Lipid analysis of tissues from camel (Camelus dromedaries) reveals unique composition in fatty acids

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Abstract— Domesticated Arabian camel, Camelus dromedarius, is the most important animal in arid and semi-arid areas, as it represents the main source of meat, milk and fat besides its high cultural and economical values. The present work was carried out to determine the compositions of fatty acids and phospholipids in the hump, plasma and liver mitochondria were studied under various physiological and nutritional conditions of this ruminant. In plasma, palmitic (C16:00), linoleic (C18:2 n-6), stearic (C18:00) and oleic (C18:1 n-9) acids represent 70% of the fatty acids identified. In liver mitochondria, a closely similar ratio was obtained (62%) but with different proportions. In the hump, palmitic, oleic, stearic and myristic acids represent 82% of the total fatty acids. Moreover, qualitative differences were observed with myristic (C14:00) acid whereas linoleic acid was lacking. The phosphatidylcholine and phosphatidylethanolamine are the major mitochondrial phospholipids with (62%) whereas sphingomyelin represent (14%), while phosphatidylinositol shows the weakest rate (5%). The composition of fatty acids (chain length and unsaturation) in plasma and in liver mitochondria did not depend on age, sex, mass and diet. These results show qualitative similarity in the composition of fatty acids between plasma and liver mitochondrial membranes but not with in the hump. These results suggest that the camel developed a particular metabolic regulatory system to maintain constant the composition of fatty acids independently of physiological and the nutritional conditions.

Index Terms— Camel, plasma, mitochondria, hump, membrane, fatty acids, phospholipids.

1 INTRODUCTION

THE camel (Camelus dromedaries; one-humped camel) belongs to the family Camelidae, genus Camelus. It has been domesticated for about 4000 years and has been long valued as a pack animal. The camel is a domesticated ruminant species of considerable biological and economical importance, especially in arid and semi-arid regions [1], [2]. To survive in these difficult conditions and thanks to these anatomical and physiological characteristics, the camel has adapted by mechanisms of resistance to protein under nutrition (urea recycling), energy (fat reserves of the hump), water (resistance to thirst), but also mineral [3], [4], [5], [6], [7], [8], [9], [10].

In the north of Africa, Middle East and Asia, camels play a critical role in providing human foods, especially meat, milk and fat [11], [12], [13], [14], [15]. Camel fats, are used to prepare many dishes, namely, the production of a chocolate butter analog [11], [16], making high-quality dry sausages, and frying [17], [18], [19]. The body lipids are the main form energy storage in mammals. Lipid deposition and mobilization cycles are essential for reproduction, lactation and ecological adaptations [20]. The adipose reserves in camels are mainly stored in the hump [21], [22], [23], [24]. However, changes in lipid reserves and related mechanisms are not well understood in this species which is able to survive long periods without feed [9], [25]. Despite these difficult survival conditions, the trans-fatty acid profile of lipids from hump adipose tissue and milk was compared in camel and cattle, and shows that there are no gross differences between the two species [26]. Moreover, D-3-hydroxybutyrate dehydrogenase, a mitochondrial inner membrane bound enzyme was discovered and purified in an unique structure in camel and involved in the conversion of ketone bodies as products of lipid metabolism [27]. Additionally, most of fatty acids in the camels are esterified as triglycerides or phospholipids and vary according to their anatomical location in the body [28], [29]. All these literature results suggest the existence of a very developed system of regulation in the dromedary which is determined probably by the environment.

Data on the composition of camel fats are relatively scarce and few available in the literature. In fact, most studies on lipid composition in camels have been reported especially in milk [30], [31], [32] and meat [33], [34], [35]. To our knowledge, no analytical work has so far been undertaken on lipid contents in camel tissues in Morocco. The present study was aimed at investigating the fatty acids and phospholipids composition in the hump, plasma and liver mitochondria under various physiological and nutritional conditions of this animal.

2 MATERIALS AND METHODS

2.1 Materials

A group of 20 camels, 13 females and 7 males, aged 7 to 14 years old and weighing between 333 to 470 kg, raised at the Bouskoura experimental station of the National Institute for Agronomic and Veterinary Sciences Hassan II (Casablanca) (see Table 1) under controlled conditions, or overfed. Blood
samples were taken at the jugular vein and were immediately centrifuged and the supernatant was frozen at -28°C until analysis.

The samples of liver and hump from 3 females and 3 males (age: from 6 months to 3 years) were collected accompanied by a veterinary hygiene inspector, after sacrificing the animals at the slaughterhouse. The livers where immediately placed in a sucrose cold solution 0.25 M pH 7, used for the preparation of homogenate and mitochondria.

2.2 Methods

2.2.1 Mitochondrial isolation and separation of the membranes

The preparation of the mitochondria was done according to the adapted method of Fleischer et al., [36]. The liver samples were immediately washed and crushed in a solution of sucrose 0.25 M pH 7 with the Ultra turrax (for 2 seconds at low speed), pottered then filtered on layers of pharmaceutical gauze then centrifuged. The mitochondria thus obtained were suspended in a minimum of Hepes buffer 10 mM pH 7.4 containing sucrose 0.25 M then frozen in nitrogen atmosphere and stored at -28°C until use. An aliquot was taken, protein concentration was determined as described by Lowry et al., [37] and phosphorus concentration was determined as described by Chen et al., [38]. The mitochondrial membranes were isolated according to the method of Kielley and Bronk, [39]. Frozen samples of mitochondria were thawed in buffer phosphate 20 mM pH 7.4, at a ratio of 0.5 ml of buffer per mg of protein. After centrifugation at 20 000 g for 10 min at 4°C, the pellet contains the inner membranes while, the supernatant contains the outer membranes and the content of the intermembranar space. The various fractions obtained were frozen in nitrogen atmosphere and stored at -28°C until use.

2.2.2. Extraction of phospholipids

After thawing the samples of plasma, hump and mitochondrial membranes, the extraction of lipids was carried out according to the method of Folch et al., [40]. The sample was crushed with the Ultra turrax, in 20 volumes of the chloroform/methanol 2/1 (v/v) mixture. The lipid extract was washed with KCl aqueous 0.2 volume by volume of a 0.8% (w/v) solution. The organic phase was then recovered and washed with the chloroform/methanol/potassium chloride mixture 0.8% (w/v) in proportions 3/48/47 (v/v/v). After centrifugation at 20 000 g for 10 min at 4°C, the pellet contains the inner membranes while, the supernatant contains the outer membranes and the content of the intermembranar space. The various fractions obtained were frozen in nitrogen atmosphere and stored at -28°C until use.

2.2.3. Preparation of fatty acids methyl esters

The transformation of the fatty acids of phospholipid esters to methyl esters was carried out according to the method of Morisson and Smith, [41]. It consisted of a transmethylation of lipids by methanol catalyzed by boron trifluoride (12% in methanol), then heated at 100°C for 60 min. According to the type of lipids. The methyl esters of the lipid phase were extracted with hexane and washed with distilled water. To separate the saturated fatty acids from the unsaturated acids according to the method of Folch et al., [40], the solvent was evaporated and total fatty acids methyl ester were submitted to fractionation by thin-layer chromatography on a silica plate impregnated with silver nitrate.

2.2.5. Identification and quantitative estimation of fatty acids

The thin-layer chromatography was carried out on Silicagel 60. After depositing the sample on the chromatography plate, a first migration was carried out in a mixture of chloroform/methanol/water 65/25/4 (v/v/v) for separation of four spots which were revealed with the 2,7-dichlorofluorescein. The three spots of weak follow reference were scraped off and the phospholipids were extracted using the mixture chloroform/methanol 2/1 (v/v). The extracts thus obtained were deposited separately on another plate. A second migration was then carried out, according to the method of Heape et al., [42], in 0.25% chloride potassium mixture/methanol/propane/methyl acetate/chloroform 3/2.7/8.6/10/8.6 (v/v/v/v/v). After identification with the 2,7-dichlorofluorescein, each phospholipid class was collected in order to prepare methyl esters as described previously, to be analyzed by gas chromatography.

The identification and quantitative estimation of fatty acids

The identification and quantification of fatty acids methyl esters derivatives previously prepared was carried out on a Carlo Erba HRGC 5300 chromatograph equipped with a flame ionization detector connected to an integrator Spectra-Physics SP 4290. The capillary column was a DB Wax, (J & W Scientific, Folsom, CA) with a length of 30 m and an inner diameter of 0.32 mm, with a film thickness of 0.5 mm. The separation was carried out under isothermal conditions at 190°C. The temperature of the injector and of the detector was 250°C. The carrier gas was helium at a pressure of 140 kPa.
3 RESULTS AND DISCUSSION

3.1 Fatty acids composition of plasmatic phospholipids

The analysis of the fatty acid composition in plasmatic phospholipids of the camel showed that palmitic (25.61±3.2%), stearic (16.47±2.13%), oleic (16.88±2.40%) and linoleic (16.62±2.05%) acids, represent more than 70% of total fatty acids. Arachidonic (C 20:4 (n-6)) acid did not exceed 3.61±1.01%. The other fatty acids were present as traces (Table 2).

The comparative study of the composition of plasmatic fatty acids did not show any significant difference between the effect of various physiological (sex, age, weight) and nutritional (food intake) conditions of the camel (Table 1). The ratio of saturated to unsaturated fatty acid was not affected (0.86). The comparison between these results and those already obtained in the rat shows that the phospholipids fatty acid composition and unsaturated/saturated ratio change with age and diet [43], [44], [45], as well as for beef [46]. In spite of our results concerning the fatty acid composition in plasmatic phospholipids of the camel, a recent study reported that only four fatty acids were widely present in the serum of camel. Palmitic acid, stearic acid, oleic acid and linoleic acid representing 89.1% of the whole fatty acids [30]. On the other hand, fatty acid profiles in erythrocyte lipid composition in sheep showed five main acids: oleic, stearic, linoleic, palmitic and arachidonic acid [47].

3.2 Fatty acids composition of total mitochondrial phospholipids

The analyses of fatty acids composition of total phospholipids of the camel liver mitochondrial membranes are presented on Table 2. Palmitic, stearic, oleic and linoleic acids are the most abundant, with more than 63% of total fatty acids, with percentages of 18.24±3.52%; 22.67±2.93%; 8.12±1.05% and 13.25±2.32% respectively. Arachidonic acid represents 7.87±1.40%.

The effect of age, sex and nutrition of the camel (Table 1) on the composition of total mitochondrial fatty acids is not significant. The comparison between the results obtained in this study and those described in the rat by Stoffel and Schiefer, [48] shows that the composition of these fatty acids in the mitochondrial membranes of the rat represents more than 76%. Indeed, the palmitic and stearic acids account for 49% of total fatty acids of the mitochondrial membranes of the rat. On the other hand, in the camel these same fatty acids only account for 39%. Arachidonic acid accounts for only 7.87±1.40% in the camel whereas in the rat, it represents more than 19.5%. This low amount of arachidonic acid may be explained by the low gamma-linolenic C18:3 n-3 acid content in the mitochondria or its total absence in the plasma since the gamma-linolenic acid is the first precursor in the enzyme conversion limiting step of linoleate into arachidonate [49]. The possible low activity of the delta-6-desaturase (E.C.1.14.99.5), the enzyme responsible for conversion of 6-linoleic acid into gamma-linolenic acid [50], [51] could also give another reason. Moreover, this metabolic process, seems to be highly affected by certain physiological processes such as the feeding [52], the hormonal statute or the age of the animal in the rat [53], the weight and nutrition in the beef [46]. This phenomenon was not seen in the camel. In spite of our results, Shibani et al., [54] reported that in camel liver, the most abundant fatty acids were oleic acid, palmitic acid and stearic acid. In the liver, relatively high levels of linoleic acid were found which is attributable to their higher percentage of phospholipids in total lipids than in the other tissues where triglycerides are the predominant lipids [54].

3.3 Fatty acids composition of hump phospholipids

The analysis of fatty acids composition in the hump showed that the myristic (8.93±1.43%), palmitic (37.2±2.65%), stearic (14.8±2.16%) and oleic (19.61±1.28%) acids represent the majority of the fatty acids (Table 2). These fatty acids constituted more than 82%. The other fatty acids were present as traces.

Our results were comparable with those reported in a recent study concerning the fatty acid composition from the hump of young camels (Hachi), where the most important acids were oleic, palmitic, stearic, palmitelaidic and myristic acid, which together accounted for approximately 88% of the total fatty acids [55]. Similarly, Shibani et al., [54] have showed that the most abundant fatty acids in lipids from camel hump were oleic acid, palmitic acid, stearic acid and myristic acid. A study performed by Kadim et al., [24] on concentrations of fatty acids in the hump and abdomen fats of three different age groups of camel reported that palmitic acid was the major fatty acid in hump fat, followed by oleic acid and stearic acid. Kadim et al., [24] have also mounted that the young camels have less total fatty acids than older camels in the hump.
3.4 Fatty acids composition of mitochondrial phospholipids

Analysis of the results, represented in Table 3, showed that PE and PC constituted the major fraction of total liver mitochondrial phospholipids, with percentages of 31.6% and 30.6% respectively. The class of PI had weakest fraction with 5.1%.

The same qualitative composition was obtained by Stoffel and Schiefer, [48] in the rat where PC and PE account for 41% and 35% respectively of total mitochondrial phospholipids. PI accounts for only 2%. However, the SM fraction which represented 14% of total phospholipids in the camel seems occur only as traces in the rat and in bovine as described by Fleischer et al., [62] and by Stoffel and Schiefer, [48].

The distribution of the fatty acids in the various phospholipid classes shows that linoleic acid is the major component in PC and PS, with 12.3%, followed by stearic acid, with 11.5%. In PE and PI, the stearic acid accounts for 28.6 and 32% respectively. For the CL the linoleic acid represents more than one-half of the total fatty acids with 52%. Whereas, for SM the palmitic acid is the major fatty acid with 13.8%, followed by stearic acid with 12.1%. The comparison between the results obtained here and those already obtained by Stoffel and Schiefer, [48] in the rat, shows a quantitative and qualitative difference in the levels of the fatty acids and of the various classes of phospholipids and especially at the level of the arachidonic acid and SM.

The analysis of the composition of fatty acids from inner membrane lipids (Table 3) of the camel liver mitochondria shows that, in PC, PE and PI, the stearic acid is the major fatty acid with 28.8%, 40.7% and 51.4% respectively, followed by palmitic acid with 20.1%, 12.4% and 14% respectively. For the CL the linoleic (C18:2 (n-6)) acid represents more than 44%. For SM, palmitic acid is the major fatty acid with more than 33% followed by oleic acid 25.5%. The study of the composition in fatty acids of the inner membrane compared to the whole mitochondria shows a clear predominance of stearic acid in PC, PS, PE. But for PI, CL and SM qualitative and quantitative differences were observed for the composition of phospholipid fatty acids.

Table 2 shows the ratios of saturated/unsaturated fatty acids change, it was 0.88, 0.85 and 1.74 respectively in plasma, in mitochondrial phospholipids and in hump. The distribution of this ratio in the different classes of mitochondrial phospholipids. The composition of total fatty acids in the hump was characterized by a high rate of saturated fatty acids: Myristic, palmitic and stearic represent the majority of total fatty acids. In the other hand, the ratio of saturated to unsaturated fatty acids is similar in plasma and in mitochondria. The observation in the hump explain by the higher level of saturated fatty acids.

In mitochondria, the distribution of this ratio was function of the different class of phospholipids studied. According to our results, we distribute the ratio of different phospholipids in three groups: The first group of PI that represents the highest ratio the stearic and palmitic acids are major in this composition. The second group of PC, PE, PS and SM that represent the means ratio being given the equivalence in saturated and unsaturated fatty acids and the third group of CL that represents a weak rate. In comparison with the results found in the rat, as already described [48], the analysis of the results obtained with camel reveal that these ratios are higher in the rat. Therefore, the rate of unsaturation is higher in the camel than in the rat. Only the cardiolipins showed a higher ratio. This could be explained by the low amount of linoleic acid in the camel compared to the rat.

Warda and Zeisig, [63] reported that the most frequently occurring lipids of the membrane of erythrocytes from the one-humped camel are sphingomyelin, phosphatidylcholine and phosphatidylserine. On the other hand, our results of total liver mitochondrial phospholipids in camel were in the range reported in the literature for cattle milk: PE (19.8-42.0%, w/w), PC (19.2-37.3%, w/w), PS (1.9-10.5%, w/w), PI (0.6-11.8%, w/w) and SM (18.0-34.1%, w/w) [64], [65], [66], [67], [68].

On the other hand, in camel meat, the major fatty acids are palmitic, oleic and linoleic, with smaller amounts of other fatty acids, both normal and branched [33], [34], [59], [69]. Kadim et al., [70] indicates that the fat content of camel meat may increase with age. For against, the comparison by age showed no significant effect on the content of fatty acids [35]. Also, the main milk fatty acids in camel milk were myristic acid, palmitic acid, palmitoleic acid, stearic acid and oleic acid representing as the whole 86.7% of the milk fatty acids [30]. The variation in fatty acids concentrations in milk could be due to differences in the type of feed, breed and stage of lactation.
amongst animals [31]. Palmquist et al., [71] reviewed other factors, which affect fatty acid composition like animal genetic, grain, amount and composition of dietary fat, dietary protein, seasonal and regional effects.

4 Conclusion

The study of the qualitative and quantitative composition of phospholipids and fatty acids of the camel showed that palmitic, stearic and oleic and linoleic acids, are the major fatty acids of plasma and liver mitochondrial inner and outer membranes with equivalent proportions, in contrast to another species, the rat. The composition of fatty acids in the hump was characterized by higher level of saturated fatty acids with a majority of myristic, palmitic and stearic acids. Plasma and liver mitochondrial membranes of camel are characterized by a low level of arachidonic acid. This low content may be due to the low levels of it precursors or to the low expression of the arachidonic acid biosynthesis enzymes. The comparison of the ratios between saturated and unsaturated fatty acids shows that the camel is characterized by membranes much less rigid than the rat.

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Abbreviations


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