in healthy rats, together with a decreased glucose uptake under insulin stimulation (41). However, the *trans* MUFA diet in this study was also rich in palmitate (38.5 g/100 g fat vs. 22.7 g/100 g fat), which is known to induce insulin resistance in myocytes (42). Furthermore, in the Nurses' Health Study, Salmeron et al. (11) showed that replacing an industrial *trans* MUFA–based energy intake with polyunsaturated fatty acids led to a 40% decrease in the risk

TABLE 3. Effects of an 8-week supplementation with either dairy *trans* (DF), industrial *trans* (IF) or *cis* (control) MUFA on mitochondrial respiration and ATP production rates in *soleus* and *tibialis anterior* muscles

	Oxygen Uptake					
Group	State 2	State 3	State 4	RCR	ATP Synthesis Rate	ATP/O
	nmolO_2/min/mg				*nmol ATP/min/mg*	
Soleus						
DF	50.4 ± 9.3	203.6 ± 40.3	34.8 ± 11.6	6.4 ± 2.2	498.5 ± 111.9^{a}	2.66 ± 0.56^{a,b}
IF	50.5 ± 9.0	224.9 ± 31.2	33.8 ± 17.5	8.1 ± 4.6	479.2 ± 126.9^{a}	2.21 ± 0.47^{b}
Control	54.7 ± 7.4	214.1 ± 25.2	40.1 ± 9.4	5.6 ± 1.3	655.9 ± 142.0^{b}	3.06 ± 0.61^{a}
ANOVA	NS	NS	NS	NS	$P < 0.05$	$P < 0.05$
Tibialis anterior						
DF	54.6 ± 11.3	314.3 ± 88.6	45.0 ± 15.1	7.3 ± 3.0	727.1 ± 104.5	2.47 ± 0.59
IF	59.7 ± 17.0	314.2 ± 51.3	37.9 ± 11.9	8.4 ± 3.1	572.6 ± 150.5	1.97 ± 0.70
Control	61.6 ± 13.6	289.6 ± 52.6	41.3 ± 13.1	7.6 ± 2.4	624.3 ± 185.7	2.17 ± 0.53
ANOVA	NS	NS	NS	NS	NS	NS

Values are given as means ± SD. Means within the same column sharing the same superscript letter or no superscript letter are not statistically different ($P > 0.05$). Oxygen uptake is expressed in nmolO_2/min/mg of mitochondrial protein. ATP production is expressed in nmolATP/min/mg of mitochondrial protein. RCR, respiratory control ratio (state 3/state 4).

TABLE 4. Effects of an 8-week supplementation with either dairy *trans* (DF), industrial *trans* (IF) or *cis* (control) MUFA on mitochondrial superoxide anion radical production rate and maximal activity of mitochondrial oxidative enzymes in *soleus* and *tibialis anterior* muscles

	Soleus				*Tibialis Anterior*			
	MSR				MSR			
Group	Rotenone−	Rotenone+	CS	COX	Rotenone−	Rotenone+	CS	COX
	min AU/μg		*mmol/min/μg*		*min AU/μg*		*mmol/min/μg*	
DF	37.4 ± 14.0^{a}	42.2 ± 16.1^{a}	1.56 ± 0.12^{a}	3.16 ± 0.41	55.4 ± 8.5	49.2 ± 10.8	1.72 ± 0.29^{a}	2.86 ± 0.62
IF	42.7 ± 14.9^{a}	49.1 ± 18.0^{a}	1.61 ± 0.29^{a}	3.05 ± 0.68	59.1 ± 19.3	48.1 ± 21.8	1.76 ± 0.55^{a}	2.95 ± 0.53
Control	65.7 ± 23.4^{b}	73.6 ± 22.8^{b}	2.06 ± 0.53^{b}	3.27 ± 1.17	71.3 ± 18.9	46.3 ± 18.4	2.42 ± 0.34^{b}	3.44 ± 0.62
ANOVA	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS	NS	NS	$P < 0.05$	NS

Values are given as means ± SD. Means within the same column sharing the same superscript letter or no superscript letter are not statistically different ($P > 0.05$). MSR (mitochondrial superoxide anion radical) production is expressed in 40 min AU/μg mitochondrial proteins. CS (citrate synthase) and COX (cytochrome C oxidase) activities are expressed in mmol/min/μg mitochondrial proteins.

of developing type 2 diabetes. However, this study carried certain methodological limitations that may have affected the interpretation of *trans* MUFA data. Notably, over the 20 year experimental period, there were foods rich in *trans* MUFA that were also rich in refined carbohydrates, the *trans* fat content of the foods changed significantly, and the techniques used to assess *trans* fat food content also improved (43).

A novel feature of our study is that it considered the effects of naturally sourced *trans* MUFA on the development of insulin resistance. Our results showed no beneficial or adverse effect of vaccenic acid-rich DF on insulin sensitivity. This is in agreement with a nutritional intervention study performed on healthy young men that did not find any effects of vaccenic acid-rich DF on fasting insulin and glucose (18). The lack of in vivo effect of vaccenic acid could be due in part to its bioconversion in CLA by the Δ9-desaturase (44, 45), inasmuch as the CLA mixture did not alter insulin sensitivity in healthy subjects (46–48). The technical limitation of our in vivo study was that we used an IPGTT, which does not give the most precise evaluation of muscle insulin sensitivity. To validate that muscle insulin sensitivity was not impaired in the presence of *trans* MUFA, we studied C2C12 myotubes, which have been widely used for investigating the effects of fatty acids on insulin-stimulated signaling and glucose metabolism (23, 49, 50). In agreement with previous reports (23, 49), our experiments indicated that insulin-stimulated Akt phosphorylation was inhibited by palmitic acid and was similar to controls after incubation with oleic acid. Furthermore, elaidic acid and vaccenic acid did not modify insulin-stimulated Akt activation, in comparison with control C2C12 myotubes. Because neither in vitro nor in vivo studies found any adverse effects of both *trans* MUFA, we concluded that *trans* MUFA had not impaired muscle insulin sensitivity.

The hypothesis of study 2 was that the distinction between *trans* MUFA chemistry and conformation may result in different cellular fates, such as oxidative rate in skeletal muscle. Data on the whole human body suggest that elaidic acid is slightly more oxidized than oleic acid (51), but a tissue-focused approach is required to distinguish the

TABLE 5. Fatty acid composition of *soleus* and *tibialis anterior* mitochondrial membrane from rats fed for 8 weeks with either dairy *trans* (DF), industrial *trans* (IF) or *cis* (control) MUFA

	Soleus			*Tibialis Anterior*		
	DF	IF	Control	DF	IF	Control
16:0	17.21 ± 1.47$^{\#}$	15.90 ± 1.90	17.64 ± 0.75	20.76 ± 0.33	17.52 ± 3.00	19.06 ± 2.22
18:0	15.50 ± 0.76$^{a\#}$	15.51 ± 0.65^{a}	16.76 ± 0.35^{b}	14.05 ± 0.50^{a}	16.73 ± 1.51^{b}	18.65 ± 1.47^{b}
Σ 16:1 (n-7 and n-9)	1.28 ± 0.33	0.94 ± 0.26	0.74 ± 0.19	1.10 ± 0.20	0.80 ± 0.33	0.51 ± 0.19
18:1 n-9	8.81 ± 0.35$^{a\#}$	10.21 ± 1.12$^{a\#}$	12.51 ± 0.02$^{b\#}$	5.49 ± 0.77^{a}	6.47 ± 0.76^{a}	8.94 ± 1.19^{b}
Other *cis* 18:1	2.86 ± 0.03$^{\#}$	2.57 ± 0.21$^{\#}$	2.96 ± 0.16$^{\#}$	3.39 ± 0.14	3.45 ± 0.29	4.12 ± 0.03
trans 18:1						
9t, 18:1	0.34 ± 0.25^{a}	2.74 ± 0.26^{b}	0.07 ± 0.02^{c}	0.31 ± 0.14^{a}	2.53 ± 0.24^{b}	0.09 ± 0.03^{c}
10t, 18:1	0.00 ± 0.00^{a}	0.20 ± 0.02$^{b\#}$	0.00 ± 0.00^{a}	0.02 ± 0.04	0.00 ± 0.00	0.02 ± 0.03
11t, 18:1	1.61 ± 0.20^{a}	0.18 ± 0.14^{b}	0.00 ± 0.00$^{c\#}$	1.38 ± 0.21^{a}	0.30 ± 0.03^{b}	0.04 ± 0.02^{c}
Total *trans* 18:1	*1.95 ± 0.40*^{a}	*3.12 ± 0.40*^{b}	*0.07 ± 0.02*$^{c\#}$	1.71 ± 0.12^{a}	2.83 ± 0.21^{b}	0.16 ± 0.08^{c}
n-6 PUFA						
18:2 n-6	21.26 ± 1.52$^{\#}$	18.76 ± 1.39$^{\#}$	21.66 ± 0.03$^{\#}$	24.22 ± 0.69^{a}	23.05 ± 1.88^{a}	17.63 ± 0.73^{b}
20:4 n-6	11.75 ± 1.27$^{\#}$	12.01 ± 0.91	11.11 ± 0.69$^{\#}$	13.56 ± 0.36	13.30 ± 0.81	13.24 ± 0.34
Other n-6 PUFA	1.44 ± 0.13^{a}	1.18 ± 0.18^{a,b}	0.99 ± 0.12$^{b\#}$	1.50 ± 0.20^{a}	1.01 ± 0.15^{b}	0.77 ± 0.07^{b}
22:5 n-3	10.69 ± 3.75$^{\#}$	6.52 ± 1.15$^{\#}$	7.35 ± 1.68$^{\#}$	1.32 ± 0.07^{a}	1.06 ± 0.10^{b}	1.80 ± 0.06^{c}
22:6 n-3	5.65 ± 0.52$^{a\#}$	6.64 ± 0.46$^{b\#}$	7.80 ± 0.60$^{b\#}$	11.90 ± 0.54	12.38 ± 1.21	14.00 ± 0.70
Others[1]	1.61 ± 1.73	2.05 ± 2.25	0.41 ± 0.03	1.00 ± 0.07	1.43 ± 0.29	1.15 ± 0.16

PUFA, polyunsaturated fatt acid. Values are given as means ± SD and are expressed in g fatty acid/100 g fat. Means within the same column sharing the same superscript letter or no superscript letter are not statistically different ($P > 0.05$). $^{\#}$ Comparison between *soleus* and *tibialis anterior* for each diet ($P < 0.05$).

[1] Including mainly C14:0, C15:0, C16:0, C17:0, C14:1, C20:1, c9,11t C18:2, C18:3 n-3, and C20:5 n-3.

relative contribution of each organ to whole-body oxidation rate. Oleic acid (C18:1 9 *cis*) oxidation rates have been studied in rat liver, muscle, and heart (52), but there are few data on the oxidation rate of *trans* MUFA available. Rat heart homogenate showed 35–40% higher oleic acid oxidation than elaidic acid oxidation (53). In contrast, in hepatocytes, elaidic acid was metabolized at a higher rate than oleic acid (54). To our knowledge, there are no documented data on the ability of muscle mitochondria to oxidize *cis* and *trans* C18-MUFA. Our results showed that the in-muscle oxidative rates of *cis* and *trans* MUFA were not significantly different between *soleus* and in *tibialis anterior*, although the 12% higher oxidation rate of both *trans* MUFA in soleus compared with oleic acid might have some physiological significance. Whole-body human studies (51) and in vitro studies on muscle homogenates (52) showed that the oxidation rate of unsaturated fatty acids increases proportionally to the number of double bonds, independently of their position on the carbon chain. Our results thus suggest that the geometrical MUFA configuration (*cis* or *trans*) does not heavily influence MUFA oxidation rate. Thus, any *trans* MUFA-induced alteration in muscle metabolism is not due to differences in oxidation rates. In particular, this can be related to the absence of *trans* MUFA effects on muscle insulin sensitivity. Indeed, any decrease in fatty acid oxidation rate is thought to at least partly induce an accumulation of fatty acid metabolites within the muscle cell (55), which in turn induces muscle insulin resistance through the activation of isoforms of protein kinase C (56, 57). Because *trans* MUFA are oxidized in patterns similar to those of *cis* MUFA, they might not alter intramyocellular fatty acid trafficking.

The hypothesis of study 1 was that IF and DF diets could modify muscle mitochondrial functions. To our knowledge, this is the first study to assess the effects of elaidic and vaccenic acid-enriched diets on mitochondrial oxidative functions. In *tibialis anterior*, neither dairy nor industrial *trans* MUFA–modified mitochondrial oxidative phosphorylation functions. This corroborates previous reports that mitochondrial oxidative functions were less affected by dietary manipulations (e.g., high-fat diet) in *tibialis anterior* than *soleus* muscle (28, 58). In *soleus*, *trans* MUFA of industrial and dairy origin did not alter mitochondrial state-3 and state-4 respiration rates but induced a decrease in the rate of ATP production. This may be the result of an altered mitochondrial ATP synthase activity or of slight uncoupling due to changes in mitochondrial membrane fatty acid composition and fluidity. Uncoupling is the mechanism that allows H^+ backflow into the mitochondrial matrix, bypassing the ATP synthase. Our study showed that elaidic acid was more incorporated into muscle mitochondrial membrane than vaccenic acid. However, this difference cannot explain the modification of ATP synthase activity, because ATP production was similar between the two *trans* diets in *soleus*. Alterations in fatty acid incorporation within mitochondrial membranes (e.g., stearic acid, oleic acid) may provide a possible explanation, but it remains difficult to identify the fatty acid(s) driving the process, especially because we compared the complex results obtained between *soleus* and *tibialis anterior*. Another possible explanation could be a specific *trans* MUFA-induced expression of uncoupling proteins in *soleus*. It has been shown that a weak uncoupling would be sufficient to reduce ROS production by mitochondria (59). This would be in agreement with the lower ROS production by *soleus* mitochondria in the IF and DF groups, compared with control rats. Thus, *trans* MUFA of both origins might induce slight mitochondrial uncoupling in oxidative muscle. However, further studies are required to examine this issue.

A decrease in CS activity was found in *soleus* and *tibialis anterior* of rats fed with *trans* diets, compared with the control group. In contrast, COX activity was unmodified. The same pattern has been previously described in the muscle of elderly adults compared with young adults, but the mechanisms responsible for this adaptation are still poorly understood (4). One possibility might be a fatty acid regulation of CS expression.

In conclusion, in vitro and in vivo experimentations demonstrated that *trans* MUFA of dairy or industrial origin do not alter muscle insulin sensitivity. This may be due to the fact that *trans* MUFA have an oxidative capacity similar to that of *cis* MUFA and do not induce alterations in muscle mitochondrial oxidative capacity in either oxidative or glycolytic muscles. Therefore, *trans* MUFA might not promote in-cell accumulation of fatty acid metabolites, which is known to induce insulin resistance. However, *trans* MUFA significantly alter mitochondrial ATP and superoxide anion production in *soleus* muscle, suggesting a slight *trans* MUFA-induced uncoupling in oxidative muscles.

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