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Original article

In vitro metabolism of rumenic acid in bovine liver slices

Anne DE LA TORRE[a], Dominique GRUFFAT[a]*, Jean-Michel CHARDIGNY[b], Jean-Louis SEBEDIO[b], Denys DURAND[a], Olivier LOREAU[c], Dominique BAUCHART[a]

[a] INRA, Research Unit on Herbivores, Nutrients and Metabolisms Group, 63122 Saint-Genès-Champanelle, France
[b] INRA, Lipid Nutrition Unit, 21065 Dijon, France
[c] CEA Saclay, 91191 Gif/Yvette Cedex, France

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Abstract – Ruminant products are the major source of CLA for humans. However, during periods of fat mobilization, the liver might play an important role in CLA metabolism which would limit the availability of the latter for muscles and milk. In this context, rumenic acid (*cis*-9, *trans*-11 CLA) metabolism in the bovine liver ($n = 5$) was compared to that of oleic acid ($n = 3$) by using the in vitro liver slice method. Liver slices were incubated for 17 h in a medium containing 0.75 mM of FA mixture and 55 μM of either [1-^{14}C] rumenic acid or [1-^{14}C] oleic acid at 37 °C under an atmosphere of 95% O_2-5% CO_2. Rumenic acid uptake by liver slices was twice ($P = 0.009$) that of oleic acid. Hepatic oxidation of both FA (> 50% of incorporated FA) led essentially to the production of acid-soluble products and to a lower extent to CO_2 production. Rumenic acid was partly converted (> 12% of incorporated rumenic acid) into conjugated C18:3. CLA and its conjugated derivatives were mainly esterified into polar lipids (71.7%), whereas oleic acid was preferentially esterified into neutral lipids (59.8%). Rumenic acid secretion as part of VLDL particles was very low and was one-fourth lower than that of oleic acid. In conclusion, rumenic acid was highly metabolized by bovine hepatocytes, especially by the oxidation pathway and by its conversion into conjugated C18:3 for which the biological properties need to be elucidated.

rumenic acid / oleic acid / metabolism / liver / bovine

Abbreviations: ASP, acid-soluble products; BSA, bovine serum albumin; CLA, conjugated linoleic acid; FA, fatty acids; NL, neutral lipids; PL, polar lipids; VLDL, very-low density lipoproteins.

1. INTRODUCTION

Conjugated Linoleic Acid (CLA) is a collective term that designates a group of positional and geometrical isomers of linoleic acid (*cis*-9, *cis*-12 C18:2) with two conjugated double bonds. Many studies have been carried out on synthetic CLA (mainly *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) because of its numerous beneficial properties suggested for human health including anticarcinogenic, antiatherogenic, antiadipogenic

* Corresponding author: gruffat@sancy.clermont.inra.fr

and antidiabetogenic effects [1, 2]. Additionally, CLA may reduce the rate of bone formation [3] and enhance immunological responses [4, 5]. Natural CLA is mainly present in ruminant products such as meat, milk and dairy products which constitute the major source of dietary CLA for humans. Typically, the mean daily intake of CLA by humans in Western countries ranges between 150 and 210 mg [6]. The incorporation of CLA into lipids in ruminant tissues or in milk results, for a low part, from bacterial biohydrogenation and trans isomerisation of dietary polyunsaturated fatty acids (PUFA) (mainly linoleic acid from forages, cereals or oleaginous seeds) in the rumen [7] and, for a large part, from endogenous synthesis [8]. Synthesis of CLA can take place in the tissues of ruminants where the Δ9 desaturase enzyme is expressed i.e., the mammary gland in lactating ruminants and adipose tissues in growing ruminants [9, 10]. Likewise, it has been demonstrated that vaccenic acid (another intermediate of bacterial biohydrogenation of PUFA, *trans*-11 C18:1) is desaturated to CLA in the mammary gland of lactating cows, and has been estimated to represent about 60 to 90% of CLA deposited in milk fat [8, 11, 12], whereas no desaturation of VA into CLA occurred in the liver of ruminants [13]. These processes lead to the production of many distinct isomers of CLA for which the chemical structures of at least 24 have been characterized [14]. Quantitatively, isomers of CLA incorporated into lipids of ruminant products are predominantly rumenic acid (*cis*-9, *trans*-11 C18:2) since it represents 80–90% of total CLA [8].

Although de novo synthesis of CLA has been widely studied in ruminants, its metabolism in tissues and/or organs has been poorly documented. Ruminants are frequently subjected to periods of fat mobilization, which occur in several nutritional and physiological situations e.g. restricted feed intake or during periparturient periods [15]. In such situations, large amounts of FA, including CLA which are stored in adipose tissues, are released into the blood and taken up by the liver. The intensity of the different CLA metabolic pathways in the liver is still poorly documented except for the fact that in the liver of different species such as the rat [16–18], lamb [19] and mouse [20] given CLA-supplemented diets, CLA is converted into conjugated C18:3 by Δ6 desaturase activity [2, 17]. Although these studies reported the presence of CLA derivatives in the liver of animals, only Gruffat et al. [21] recently demonstrated, by using rat liver slices incubated with *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers, that CLA conversion into conjugated C18:3 actually occurs in this organ. However, the biological properties of the conjugated derivatives of CLA are still to be determined.

With this in mind, the aim of the present study was to determine the metabolism of rumenic acid in bovine liver cells (as a species that produces CLA). The different steps of the hepatic metabolism of rumenic acid were compared with those of oleic acid (a plasma FA representative) i.e., uptake, oxidation, desaturation, esterification and finally secretion as part of VLDL particles, by using an in vitro experimental approach of incubated liver slices. These data are of great interest since the partition of CLA between the different metabolic pathways may determine its availability for peripheral tissues such as muscles and milk during periods of fat mobilization.

2. MATERIALS AND METHODS

2.1. Chemicals and materials

The medium used for incubation of liver slices (RPMI-1640), bovine serum albumin (BSA) free of FA, glutamine (300 $mg \cdot L^{-1}$), FA and antibiotic-antimycotic cocktail (100 $U \cdot mL^{-1}$-penicillin, 0.1 $mg \cdot mL^{-1}$-streptomycin and 0.25 $\mu g \cdot mL^{-1}$-amphoterin B) were purchased from Sigma Chemicals (St Louis, MO, USA). Tri [9,10 ^{3}H] olein (185 $MBq \cdot mL^{-1}$), L-3-phosphatidyl[N-methyl-^{3}H]choline 1,2 dipalmitoyl (37 $MBq \cdot mL^{-1}$) and [1-^{14}C] oleic acid (2.06 $GBq \cdot mmol^{-1}$) were purchased from Amersham International

(Bucks, UK). [1-^{14}C] rumenic acid (1.97 Gbq·mmol^{-1}) was synthetized by Loreau et al. [22]. Hyamine hydroxide was purchased from ICN Biochemicals (Irvine, CA, USA). Perchloric acid and organic solvents (chloroform, methanol, propanol, diethyl ether and acetic acid) were purchased from Polylabo (Paris, France). The Ready Safe® scintillation cocktail was purchased from Beckman Instruments (Fullerton, CA, USA). Plastic organ culture Petri dishes were purchased from Beckton Dickinson (Cockeysville, MD, USA). Plastic center wells were purchased from Kontes (Vineland, NJ, USA) and aminopropyl-activated silica Sep-Pak® cartridges were purchased from Waters (Milford, MA, USA).

2.2. Liver tissue preparation and liver slice incubation

All experiments were conducted in a manner compatible with the national legislation on animal care (Certificate of Authorisation to Experiment on Living Animals No. 7740, Ministry of Agriculture and Fish Products). Five Charolais steers (age: 25 ± 1 months, live weight: 713 ± 39 kg) were fed a conventional diet (hay and cereal concentrate, 45 and 55% of dry matter, respectively) and were used to study the hepatic metabolism of rumenic acid. To study hepatic metabolism of oleic acid, five other steers were subjected to similar conditions of rearing and breeding. However, two steers were removed from the experiment due to ill-health. Consequently, only three animals were used to study oleic acid metabolism. Representative liver samples of the whole liver, taken from steers just after slaughtering, were prepared for metabolic labelling as previously described by Graulet et al. [23]. Briefly, liver samples were immediately rinsed in ice-cold saline solution (9 g·L^{-1}-NaCl) and cut into 0.5 mm thick slices. Approximately, 200 mg of fresh liver were placed on stainless steel grids positioned either on a plastic organ culture Petri dish or in a 25 mL flask equipped with suspended plastic center wells (for CO_2 measurements) with RPMI-1640 medium (0.9 mL per dish ; 1.4 mL per flask) supplemented with the antibiotic-antimycotic cocktail. Hepatic tissue samples were placed in an incubator for 3 h at 37 °C in a water-saturated atmosphere (95% O_2/5% CO_2) in order to deplete hepatocytes of intracellular FA. Subsequently, to approximate physiological conditions, a mixture of FA representative of bovine plasma non-esterified FA (0.75 mM final concentration) was added to the medium [24]. The FA mixture was composed of 16 μM-caprylic acid, 16 μM-capric acid, 58 μM-myristic acid, 200 μM-palmitic acid, 220 μM-stearic acid, 213 μM-oleic acid and 26 μM-linoleic acid, supplemented with 55 μM-[1-^{14}C] rumenic acid (1.97 GBq·mmol^{-1}) complexed to BSA (FA: albumin molar ratio of 4:1) in order to test rumenic acid metabolism. In the control assay, the same mixture of FA, except for oleic acid, was added to the medium in the presence of 55 μM-[1-^{14}C] oleic acid (2.06 GBq·mmol^{-1}) complexed to BSA. Previous kinetic experiments [25] had been performed to verify the viability of liver slices up to 24h. Consequently, liver slice incubations of 17h were maintained. At the end of incubation, the media (2.5 mL) were collected and liver slices were washed twice with 1 mL of buffered solution (0.4 g·L^{-1}-KCl, 0.8 g·L^{-1}-$NaHPO_4$, pH 7.4 and 2 g·L^{-1}-D-glucose) and then homogenized in 2 mL of 25 mM-Tris-HCl (pH 8.0), 50 mM-NaCl buffer with a Dounce homogenizer. For each analysis, except for CO_2 determination, an aliquot of medium and/or liver homogenate from the same incubation flask was taken. Since the specific activity of each [1-^{14}C] FA was known and the weight of the liver in each flask was determined, the results were expressed in nmol of FA per g of liver or in percent of FA added to the medium.

2.3. Determination of FA oxidation

CO_2 produced by liver slices was complexed to hyamine hydroxide (500 μL) introduced into suspended plastic center wells inside flasks at the beginning of the incubation period. At the end of incubation,

the center wells were placed in scintillation vials containing 4 mL of Ready-Safe® scintillation cocktail and the radioactivity was counted using a Liquid Scintillation Analyzer (Tri-carb 2100 TR, Packard, USA). Production of acid perchloric soluble products (ASP), mainly represented by ketone bodies, was determined by using the method of Williamson and Mellanby [26]. Briefly, aliquots of medium (500 μL) and of liver homogenates (250 μL) were treated for 20 min at 4 °C with perchloric acid (0.2 M). The precipitated lipids and proteins were pelleted by centrifugation (1.850 *g* for 20 min at 4 °C). An aliquot of the supernatant containing ASP was placed in a scintillation vial containing a scintillation cocktail and the radioactivity was counted.

2.4. Determination of FA bioconversion

Total lipids of liver slices were extracted according to the method of Folch et al. [27]. FA methyl esters (FAME) were prepared from total lipids at room temperature by using sodium methylate (1 M) followed by boron trifluoride in methanol (14% v/v) as previously described by Sébédio et al. [17]. FAME containing [1-^{14}C] FAME were then analysed by gas-liquid chromatography using a Hewlett-Packard 5890 series II gas chromatograph (Palo Alto, CA) equipped with a splitless injector and fused Stabilwax wide-bore silica column (60 m × 0.53 mm i.d., film thickness: 0.50 μM, Restek, Evry, France). The outflow from the column was split between a flame-ionization detector (10%) and a copper oxide oven heated at 700 °C in order to transform labelled FA into [1-^{14}C] CO_2 (90%). The radioactivity was determined with a radiodetector (GC-RAM, Lablogic, Sheffield, UK) by counting [1-^{14}C] CO_2 as previously described by Bretillon et al. [28]. Data were computed using Laura software (Lablogic, Sheffield, UK).

2.5. Determination of FA esterification

Total lipids were extracted from liver homogenates according to the method of Folch et al. [27] after addition of non radioactive liver homogenate (850 μL) containing 10 mg of lipids used as a lipid carrier and of [1-^{3}H] trioleyl glycerol (67 Bq) and [1-^{3}H] phosphatidylcholine (100 Bq) respectively used as external triacylglycerol and phospholipid standards in order to calculate the extraction efficiency. Neutral (NL) and polar lipids (PL) were isolated by affinity-liquid chromatography using aminopropyl-activated silica Sep-Pack® cartridges as described by Kaluzny et al. [29]. Briefly, NL were eluted by 6 mL of chloroform/propanol (2/1, v/v). Free FA were then eluted by 6 mL of acetic acid (2% in diethyl ether). Finally, PL were eluted by 6 mL of pure methanol. NL and PL fractions were collected directly into scintillation vials, evaporated under an air stream and counted for radioactivity.

2.6. Determination of secreted VLDL in medium

Three millilitres of medium were supplemented with purified calf plasma VLDL (0.3 mg of VLDL-TG·12 mL^{-1} tube) used as a VLDL carrier [23]. The medium was brought to a density of 1.063 $g \cdot mL^{-1}$ with potassium bromide and overlaid with 9 mL of saline solution (with a density of 1.006 $g \cdot mL^{-1}$). VLDL particles were isolated and purified by ultracentrifugal flotation at 100 000 *g* for 16 h at 15 °C in a Kontron Centrikon T-2060 ultracentrifuge with a TST 41-14 rotor (Kontron, Zurich, Switzerland). Two milliliters from the top of each tube were recentrifuged in the same conditions except that BSA (50 $mg \cdot tube^{-1}$) was added in order to remove traces of [1-^{14}C] labelled free FA absorbed onto VLDL particles. Finally, purified VLDL were collected at the top of each tube (2.5 mL) and counted for radioactivity in scintillation vials.

2.7. Statistical analysis

The values are expressed as means ± SEM for the five (rumenic acid isomer metabolism) or three (oleic acid metabolism) independent experiments. A comparison of means between the two FA was

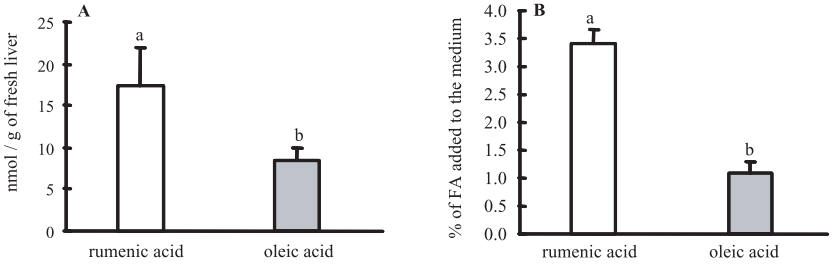

Figure 1. Uptake of rumenic and oleic acids by bovine liver slices. Liver slices from bovine animals were incubated in a medium containing a FA mixture (0.75 mM) and [1-^{14}C] rumenic acid or [1-^{14}C] oleic acid for 17 h. The FA uptake was calculated as the sum of FA converted into CO_2, into ASP, and incorporated into total cellular lipids. The values are expressed in nmol per g fresh liver (**A**) or as % FA added in the medium (**B**). The data are means ± SEM of 5 animals per group for rumenic acid uptake and 3 animals per group for oleic acid uptake. Mean values with different superscripts were significantly different (^{a,b} $P < 0.01$).

Table I. Oxidation of rumenic acid and oleic acid into acid-soluble products and into CO_2 in bovine liver slices.

	Rumenic acid ($n = 5$)	Oleic acid ($n = 3$)
Extent of FA oxidation		
% of FA incorporated by cells	64.8 ± 3.90	53.4 ± 8.30
Acid soluble products		
% of oxidized FA equivalents	99.7 ± 0.07^a	98.6 ± 0.40^b
CO_2		
% of oxidized FA equivalents	0.33 ± 0.07^a	1.40 ± 0.40^b

Values are expressed as means ± SEM. ^{a,b} $P < 0.01$ (ANOVA).

tested by analysis of variance (ANOVA) using the mixed procedure of SAS [30].

3. RESULTS

3.1. FA uptake

The amount of FA taken up by the liver slices prepared from steers corresponded to the sum of radiolabelled FA incorporated into total lipids in homogenates (neutral plus polar lipids), of FA partly oxidized as ASP and secreted into the medium and of FA totally oxidized and released as CO_2 into the atmosphere. After 17 h of incubation, the rate of uptake of rumenic acid by hepatocytes was double ($P = 0.009$) that of oleic acid (17.2 vs. 8.4 nmol·g^{-1} fresh liver, respectively) (Fig. 1A). Similar results were observed when expressed as the percentage of radioactivity introduced into the incubation medium (Fig. 1B).

3.2. FA oxidation

The extent of oxidation of rumenic and oleic acids in bovine liver slices (expressed as the percentage of incorporated FA converted into ASP and CO_2) was similar and represented more than 50% of incorporated FA (Tab. I). The oxidation of both FA led mainly to the formation of ASP (> 98% of oxidized FA equivalents, $P = 0.002$). The oxidation of rumenic and oleic acids into

CO_2 was of minor importance but that of oleic acid was 3-fold higher (P = 0.008) than that of rumenic acid (1.4 vs. 0.33% of oxidized FA equivalents, respectively) (Tab. I).

3.3. Bioconversion of rumenic acid

Gas-liquid chromatography analysis of FAME prepared from total lipids of cell homogenates showed a net conversion of rumenic acid into conjugated C18:3 in bovine liver slices (Fig. 2). Conjugated C18:3 was identified as the *cis*-6, *cis*-9, *trans*-11 C18:3 isomer on the basis of its retention time, compared with that of purified *cis*-6, *cis*-9, *trans*-11 C18:3 used as an external standard. The conversion of rumenic acid into conjugated C18:3 corresponded to 13% of the fraction of *cis*-9, *trans*-11 CLA isomer escaping from the oxidative pathway.

3.4. FA esterification

The esterification of rumenic and oleic acids (expressed as a percentage of FA converted into neutral (NL) and polar lipids (PL) out of the total FA incorporated into the cells) represented 35.2 and 46.5% of FA incorporated by bovine hepatic cells, respectively (Tab. II). More than 71% of esterified rumenic acid equivalents were incorporated into PL whereas esterification of oleic acid led predominantly (59.8% of esterified oleic acid equivalents) to NL.

3.5. FA secretion as part of VLDL particles

The amount of rumenic and oleic acids secreted into the medium as part of VLDL particles by hepatic cells was low (0.015 and 0.033 nmol secreted as part of VLDL·g^{-1} fresh liver/17h for rumenic and oleic acids, respectively) (Fig. 3A). When expressed as the percentage of FA incorporated into hepatocytes (Fig. 3B), the secretion rate of rumenic acid was one-fourth lower (P = 0.005) than that of oleic acid (0.091 vs. 0.365% of FA incorporated into cells for rumenic and oleic acids, respectively) (Fig. 3B).

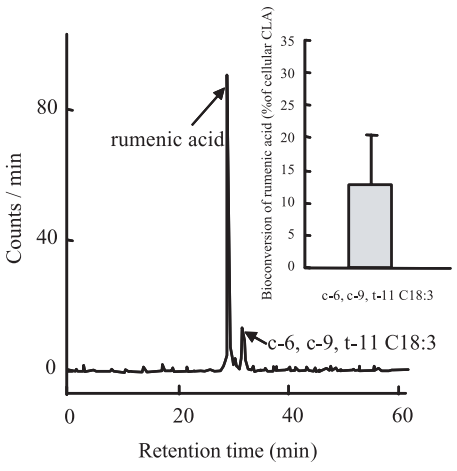

Figure 2. A representative radiochromatogram of [1-^{14}C] rumenic acid and proportion of conjugated C18:3 resulting from its bioconversion in bovine liver slices. Liver slices from bovine animals were incubated for 17 h in a medium containing a FA mixture (0.75 mM) and [1-^{14}C] rumenic acid or [1-^{14}C] oleic acid. At the end of incubation, the cellular lipids were extracted, transformed into methyl esters and analyzed by GLC. The outflow from the column was split between a flame-ionization detector (10%) and a copper oxide oven in order to transform the labelled FA into [1-^{14}C] CO_2 (90%). The radioactivity was determined with a radiodetector by counting [1-^{14}C] CO_2. The proportion of rumenic acid converted into conjugated C18:3 was calculated as the ratio between the radioactivity corresponding to the conjugated C18:3 and the sum of the radioactivity present in peaks of rumenic acid and its conjugated C18:3 derivative. Values are expressed as means ± SEM.

4. DISCUSSION

The liver plays an important role in lipid metabolism of ruminants especially during periods of fat mobilization which occur in several nutritional and physiological situations

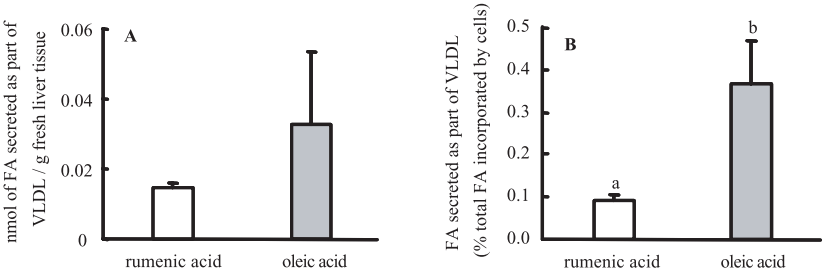

Figure 3. Secretion of rumenic and oleic acids as part of VLDL particles by bovine liver slices. Bovine liver slices were incubated in a medium containing a FA mixture (0.75 mM) and [1-^{14}C] rumenic acid or [1-^{14}C] oleic acid for 17 h. VLDL particles were purified by ultracentrifugal flotation. The values are expressed in nmol per g fresh liver (**A**) or as % FA incorporated into liver slices (**B**). Data are means ± SEM of 5 animals for the rumenic acid group and of 3 animals for the oleic acid group. Mean values with different superscripts were significantly different ($^{a, b}$ $P < 0.01$).

Table II. Esterification of rumenic acid and oleic acid into phospholipids and neutral lipids in bovine liver slices.

	Rumenic acid (n = 5)	Oleic acid (n = 3)
Extent of FA esterification		
% of FA incorporated by cells	35.2 ± 3.9	46.5 ± 8.3
Phospholipids		
% of esterified FA equivalents	71.7 ± 3.1^{a}	40.2 ± 4.2^{b}
Neutral lipids		
% of esterified FA equivalents	28.3 ± 3.1^{a}	59.8 ± 4.2^{b}

Values are expressed as means ± SEM. ^{a,b} $P < 0.01$ (ANOVA).

such as restricted feed intake or periparturient periods [15] and its regulation may affect the amount of CLA in the lipids of ruminant products. The aim of this study was to investigate the metabolic behavior of rumenic acid (as the main CLA isomer present in ruminant products) in the bovine liver (as a specific CLA producing animal). Moreover, these characteristics were compared with those obtained for oleic acid, a FA representative in bovine plasma.

From the point of view of the experimental procedure, the levels of rumenic acid added to the medium in the present study (55 μM) were similar to those used in different systems such as human cancer cells [31] and isolated rat hepatocytes [32], and were lower than those used in monolayer cultures of bovine hepatocytes [33]. In all cases, no cytotoxic effects occurred as assessed by cell viability measurement [32]. Moreover, when compared with a recent study carried out in bovine animals by Loor et al. [34], the amounts of rumenic acid added to the medium in our study were higher than those present in plasma non-esterified FA of animals (3 μM), but the values remained supraphysiological.

Under our experimental conditions, the higher uptake of rumenic acid by bovine

liver slices was in comparison to that of oleic acid confirmed by previous results obtained in rat hepatocytes by Gruffat et al. [21]. Such a difference could result from a particular conformation of the double bonds of rumenic acid (*cis*, *trans* conjugated double bond) compared to the cis conformation of the oleic acid double bond. Indeed, Bickerstaffe and Annison [35] previously showed, in the perfused chicken liver, that the incorporation of mono-unsaturated fatty acid by hepatic cells depended more on the spatial conformation of the double bonds rather than on the position along the hydrocarbon chain, the trans-monounsaturated FA being more easily incorporated than the cis-monounsaturated FA (i.e., oleic acid). Although rumenic acid was better utilized by hepatic cells than oleic acid in our experimental conditions, the efficiency of hepatic uptake of both FA by bovine hepatocytes was lower than that observed in the rat liver [21]. It is well accepted that FA uptake by the liver mainly depends on the concentration of plasma FA, the transfer of FA through biological membranes resulting from a simple diffusion [36] and/or from a facilitated diffusion [37] involving FA transport proteins. Therefore, differences in FA uptake observed between both species could be attributable to a lower activity or a lower amount of FA transporters in bovine than in rat liver. In our experimental conditions, the uptake of oleic acid by bovine liver slices amounted to about 1.08% of FA added to the medium whereas it amounted to 2.5% of FA in rat liver slices medium [21]. The incorporation of these FA was lower than that reported in previous experiments in the bovine liver in vivo (7 to 25% of circulating non esterified FA) [38] or using isolated rat hepatocytes (50% after 6 h of incubation) [39], or on perfused rat liver (90% after 2 h of incubation) [40]. These discrepancies could be explained by a lower contact between the cells and medium in the in vitro model with liver slices than in the in vivo or the in vitro model with isolated hepatocytes or in the ex vivo model with perfused liver.

Once incorporated into bovine hepatic cells, rumenic and oleic acids were highly catabolized through the β-oxidation pathway since this metabolic pathway represented more than 50% of incorporated FA on the contrary to the situation in the rat liver where the extent of oxidation did not exceed 40% [21]. One explanation might be the low efficiency of bovine animals in secreting FA from the liver [41], therefore directing FA preferentially towards the oxidative pathway. The oxidation of both rumenic and oleic acids led mainly to ASP production. The catabolism of FA in the liver is mostly directed towards the synthesis of ketone bodies for energy utilisation by tissues [42] as shown in the present study where products of rumenic and oleic acid oxidation were predominantly ASP (more than > 98% of total oxidation products).

The conversion of non-oxidized rumenic acid into the conjugated C18:3 derivative by desaturation activity in the liver has been previously suggested [20]. The presence of these derivatives has been mentioned in the liver of different species such as the rat [16–18, 21], lamb [19] and mouse [20]. Moreover, it has been recently demonstrated in the rat that the liver is able to convert rumenic acid into a conjugated derivative identified on the basis of its retention time as the *cis-6, cis-9, trans-11* C18:3 [21]. In isolated rat microsomes, this conversion was shown to result from the action of Δ6 desaturase [43]. Furthermore, Juanéda and Sébédio [44] demonstrated in the rat liver that rumenic acid is converted not only into C18:3 but also into C20:3 derivatives, the latter being identified as Δ8, 11, 13 C20:3. The lack of bioconversion of CLA into a conjugated C20:3 derivative by the bovine liver in our experimental conditions could be explained by an incubation time of liver slices too short to allow subsequent elongation of the C18:3 derivative into the C20:3 derivative or by a lack of sensitivity in our analytical procedure. However, the fact that bovine hepatocytes converted rumenic acid into a C18:3 derivative two-fold less efficiently than rat liver slices [21] could be explained

by a higher hepatic Δ6 desaturase content and/or activity in the rat. A recent study on CLA-fed mice showed that CLA increases mRNA expression of Δ6 desaturase in comparison to linoleic-fed mice [45]. As previously discussed, the efficiency of CLA uptake by the rat liver was higher than that of the bovine liver suggesting a possible higher expression of Δ6 desaturase in the rat than in the bovine liver. Moreover, properties of the C18:3 conjugated derivative are still unknown.

Oleic acid, rumenic acid and its derivatives that escaped from β-oxidation were esterified in bovine liver slices. The preferential esterification of rumenic acid and its derivatives into polar lipids and oleic acid into neutral lipids confirmed previous results in the lamb liver showing that CLA and its conjugated derivatives such as C18:3, C20:3 and even C20:4 are mainly associated with polar lipids [18]. This preferential path of conjugated FA esterification appeared to be specific to ruminant animals since in the rat liver rumenic acid was mainly incorporated into neutral lipids [21]. The synthesis of neutral and polar lipids is known to occur from a common step which is the formation of a diacylglycerol [46], and it may be a possible explanation for the difference in the esterification pathway of rumenic acid. However, the mechanisms regulating the conversion of diacylglycerols into triglycerides or phospholipids are still unknown. The more obvious hypothesis seems to be the selectivity of the substrate by enzymes (diacylglycerol acyltransferase for NL synthesis and phosphocholine or phosphoethanolamine acyltransferase for PL synthesis) [46]. Furthermore, it has been shown, in the rat liver, that linoleic acid competes with CLA for its incorporation into polar lipids leading to CLA esterification into storage lipids such as neutral lipids [47]. We can thus speculate that the enzymes responsible for the incorporation of PUFA into polar lipids are more active or abundant in bovine than in the rat liver and therefore are not limiting for the esterification of rumenic acid into polar lipids.

In our experimental conditions, the products of esterification were secreted as part of VLDL particles by liver slices to a very low extent. The hepatic synthesis of VLDL is a complex process requiring the coordinated synthesis of lipids and constitutive proteins (mainly apoprotein B) and their assembly into lipoprotein particles [48]. The time necessary to allow the synthesis and secretion of VLDL particles is probably more than 17 h of liver slice incubation in our experimental conditions. When the secretion of rumenic and oleic acids as part of VLDL particles by the bovine liver was expressed as $nmol \cdot g^{-1}$ fresh liver, the extent of secretion was quite similar for the two FA. However, when expressed as the percentage of incorporated FA, the secretion of oleic acid was 3-fold higher than that of rumenic acid. This discrepancy, already observed in the rat liver [21], was probably due to the higher intensity of liver uptake of the CLA isomer compared to that of oleic acid. Furthermore, the fact that the bovine liver in our experimental conditions secreted a lower amount of rumenic acid than the rat liver [21] could be explained by the preferential esterification pathway in the former, directing the CLA isomer mainly into polar lipids, which are preferentially associated to membranes as part as lipoproteins for export.

In conclusion, the in vitro model of liver slices allows us to compare the main metabolic pathways of the more abundant CLA isomer present in ruminant products and of oleic acid in the bovine liver. Thus, under our experimental conditions, we have clearly demonstrated that rumenic acid incorporated into bovine hepatocytes was highly catabolized (more than 50%), a non negligible part was converted into conjugated C18:3, and CLA and its derivatives were preferentially incorporated into the polar lipid fraction. Moreover, further investigations concerning conjugated derivatives arising from the hepatic conversion will be therefore necessary in order to determine their biological properties.

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