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Lipolysis in muscles during refrigerated storage as related to the metabolic type of the fibres in the rabbit

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Abstract

The relation between lipolysis and the metabolic fibre type was investigated during refrigerated storage of rabbit muscles. Free fatty acid, monoacylglycerol and diacylglycerol contents and free fatty acid composition were compared in five muscles immediately after slaughter and after a 7-day-storage at 4°C. The results showed that. (1) The amount of free fatty acids sharply increased during the refrigerated storage (from 2–10 to 11–32 mg/100 g of muscle), especially that of long chain polyunsaturated fatty acids (from less than 0.1 to 1.4-3.3 mg/100 g of muscle). (2) The glycolytic muscles contained 1.5 times less free fatty acids than the oxidative ones. However, the rates of phospholipid and triacylglycerol hydrolysis were not related to the metabolic type of the fibres. (3) The contribution of phospholipids to free fatty acid fraction was twice that of triacylglycerols in the glycolytic muscles whereas it was similar or lower to that of triacylglycerols in the oxidative muscles.

1. Introduction

The metabolic type of the fibres is a major factor involved in the heterogeneity of muscle quality within a carcass. The oxidative muscles are more tasty and juicy and redder than the glycolytic ones (Valin, Touraille, Vigneron & Ashmore, 1982). They are more sensitive to oxidation than the glycolytic ones (Renerre and Labas, 1987; Wilson, Pearson & Shortland, 1976). This is partly related to their higher phospholipid content (Igene, Pearson, Merkel & Coleman, 1979) and to their higher long chain polyunsaturated fatty acid content (Alasnier & Gandemer, 1998) because phospholipids rich in long chain polyunsaturated fatty acids such as phosphatidyl ethanolamine are very sensitive to oxidation (Gandemer, 1990; Moerck & Ball, 1974). Lipolysis which releases free fatty acids (FFA) from both triacylglycerols and phospholipids is also suspected to favour lipid oxidation because FFA are very sensitive to lipid oxidation (Nawar, 1996). However, the relationship between lipolysis and the metabolic type of the fibres is not clearly established because no study was devoted to lipolysis in pure oxidative muscles. Lipolysis in fresh muscles was poorly studied. Some data are available in breast, thigh or leg muscles of chicken and turkey during refrigerated storage (Currie & Wolfe, 1977; Sklan, Tenne & Budowski, 1983). Both studies indicated that FFA content was lower in breast muscle (glycolytic) than in thigh or leg muscles (intermediate), suggesting that lipolysis was less intense in glycolytic muscles than in more oxidative ones.

The aim of this study was to evaluate lipolysis in muscles as related to the metabolic type of the fibres during refrigerated storage in the rabbit. Five muscles were chosen: 2 glycolytic ones (*Psoas major* and *Longissimus lumborum*), 2 oxidative ones (*Soleus* and *Semimembranosus proprius*) and an intermediate one (*Gastrocnemius laterale*). Lipid hydrolysis in muscles was evaluated immediately after slaughter and after 7 days of storage at +4°C by estimating the FFA, mono and diacylglycerol contents and FFA composition.

2. Material and methods

2.1. Animals

Sixteen rabbits (8 males and 8 females, New Zealand) were fed a standard commercial diet (containing 3.7%

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of lipids). They were 10 week-old and weighted 2.3 kg at slaughter. They were bled, skinned and eviscerated. The muscles of eight carcasses were immediately analysed (*T*0) and the eight other carcasses were covered with a food-grade plastic film and stored at +4°C under UV light (*T*7) for 7 days.

Five muscles were dissected from both sides of the carcasses: the *Longissimus lumborum* (LL) at the level of the 1st–4th lumbar vertebra, the *Psoas major* (PM), the *Semimembranosus proprius* (SM), the *Soleus* (SO) and the *Gastrocnemius* laterale (GL).

2.2. Lipid extraction

Muscles were carefully trimmed to remove adipose tissue and were minced in a blender. Lipids were extracted from 2 to 10 g of muscle with chloroform/methanol (2:1) according to the method of Folch, Lees and Sloane-Stanley (1957). The extracts were dried under vacuum on a rotary evaporator. The total lipid content was weighed and expressed in g/100 g of muscle. The phospholipid content was calculated (P × 25) after phosphorus was determined in the total lipid extract by the method of Bartlett (1959). The neutral lipid content was estimated by the difference between total lipid and phospholipid contents. Phospholipids and neutral lipids were expressed in g/100 g of muscle.

2.3. Lipid extract fractionation

For further lipid characterisation, the total lipid extracts were fractionated into neutral lipids and phospholipids on silica cartridges (Sep-Pack, Waters) following the procedure described by Juaneda and Rocquelin (1985).

2.4. Neutral lipid composition

Triacylglycerols, diacylglycerols, monoacylglycerols and free fatty acids were quantified according to a method adapted from that of Myher and Kuksis (1984). An aliquot of 50–200 μg of the neutral lipid fraction was introduced into a conic vial. The solvent was evaporated under N_2 and the lipids were silylated using 100 μl of a mixture of Tri-Sil/BSA (composed of pyridine, trimethylchlorosilane and *N*-*O*-bis trimethylsilylacetamide) (Formula ''P'', No. 49011, Pierce Chem., Co., Rockford, USA). The vial was immediately closed with a Teflon cap. The reaction was achieved in 30 min at room temperature. The silylated components were then analysed by gas chromatography. The chromatograph was a Hewlett–Packard (HP 5890) equipped with a flame ionisation detector and an ''on-column'' injector. One microliter sample was injected in a non-polar capillary column DB5 of 12 m length (0.32 mm internal diameter, 0.1 μm film thickness). The oven temperature was maintained at 100°C for 3 min, increased from 100 to 200°C at 30°C/min, from 200 to 250°C at 20°C/min, and from 250 to 335°C at 7.5°C/min and then held at 335°C for 5 min. The detector temperature was maintained at 350°C. The carrier gas was hydrogen at 0.6 bar head pressure. Lipids were eluted according to their molecular weight in 22 min. They were quantified using the tricaprin as the internal standard for which the response coefficient was set at 1. The response coefficients were 1.12 for FFA, 1.17 for the monoacylglycerols, 1.1 for the diacylglycerols and 0.97 for the triacylglycerols. Data were collected with an Apex workstation including an acquisition interface, software and a computer (Stang, France). Results were expressed as mg/100 g of fresh muscle.

2.5. Isolation of free fatty acids

FFA were purified from neutral lipids according to the method of Gandemer, Morvan-Mahi, Meynier and Lepercq (1991) using an anionic exchange resin (Amberlyst). An aliquot of 40–50 mg of neutral lipids were dissolved in 20 ml of a mixture of acetone:methanol 2:1 (v:v). After addition of 100–200 mg of the resin, the mixture was shaken for 30 min. Non-resin bound lipids were removed by washing the resin with acetone: methanol 2:1 (v:v). Resin was then transferred into a dry tube for the direct FFA methylation.

2.6. Fatty acid composition

The fatty acid composition of triacylglycerols, FFA and phospholipids was determined by gas liquid chromatography (GLC). Triacylglycerol and FFA methyl esters were prepared following the method of Morrison and Smith (1964). To ensure a simultaneous derivatisation of aldehydes and fatty acids, derivatives of phospholipids were prepared according to the method of Berry, Cevallos and Wade (1965). The gas chromatograph (Hewlett–Packard 5890) was equipped with split and on-column injectors and a flame ionisation detector. The derivatives were separated using a capillary column (DB 225, J&W, 30 m long, 0.32 mm internal diameter, 0.25 μm film thickness) containing a polar stationary phase (cyanopropylphenyl-methylpolysiloxane). For the methyl esters of triacylglycerols, split injection mode was used. The split flow rate was set at 30 ml/min. The oven temperature was held at 150°C for 4 min, increased to 200°C at 10°C/min, and then maintained at 200°C until the end of the analysis. The injector and detector temperatures were held at 250°C. For the methyl esters of phospholipids, on-column injection mode was used. The oven was first held at 50°C for 3 min and then programmed from 50 to 180°C at 15°C/min, held at 180°C for 10 min, increased from 180 to 210°C at 20°C/min and then maintained at 210°C for

10 min. The detector temperature was set at 250°C. Data were collected as described above. The individual fatty acid esters and aldehyde dimethylacetals (DMA) were identified by mass spectrometry using a bench mass spectrometer (HP MSD 5971A). Fatty acid and DMA compositions were respectively expressed as percentage of total fatty acid methyl esters present and as total DMA present. DMA proportion was expressed as percentage of the sum of fatty acid methyl esters + DMA.

2.7. Statistical analysis

Data were subjected to a two-way variance analysis using the GLM procedure. The model with fixed effects included metabolic type of the muscles (five levels) and storage time (two levels) and the interaction metabolic type × storage time. The calculations were performed with Statgraphics software.

3. Results

As previously reported, the total lipid, phospholipid and triacylglycerol contents depend on the metabolic type of the muscles (Alasnier, Remignon & Gandemer, 1996) (Table 1). Phospholipid content was 0.69 g/100 g of muscle in the PM, 0.71 in the LL, 0.87 in the GL, 0.93 in the SO and 0.92 in the SM. The content of neutral lipids (triacylglycerols + diacylglycerols + monoacylglycerols + FFA) was 0.47, 0.50 and 0.59 g/100 g of muscle in the PM, LL and GL, respectively, and 2.94, 3.40 in the SO and SM, respectively. Total lipid, phospholipid and triacylglycerol contents of muscles of the two rabbit groups were not significantly different even if the triacylglycerol content of muscles from rabbits whose carcasses were stored 7 days at 4°C tended to contain less triacylglycerols.

Fatty acid compositions of neutral lipids and phospholipids were related to the metabolic type of the muscles but they were similar in both groups of rabbits. According to the muscle, the neutral lipids contained 22.9–25.8% of polyunsaturated fatty acids (PUFA) whereas the phospholipids contained 37.3–45.9% of PUFA including 17.4–24.1% of long chain PUFA (results not shown, see Alasnier et al., 1996).

3.1. Effect of refrigerated storage on post-mortem lipolysis in the five muscles

The refrigerated storage largely affected FFA and monoacylglycerol amounts and the FFA composition (explaining more than 90% of the variability of these parameters). The amount of FFA increased significantly in the five muscles during the storage (Table 1). Immediately after slaughter, lipids were slightly hydrolysed and FFA represented 2–10 mg/100 g of muscle and only 0.2–0.6% of total lipids. After 7 days of storage, FFA amount was multiplied by 3–9 and reached 11.5–32.4 mg/100 g of muscle.

A concomitant increase in monoacylglycerol amount was observed in all muscles (from traces to 0.1–0.8 mg/100 g). Diacylglycerol amount was only slightly affected by the storage. This effect was associated with an interaction storage time × muscle because the amount of diacylglycerols was similar in all muscles except in SM where it decreased during storage. The lower diacylglycerol amount in SM of the second group of rabbits was largely related to the lower amount of triacylglycerols observed in the SM of these rabbits. Indeed, when expressed as % of triacylglycerol amount, the diacylglycerol proportion was similar in both groups of rabbits (0.6–0.5%).

The changes in FFA amount in muscles during the storage were associated with dramatic changes in FFA

Table 1
Changes in lipid fractions in rabbit muscles stored at +4°C for 7 days

Muscles	*Psoas major*		*Longissimus lumborum*		*Gastrocnemius laterale*		*Soleus*		*Semimembranosus proprius*		Statistical effects			
Time (days)	0	7	0	7	0	7	0	7	0	7	Muscle	Storage	Muscle × storage	SEE[a]
Number of rabbits	8	8	4	8	8	8	8	8	8	8				
Components[c] (mg/100 g of fresh muscle)														
Total lipids	1240	1000	1240	1140	1530	1230	3970	3970	4420	4100	***[d]	ns[b]	ns	489.7
Phospholipids	710	730	690	670	870	900	930	940	920	930	***	ns	ns	65.8
Triacylglycerols	467	242	492	414	582	270	2916	2898	3375	3049	***	ns	ns	473.3
Diacylglycerols	4.5c	4.0c	4.0c	4.8c	7.2c	4.3c	16.9ab	16.4ab	19.3a	13.8b	***	**	*	2.71
Monoacylglycerols	0.0d	0.1cd	0.0d	0.3bc	0.0d	0.4bc	0.0d	0.8a	0.0d	0.6ab	***	***	***	0.21
Free fatty acids	2.3ef	21.0c	1.9f	11.5d	3.1ef	25.1bc	10.5d	32.4a	7.2def	29.0ab	***	***	ns	6.33

[a] SEE: standard error of estimation.
[b] ns: not significant.
[c] Within rows, the means with different letters differ significantly.
[d] Significant effects: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

composition. Immediately after slaughter, FFA contained a large proportion of saturated fatty acids (59.5–63.9%) and low proportions of monounsaturated fatty acids (24.1–26.6%) and PUFA (11.0–14.7%) in all muscles (Table 2). After 7 days of storage, the proportion of saturated fatty acids sharply decreased in all muscles (from 32.8–42.2% to 20.6–32.3% for the C16:0 and from 15.2–21.3% to 8.6–13.7% for the C18:0). This drop was associated with a sharp increase of PUFA proportion in all muscles. The proportion of C18:2 *n*-6 rose from 8.7–11.2% to 16.4–20.8%, that of C20:4 *n*-6 from 0.6–1.5% to 4.4–7.4%. Similarly, other long chain PUFA (C20:2 *n*-6 + C20:3 *n*-6 + C20:5 *n*-3 + C22 *n*-6 and *n*-3), which were in trace amounts in FFA fraction immediately after slaughter, reached 2.9–5.0% after 7 days of storage.

After 7 days of storage, the fatty acid composition of the FFA fraction was intermediate between that of phospholipids and that of triacylglycerols attesting that both phospholipids and triacylglycerols contributed to FFA formation. For example, in PM, monounsaturated fatty acids represented 18.2% in phospholipids, 23.1% in FFA and 36.6% in triacylglycerols, PUFA represented 42.3% in phospholipids, 35.5% in FFA and 23.7% in triacylglycerols. Long chain PUFA accounted for 11.1% in FFA while they accounted for 18.4% in phospholipids and a very small proportion in triacylglycerols (1.2%). We can estimate the proportion of phospholipids and triacylglycerols hydrolysed during the storage from the amounts of some fatty acids accumulated in FFA fraction. On one hand, considering long chain PUFA are almost exclusively esterified in phospholipids and, consequently, those found in FFA fraction arose from phospholipid hydrolysis, we can estimate the proportion of phospholipids hydrolysed after 7 days of storage by the ratio of the amounts of these fatty acids in FFA and in phospholipids (Table 3). On the other hand, considering C14:0 and C16:1 are mainly esterified in triacylglycerols and those found in FFA arose from triacylglycerol hydrolysis, we can estimate the proportions of triacylglycerols hydrolysed by the ratio of the amounts of these fatty acids in FFA and in triacylglycerols (Table 3). Based on these calculations, the proportions of phospholipids hydrolysed during the storage ranged from 1.6 to 3.1% and that of triacylglycerols hydrolysed from 1 to 6.7%. The validity of these calculations is based on the hypothesis that lipolysis of both triacylglycerols and phospholipids was not fatty acid specific. To validate this hypothesis, we calculated the amount of individual FFA in muscles by

Table 2
Changes in free fatty acid (FFA) composition in rabbit muscles stored at +4°C for 7 days

Muscles	*Psoas major*		*Longissimus lumborum*		*Gastrocnemius laterale*		*Soleus*		*Semimembranosus proprius*		Statistical effects			
Time (days)	0	7	0	7	0	7	0	7	0	7	Muscle	Storage	Muscle × storage	SEE[a]
Number of rabbits	7	5	7	8	5	7	8	5	7	8				
Fatty acids (% of total methyl esters present)[c]														
C14:0	3.7	2.5	4.0	2.3	4.8	3.0	4.2	3.1	4.7	2.8	ns[b]	***[d]	ns	0.98
C15:0	1.9	0.8	1.6	1.1	1.6	1.4	1.7	1.3	1.8	0.9	ns	***	ns	0.51
C16:0	32.8cd	26.1e	34.5bc	28.6de	39.0ab	32.3cd	42.2a	24.1ef	40.4a	20.6f	**	***	***	4.15
C18:0	21.3	12.0	19.4	13.7	15.2	11.3	15.8	10.3	15.7	8.6	*	***	ns	4.10
Saturated	59.7a	41.4b	59.5a	45.7b	60.6a	48.0b	63.9a	38.8bc	62.6a	32.9c	ns	***	**	7.09
C16:1	4.2bc	4.3bc	4.2bc	3.0c	4.6bc	3.9bc	5.5b	5.8b	4.5bc	8.6a	**	ns	**	2.10
C18:1	21.7bc	18.8c	21.6bc	20.9c	22.0bc	17.7c	18.6c	25.6b	21.9bc	30.1a	***	ns	***	3.93
Monounsaturated	25.9bc	23.1c	25.8bc	23.9c	26.6bc	21.6c	24.1bc	31.4b	26.4bc	38.7a	***	ns	***	5.28
C18:2 *n*-6	10.9	20.8	11.2	16.4	10.2	17.0	9.7	17.9	8.7	17.8	ns	***	ns	2.51
C20:4 *n*-6	1.5d	6.5ab	1.5d	7.0a	1.0d	7.4ab	0.6d	5.4bc	0.8d	4.4c	***	***	*	0.96
Other long chain *n*-6[e]	0.4d	2.6ab	0.3d	2.8a	0.2d	2.4b	0.2d	2.3b	0.2d	1.4c	***	***	***	0.30
n-6	12.8	30.0	13.0	26.3	11.4	26.8	10.5	25.6	9.7	23.5	***	***	ns	1.01
C18:3 *n*-3	1.6c	3.6a	1.7c	1.9cd	1.4c	2.3bc	1.5c	2.5bd	1.3c	3.5a	**	***	***	0.60
Long chain *n*-3[f]	0.0d	1.9ab	0.0d	2.2a	0.0d	1.4c	0.0d	1.6bc	0.0d	1.5bc	*	***	*	0.34
n-3	1.6d	5.5a	1.7d	4.1bc	1.4d	3.6c	1.5d	4.2bc	1.3d	4.9ab	*	***	*	0.72
Polyunsaturated	14.4	35.5	14.7	30.4	12.8	30.4	12.0	29.8	11.0	28.4	**	***	ns	1.18

[a] SEE: standard error of estimation.
[b] ns: not significant.
[c] Within rows, the means with different superscripts differ significantly.
[d] Significant effects: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.
[e] C20:2 *n*-6 + C20:3 *n*-6 + C22:4 *n*-6 + C22:5 *n*-6.
[f] C20:5 *n*-3 + C22:5 *n*-3 + C22:6 *n*-3.

multiplying the amount of individual fatty acids in triacylglycerols or phospholipids by the rate of hydrolysis of the corresponding lipid class estimated as described above. The results of these calculations were compared to the amouts of FFA measured experimentally by the method of Gandemer et al. (1991). We found a good agreement between the values calculated and measured. For long chain PUFA, the values were, respectively, 1 and 1.4 mg/100 g in the LL, and 2 and 2.1 mg/100 g in the SM. For the sum C14:0 + C16:1, they were respectively 0.4 and 0.6 mg/100 g in the LL, and 3.2 and 3.3 mg/100 g in the SM. So we can conclude that the hypothesis was valid.

3.2. Influence of the metabolic type of the muscles on post-mortem lipolysis

Only diacylglycerol amount was strongly related to the metabolic type of the muscles which explained between 40 and 90% of the variability of this parameter. Other parameters such as FFA and monoacylglycerol amounts and PUFA proportions in FFA were significantly affected by muscle but this factor explained less than 10% of the variability of these parameters which were not always related to the metabolic type.

The diacylglycerol amount was higher in the oxidative muscles than in the glycolytic ones (13.8–19.3 mg/100 g versus 4–4.8 mg/100 g) (Table 1). FFA amount was 2–5 times higher in the oxidative muscles than in the glycolytic ones (Table 1). The oxidative muscles contained twice as manymonoacylglycerols as the glycolytic ones. This effect was associated with an interaction muscle × storage time because the effect of the metabolic type was marked only after 7 days of storage (0.6–0.8 versus 0.1–0.3 mg/100 g of muscle), monoacylglycerols being present in traces in all the muscles immediately after the death.

The FFA composition depended on muscles but only the proportions of monounsaturated fatty acids and long chain *n*-6 PUFA were related to the metabolic type of the muscles (Table 2). The effect of muscle was associated with an interaction muscle × storage time because the differences in FFA composition between the five muscles were not significant immediately after slaughter and became significant only after 7 days of storage. Thus, after 7 days of storage, the proportions of C16:1, C18:1, C20:4 *n*-6 and long chain *n*-6 PUFA were higher in the oxidative muscles than in the glycolytic ones. The proportions of the other FFA such as C16:0 and *n*-3 PUFA varied according to the muscles but they were not related to the metabolic type. Thus, the higher proportion of C16:0 was observed in the intermediate muscle (GL) and the lower in both glycolytic (LL, PM) and in one of the oxidative muscles (SM).

The proportions of phospholipids and triacylglycerols hydrolysed during the refrigerated storage varied from one muscle to another but they were not related to the metabolic type. The proportion of phospholipids hydrolysed was 1.6–2.3% in SM, LL, GL and SO and only that in PM was significantly higher than in the

Table 3
Contribution of phospholipids and triacylglycerols to lipolysis in five rabbit muscles stored at +4°C for 7 days

Muscles	*Psoas major*	*Longissimus lumborum*	*Gastrocnemius laterale*	*Soleus*	*Semimembranosus proprius*	Statistical effect Muscle	SEE[c]
			Proportion of phospholipids hydrolysed				
Long chain FA in FFA (1)[a] (mg/100 g of fresh muscle)	2.3bc[d]	1.4c	2.8a,b	3.3a	2.1bc	***[e]	0.76
Long chain FA in PL (2) (mg/100 g of fresh muscle)	77.7b	76.2b	126.4a	142.3a	129.9a	***	14.86
(1)/(2) × 100 (%)	3.1a	1.8b	2.2b	2.3b	1.6b	**	0.68
			Proportion of triacylglycerols hydrolysed				
14:0 + 16:1 in FFA (3) (mg/100 g of fresh muscle)	1.4b	0.6b	1.8b	3.7a	3.5a	***	1.14
14:0 + 16:1 in TG (4) (mg/100 g of fresh muscle)	22.3b	40.2b	32.4b	376.8a	356.4a	***	78.75
(3)/(4) × 100 (%)	6.7a	1.6b	5.9a	1.0b	1.0b	***	1.31
			Contribution of phospholipids and triacylglycerols to lipolysis				
Long chain PUFA/ (14:0 + 16:1) in FFA	1.7b	2.6a	1.6b	1.0bc	0.7c	***	0.69
Relative contributions of PL[b] and TG to lipolysis (%)	62:38	70:30	60:40	48:52	40:60	***	7.7

[a] FFA: free fatty acids; FA: fatty acids.
[b] PL: phospholipids; TG: triacylglycerols.
[c] SEE: standard error of estimation.
[d] Within rows, the means with different letters differ significantly.
[e] Significant effects: *** $p < 0.001$; ** $p < 0.01$.

others (3.1%) (Table 3). The proportion of triacylglycerols hydrolysed was 1% in the SO and SM, 1.6% in the LL and 5.9–6.7% in the GL and PM (Table 3).

The relative contributions of phospholipids and triacylglycerols to lipolysis was estimated by calculating the ratio of the amount of the FFA arising from phospholipids to those arising from triacylglycerols. The contribution of phospholipids to FFA formation was higher in the glycolytic muscles than in the oxidative ones (ratio 1.7–2.6 versus 0.7–1, Table 3) although the difference between those in PM and SO was not significant (1.7 versus 1).

4. Discussion

4.1. Post-mortem changes in the regulation of lipolysis

The FFA amount increased in all rabbit muscles during refrigerated storage as previously reported in chicken and turkey muscles (Currie & Wolfe, 1977; Sharma, Kowale & Joshi, 1982a,b; Sklan et al., 1983). The changes in FFA composition confirmed most of the previous data obtained in fresh pig muscles (Flores, Nieto, Bermell & Alberola, 1987; Motilva, Toldra, Nadal & Flores, 1994) and in turkey (Sklan et al., 1983) during refrigerated storage.

The increase in FFA amount and the changes in FFA composition during 7 days of refrigerated storage illustrate the drastic changes occurring in the regulation of lipolysis after the death. In vivo, FFA pool size and composition depend on the equilibrium between FFA arising from de novo synthesis, lipid captation from blood circulation, lipolysis of intracellular triacylglycerols and phospholipids and FFA used for biosynthesis of triacylglycerols and phospholipids, for energy supply through β-oxidation and for synthesis of eicosanoïds. Ten minutes after the death, the high proportions of saturated fatty acids and low proportions of PUFA could be a consequence of a rapid and preferential esterification of PUFA into phospholipids and triacylglycerols as compared to saturated fatty acids (Choy, Tran, Hatch & Kroeger, 1997; Entressangles, 1992; Merkl & Lands, 1963). Post-mortem, esterification of FFA into triacylglycerols and phospholipids and β-oxidation of FFA no longer occur because of a rapid depletion of ATP which is a cofactor required for FFA activation into acyl-coA before entering these metabolic pathways. Consequently, FFA arising from lipolysis accumulate in muscles causing both an increase in FFA amount and progressive changes in their fatty acid composition.

FFA were generated through the hydrolysis of both phospholipids and triacylglycerols. Most of the previous studies indicated that phospholipids were the main substrates of lipolysis in poultry and pig muscles (Buscailhon, Gandemer & Monin, 1994; Currie & Wolfe, 1977; Sklan et al., 1983). This conclusion established from glycolytic or intermediate muscles in pig and poultry muscles can not be extended to rabbit muscles, specially oxidative ones in which the contribution of triacylglycerols to lipolysis is significant.

As previously shown in dry-cured ham (Buscailhon et al., 1994) and in refrigerated poultry and pig meat (Currie & Wolfe, 1977; Flores et al., 1996; Rosell & Toldra, 1996; Sklan et al., 1983), the hydrolysis of phospholipids in rabbit muscles was established by the increase in the amount of long chain PUFA in FFA. Phospholipid hydrolysis is generally attributed to phospholipases and lysophospholipases. Up to now, very little is known about the activities of these enzymes in skeletal muscles. Recently, we have established that rabbit muscles contained both phospholipase A and lysophospholipase activities with basic optimum pH and that these enzymes retained more than 50% of their potential activity at the ultimate pH of muscles (5.5–6) (Alasnier & Gandemer, 1999). In the present study, the hydrolysis of triacylglycerols in rabbit muscles was demonstrated by the increase in the amounts of C14:0 and C16:1 in FFA and in the amount of monoacylglycerols during refrigerated storage. In previous studies, the hydrolysis of triacylglycerols was supported by the fact that fatty acid composition of FFA was intermediate between those of triacylglycerols and phospholipids (Sharma et al., 1982a,b; Sklan et al., 1983). Motilva, Toldra and Flores (1992) established that three triacylglycerol lipases are present in pig skeletal muscles and that their activities were detected at least 6 months during dry-cured ham processing. Even if very little is known on the post-mortem regulation of the activities of lipases and phospholipases in skeletal muscles, the present data strongly suggested that these enzymes remain active post-mortem and both contribute to FFA production.

4.2. Lipolysis and metabolic type of muscles

The present data indicated that FFA amount was lower in glycolytic muscles than in oxidative ones in rabbit. This result is consistent with those previously published on lipolysis in chicken and turkey which showed that FFA amount was lower in glycolytic muscles such as breast than in more oxidative ones such as thigh or leg muscles (Currie & Wolfe, 1977; Sklan et al., 1983). The higher amount of FFA in rabbit oxidative muscles was related to the higher amount of triacylglycerols in these muscles. Indeed oxidative muscles contained 7–8 times more triacylglycerols than glycolytic ones. So, even if the rate of triacylglycerol hydrolysis was lower in oxidative muscles than in glycolytic ones, the contribution of triacylglycerols to FFA production remained double in oxidative muscles than in glycolytic ones. Moreover, the rate of phospholipid hydrolysis and the amount of long chain PUFA

released from phospholipids were similar in the five muscles studied (exception the PM).

The proportion of triacylglycerols hydrolysed during the 7 days of refrigerated storage was lower in the oxidative muscles (SO and SM) than in the intermediate one (GL) and in the glycolytic ones (PM and LL) even if that in LL was not found significantly different from that of SO and SM. This estimation is consistent with the lower proportions of monoacylglycerols and diacylglycerols (expressed as % of triacylglycerol amount) in SO and SM than in GL, PM and LL after 7 days of storage (0.45–0.56% versus 0.81–1.65% for diacylglycerols and 0.02–0.03 versus 0.04–0.15% for monoacylglycerols). The difference in triacylglycerol hydrolysis rate between muscles is difficult to explain. We can postulate that the differences in triacylglycerol hydrolysis rates between oxidative and glycolytic muscles are not related to the activities of lipases in these muscles because it has been established that the activities of these enzymes were not related to the metabolic type (Flores et al., 1996; Hernandez, Navarro & Toldra, 1998). A possible explanation of the differences could be related to the location of triacylglycerols in oxidative and glycolytic muscles. Thus, triacylglycerols are located almost exclusively in droplets in cytosol of fibres in glycolytic muscles while they are mainly stored in adipose cells located along the fibres in the oxidative muscles (Cassens & Cooper, 1971; Kauffman & Safini, 1967). The hypothesis we can put forward is that intrafibre triacylglycerols could be more readily hydrolysed than triacylglycerols of adipose cells located between fibres because they are an in situ reserve of fatty acids quickly mobilised to provide fuel for β-oxidation to supply energy (Coppack, Jensen & Miles, 1994).

Conclusion

This study demonstrates that FFA increased during refrigerated storage and that both triacylglycerols and phospholipids contributed to FFA formation in rabbit muscles. The metabolic type clearly affected the FFA amount of muscles and oxidative muscles contained more FFA than glycolytic ones. This result is explained by the high triacylglycerol content of oxidative muscles as compared to glycolytic ones. Indeed the rate of phospholipid hydrolysis was close in all muscles while that of triacylglycerol hydrolysis was slightly higher in glycolytic muscles than in oxidative ones. Consequently, the amount of free long chain PUFA arising from phospholipid hydrolysis was found similar in both oxidative and glycolytic muscles. Considering that long chain PUFA are the main substrates of lipid oxidation in muscles (Gandemer, 1990) and FFA are suspected to be more sensitive to lipid oxidation than esterified ones (Nawar, 1996), lipolysis provided similar amount of highly sensitive substrates to lipid oxidation in both oxidative and glycolytic muscles. Hence, our results strongly suggest that lipolysis should not be responsible for the higher sensitivity of oxidative muscles to oxidation.

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