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Comparison of the Phospholipid and Triacylglycerol Fatty Acid Profile of Rat Serum, Skeletal Muscle and Heart

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Summary

Although several studies have analyzed the fatty acid profile of phospholipids (PL) and, to a lesser degree, triacylglycerols (TG) in one or more tissues concurrently, a systematic comparison of the fatty acid composition of different tissues and/or lipid classes is lacking. The purpose of the present study was to compare the fatty acid composition of major lipid classes (PL and TG) in the rat serum, soleus muscle, extensor digitorum longus muscle and the heart. Lipids were extracted from these tissues and analyzed by a combination of thin-layer chromatography and gas chromatography. We found many significant differences in various tissues and lipid classes. Serum had the most distinct fatty acid profile in PL but this "uniqueness" was less apparent in TG, where differences among tissues were in general less frequent than in PL. These two skeletal muscles exhibited similar fatty acid composition in both lipid classes despite their different muscle fiber type composition, denoting that fiber type is not a major determinant of the fatty acid composition of rat skeletal muscle. The fatty acid profile of heart PL was the most different from that of the other tissues examined. PL were rich in polyunsaturated fatty acids, whereas TG were rich in monounsaturated fatty acids. Although the reasons for the differences in fatty acid profile among the tissues examined are largely unknown, it is likely that these differences have an impact upon numerous biological functions.

Key words
Fatty acid composition • Lipid metabolism

Introduction

In recent years a number of studies have addressed the effects of various stimuli (e.g. diet and exercise) on the fatty acid composition of animal and human tissues (reviewed by Bas and Morand-Fehr 2000 and Nikolaidis and Mougios 2004). This was probably motivated by partial unraveling the role of individual fatty acids in animal and human biology. Most of the relevant studies have analyzed the fatty acid profile of phospholipids (PL) and triacylglycerols (TG). The focus on these lipid classes is justified by the effect that their fatty acid profile may exert on physiological function. For example, the proportion of polyunsaturated fatty acids (PUFA) in PL positively correlated with cellular metabolic activity, whereas the level of unsaturation in PL of animals positively correlated with the lifespan (Hulbert 2003). Furthermore, infusion of glucose to rats

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for 5 days (a condition that causes insulin resistance in skeletal muscle) decreased the percentage of stearate (18:0) and increased that of palmitoleate (16:1ω7) in the TG fraction of skeletal muscle by more than twofold compared to the untreated rats (Houdali *et al.* 2003).

Several studies have analyzed the fatty acid profile of PL and, to a lesser degree, TG in one or more tissues concurrently (Andersson *et al.* 1998, Ayre *et al.* 1998, Gutiérrez *et al.* 2002, Pan and Storlien 1993). However, most of these studies have not performed a rigorous comparison (i.e. through statistical analysis) of the fatty acid composition of the different tissues and/or lipid classes. Additionally, their discussion of the relevant issues was rather fragmentary, probably because their main interest lay in the effects of the treatment such as diet (e.g. Pan and Storlien 1993) or exercise (e.g. Andersson *et al.* 1998) on the fatty acid composition. We believe that ignoring possible differences in the fatty acid profile of tissues or lipid classes might prevent the disclosure of important implications for cellular metabolism.

Therefore, the purpose of the present study was to compare statistically the fatty acid composition of the major lipid classes (PL and TG) in rat serum, skeletal muscle and heart. Since different skeletal muscle fiber types exhibit markedly different phenotypes, we analyzed two muscles lying at opposite ends of the fiber type spectrum, i.e. the soleus, consisting of oxidative fibers by 87 % and oxidative glycolytic fibers by 13 %, and the extensor digitorum longus (EDL), consisting of oxidative fibers by 2 %, oxidative glycolytic fibers by 42 % and glycolytic fibers by 56 % (Armstrong and Phelps 1984). By using these muscles we attempted to ascertain whether the different functional properties of skeletal muscles are linked to the underlying structural foundation related to their fatty acid composition.

Methods

Animals

Fourteen male Wistar rats were purchased from Charles River Laboratories (Sulzfeld, Germany) and were housed under controlled environmental conditions (21 °C, 12:12-h light-dark cycle) for two months. The rats had free access to water and standard rodent chow from Ssniff (Soest, Germany). The main dietary fatty acids were palmitate (16:0), oleate (18:1ω9) and linoleate (18:2ω6), accounting for 89 % of the total fatty acid intake (28, 21, and 40 %, respectively) (Nikolaidis *et al.* 2004). The rats were maintained according to the European Union guidelines for the care and use of laboratory animals.

Specimen collection

The animals were decapitated at the age of 15 weeks after a 6-h fast. Blood was collected promptly and left to clot at room temperature. The soleus and EDL of the right hindlimb, as well as the heart (without the great vessels), were then removed as fast as possible. The tissues were deprived of visible fat, nerves and fasciae, and were immersed in liquid nitrogen immediately. Subsequently, they were pulverized with mortar and pestle and stored at –80 °C. Upon clotting, the blood was centrifuged at 1500×*g* for 10 min. Serum was separated and also stored at –80 °C.

Fatty acid analysis

The fatty acid composition of the specimens was determined by a combination of thin-layer chromatography (TLC) and gas chromatography (GC). 0.5 ml of serum or 30 mg of tissue powder were mixed with 2.5 ml of 2-propanol: heptane: 0.5 M H_2SO_4, 40:10:1 (v/v/v), after the addition of diheptadecanoyl phosphatidylcholine and triheptadecanoyl glycerol (both from Sigma, St. Louis, MO, USA) as internal standards of PL and TG, respectively. After 10 min 1 ml of heptane and 1.5 ml of water were added, and the mixture was stirred vigorously in order to afford extraction of the lipids (Dole 1956).

The upper layer was then removed, condensed under a stream of nitrogen, and applied onto silica gel G TLC plates (Sigma no. Z12,277-7). The plates were developed with petroleum ether:diethyl ether:acetic acid, 130:20:1.5 (v/v/v), and lipid spots were located under ultraviolet light after spraying with a solution of 0.2 % dichlorofluorescein in ethanol. The spots corresponding to PL and TG were excised separately and incubated in 0.5 ml of methanolic sodium methoxide (Sigma) at 50 °C for 10 min. Then 0.5 ml of boron trifluoride (Fluka, Buchs, Switzerland) was added and incubation was repeated as before (Kramer *et al.* 1997). The fatty acid methyl esters thus produced were extracted with 1.5 ml of hexane and separated in a Hewlett Packard 5890 Series II gas chromatograph (Waldbronn, Germany) equipped with a 30-m long AT-WAX capillary column from Alltech (Deerfield, IL, USA) and a flame ionization detector. The column temperature was programmed from 160 to 250 °C at 5 °C/min. The carrier gas was helium at a flow rate of

1 ml/min (at 160 °C). Methyl esters of individual fatty acids were identified in the chromatograms by comparing their retention times to those of pure methyl esters purchased from Sigma and were quantified by comparing the area under their peaks to that of methyl heptadecanoate (derived from the internal standards) with the aid of the HP 3365 ChemStation software from Hewlett Packard.

Calculations and statistics

We have calculated the following indices of the fatty acid profile of PL and TG in each tissue: monounsaturated fatty acids (MUFA), PUFA, ω6 fatty acids, ω3 fatty acids, ω6/ω3, unsaturated-to-saturated ratio (U/S) and unsaturation index (UI; the average number of double bonds per fatty acid multiplied by 100). Additionally, we estimated fatty acid elongase and Δ^5-, Δ^6- as well as Δ^9-desaturase activities in the skeletal muscles and the heart through appropriate product-to-precursor ratios. These were 18:0/16:0 for elongase, 20:4ω6/20:3ω6 for Δ^5-desaturase, 18:3ω6/18:2ω6 for Δ^6-desaturase and 18:1ω9/18:0 for Δ^9-desaturase. The ratios were calculated from the sum of the concentrations of each fatty acid in PL and TG.

Values are expressed as means ± SD. The distribution of all dependent variables was examined by the Kolmogorov-Smirnov test and was found not to differ significantly from normal. Differences among tissues (serum taken as a tissue at large) and between lipid classes were examined by two-way ANOVA (tissue × lipid class) with repeated measures on both factors. *Post-hoc* pairwise comparisons were performed by simple main effect analysis. The level of statistical significance was set at $\alpha = 0.05$. The SPSS version 10.0 (SPSS Inc., Chicago, IL) was used for all analyses. Part of the descriptive statistics reported in this study had been presented in a paper regarding the effects of exercise on the fatty acid composition of tissues (Nikolaidis *et al.* 2004).

Results

Fifteen fatty acids were detected in considerable amounts by gas chromatography, namely, myristate (14:0), 16:0, 16:1ω7, 18:0, 18:1ω9, *cis*-vaccenate (18:1ω7), 18:2ω6, γ-linolenate (18:3ω6), α-linolenate (18:3ω3), gondoate (20:1ω9), dihomo-γ-linolenate (20:3ω6), arachidonate (20:4ω6), eicosapentaenoate (20:5ω3), docosapentaenoate (22:5ω3) and docosahexaenoate (22:6ω3).

The fatty acid profiles of PL and TG in the tissues examined are presented in Table 1. U/S and UI are plotted in Figure 1, while the estimated activities of enzymes involved in fatty acid metabolism are shown in Figure 2. We found many differences either across tissues or between lipid classes. Briefly, serum exhibited the most distinct fatty acid profile in PL but not in TG. The two skeletal muscles exhibited similar PL and TG fatty acid composition, whereas the fatty acid profile of heart PL was the most different from that of the other tissues examined. On the other hand, the fatty acid profile of heart TG resembled that of the other tissues.

Discussion

The purpose of the present study was to make every possible meaningful comparison of fatty acid composition across four rat tissues and two lipid classes. In this context, we have provided data on the fatty acid composition of the major lipid classes (PL and TG) in some very frequently examined tissues (serum, skeletal muscle and heart), especially in the fields of nutrition and exercise physiology. Regarding TG in particular, we are not aware of any study that compared their fatty acid profile across tissues or with the fatty acid profile of PL in any tissue. Our comparisons produced several relevant results, which are discussed below.

Fatty acid profile of serum

Serum had the most distinct fatty acid profile in PL. However, this "uniqueness" faded in TG, where differences among tissues were, in general, considerably fewer than in PL.

Fatty acid profile of skeletal muscle

Although there were several significant differences in the fatty acid profile of soleus and EDL in both PL and TG, these were less frequent and smaller than the differences between either of the two muscles and serum or the heart. This can be verified by an examination of the percentages of the major fatty acids 16:0, 18:0, 18:1ω9, 18:2ω6 and 20:4ω6 in Table 1. In general, the two skeletal muscles exhibited similar PL and TG fatty acid composition despite representing the two extremes of the muscle fiber type spectrum. This denotes that muscle fiber type is not a major determinant of the fatty acid composition of skeletal muscle.

In our opinion, the relatively similar fatty acid

Table 1. Molar percentage distribution of individual fatty acids and indices of the fatty acid profile in rat serum, skeletal muscle and heart phospholipids and triacylglycerols (means ± SD)

Fatty acid	Phospholipids				Triacylglycerols			
	Serum	Soleus	EDL	Heart	Serum	Soleus	EDL	Heart
14:0	0.18 ± 0.06^{cd}*	0.28 ± 0.10^{d}*	0.29 ± 0.09^{ad}*	0.09 ± 0.02^{abc}*	0.89 ± 0.21^{bc}	2.43 ± 0.29^{acd}	1.41 ± 0.37^{ab}	1.28 ± 0.50^{b}
16:0	19.04 ± 1.05^{bcd}*	10.08 ± 1.05^{acd}*	14.24 ± 0.93^{abd}*	6.97 ± 0.28^{abc}*	32.70 ± 1.67^{bc}	28.81 ± 1.28^{ac}	30.38 ± 1.77^{ab}	30.09 ± 5.29
16:1ω7	0.54 ± 0.18^{b}*	0.86 ± 0.24^{acd}*	0.59 ± 0.14^{bd}*	0.45 ± 0.15^{bc}*	3.87 ± 1.58^{bc}	8.42 ± 2.87^{ad}	9.17 ± 2.73^{ad}	5.00 ± 2.35^{bc}
18:0	23.32 ± 1.52^{bd}*	21.62 ± 1.89^{ad}*	22.08 ± 1.52^{d}*	18.40 ± 0.84^{abc}*	2.34 ± 0.41^{bcd}	3.22 ± 0.44^{acd}	5.35 ± 2.41^{ab}	6.94 ± 2.04^{ab}
18:1ω9	4.37 ± 0.57^{bcd}*	3.78 ± 0.47^{ad}*	3.77 ± 0.40^{ad}*	2.41 ± 0.46^{abc}*	19.36 ± 2.84^{c}	20.84 ± 1.37^{c}	23.01 ± 2.12^{ab}	24.40 ± 10.12
18:1ω7	4.19 ± 0.70^{bcd}	3.44 ± 0.25^{a}	3.36 ± 0.24^{ad}*	3.52 ± 0.26^{ac}	3.95 ± 0.64	3.58 ± 1.01	4.23 ± 0.30	3.78 ± 0.83
18:2ω6	19.68 ± 2.10^{bcd}*	34.41 ± 2.06^{acd}*	29.23 ± 1.76^{abd}*	46.99 ± 1.89^{abc}*	30.57 ± 3.94^{cd}	28.57 ± 3.89^{c}	22.19 ± 4.05^{ab}	24.30 ± 8.58^{a}
18:3ω6	0.60 ± 0.06^{bcd}*	0.35 ± 0.04^{ad}*	0.40 ± 0.09^{ad}	0.24 ± 0.03^{abc}*	0.47 ± 0.15^{b}	0.23 ± 0.08^{ad}	1.22 ± 1.51	0.83 ± 0.52^{b}
18:3ω3	0.11 ± 0.04^{bcd}*	0.26 ± 0.04^{a}*	0.26 ± 0.05^{a}*	0.23 ± 0.03^{a}*	1.20 ± 0.43^{b}	1.76 ± 0.22^{acd}	1.16 ± 0.24^{b}	0.94 ± 0.46^{b}
20:1ω9	0.43 ± 0.11^{bcd}*	0.17 ± 0.04^{ad}*	0.20 ± 0.07^{ad}*	0.10 ± 0.03^{abc}*	0.67 ± 0.32^{b}	0.28 ± 0.06^{ad}	0.48 ± 0.37	0.45 ± 0.17^{b}
20:3ω6	0.96 ± 0.26^{bcd}*	0.58 ± 0.10^{ad}*	0.57 ± 0.06^{ad}	0.30 ± 0.04^{abc}*	0.28 ± 0.07^{bd}	0.14 ± 0.07^{a}	ND	0.15 ± 0.07^{a}
20:4ω6	22.27 ± 2.30^{bcd}*	11.04 ± 1.25^{acd}*	9.88 ± 0.81^{abd}*	13.63 ± 0.76^{abc}*	2.07 ± 0.63^{bd}	1.22 ± 0.28^{a}	1.40 ± 0.63	1.35 ± 0.66^{a}
20:5ω3	0.21 ± 0.07^{cd}*	0.20 ± 0.10^{d}*	0.14 ± 0.03^{ad}	0.09 ± 0.03^{abc}	0.52 ± 0.17^{bd}	0.09 ± 0.05^{a}	ND	0.09 ± 0.13^{a}
22:5ω3	1.05 ± 0.22^{bc}*	3.07 ± 0.25^{acd}*	3.33 ± 0.29^{abd}	1.28 ± 0.38^{bc}*	0.43 ± 0.17^{bd}	0.19 ± 0.07^{a}	ND	0.19 ± 0.08^{a}
22:6ω3	3.04 ± 0.42^{bcd}*	9.88 ± 1.18^{acd}*	11.67 ± 0.85^{abd}	5.30 ± 1.46^{abc}*	0.68 ± 0.30^{bd}	0.21 ± 0.07^{a}	ND	0.23 ± 0.13^{a}
Sum	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
				Indices				
MUFA	9.5 ± 1.2^{bcd}*	8.2 ± 0.8^{ad}*	7.9 ± 0.6^{ad}*	6.5 ± 0.8^{abc}*	27.8 ± 4.8^{bc}	33.1 ± 3.7^{ac}	36.9 ± 4.3^{ab}	33.6 ± 11.5
PUFA	47.9 ± 1.3^{bcd}*	59.8 ± 2.7^{acd}*	55.5 ± 1.9^{abd}*	68.1 ± 0.8^{abc}*	36.2 ± 4.7^{bcd}	32.4 ± 4.2^{ac}	26.0 ± 3.4^{ab}	28.1 ± 8.7^{a}
ω6	43.5 ± 1.7^{bcd}*	46.4 ± 2.0^{acd}*	40.1 ± 1.7^{abd}*	61.2 ± 1.5^{abc}*	33.4 ± 4.4^{bcd}	30.2 ± 4.0^{ac}	24.8 ± 3.4^{ab}	26.6 ± 8.3^{a}
ω3	4.4 ± 0.6^{bcd}*	13.4 ± 1.3^{acd}*	15.4 ± 0.9^{abd}*	6.9 ± 1.7^{abc}*	2.8 ± 0.9^{cd}	2.3 ± 0.3^{cd}	1.2 ± 0.2^{ab}	1.5 ± 0.5^{ab}
ω6/ω3	10.0 ± 1.6^{bc}*	3.5 ± 0.3^{acd}*	2.6 ± 0.2^{abd}*	9.5 ± 2.9^{bc}*	13.2 ± 4.8^{c}	13.5 ± 1.9^{cd}	22.1 ± 4.6^{ab}	18.6 ± 4.5^{b}

EDL, extensor digitorum longus; MUFA, monounsaturated fatty acids; ND, not detected; PUFA, polyunsaturated fatty acids. a, b, c, d: Significantly different from serum, soleus, EDL, and heart, respectively, in the same lipid class ($P<0.05$). * Significantly different from TG in the same tissue ($P<0.05$).

pattern of soleus and EDL is a specific characteristic of skeletal muscle. In fact, differences in the fatty acid profile between muscles with divergent physiological characteristics are much smaller than the differences in the concentrations of their phosphagens, carbohydrates or enzymes. This may be one of the reasons why the changes that chronic exercise induces on the fatty acid composition of different skeletal muscles are rather similar (Helge *et al.* 1999, Nikolaidis *et al.* 2004, Petridou *et al.* 2005).

Fatty acid profile of the heart

The fatty acid profile of heart PL was the most different from that of the other tissues examined. It is interesting to note that 18:2ω6, the major dietary fatty acid, constituted almost one half of the total fatty acids in heart PL (scoring the highest value in the present study). This is probably due to the high cardiolipin content of the heart, since cardiolipin is rich in 18:2ω6 (Hoch 1992). This notion is supported by the fact that the abundance of 18:2ω6 followed the abundance of mitochondria (where cardiolipin resides) in the three tissues (heart > soleus > EDL). On the other hand, the fatty acid profile of heart TG resembled that of EDL more than that of serum or soleus.

Fatty acid profile across tissues

The principal observation emerging from the comparison of the fatty acid composition of the four tissues is their quite similar TG profile. It seems that there are no considerable differences in fatty acid selectivity as far as TG biosynthesis and/or degradation are concerned

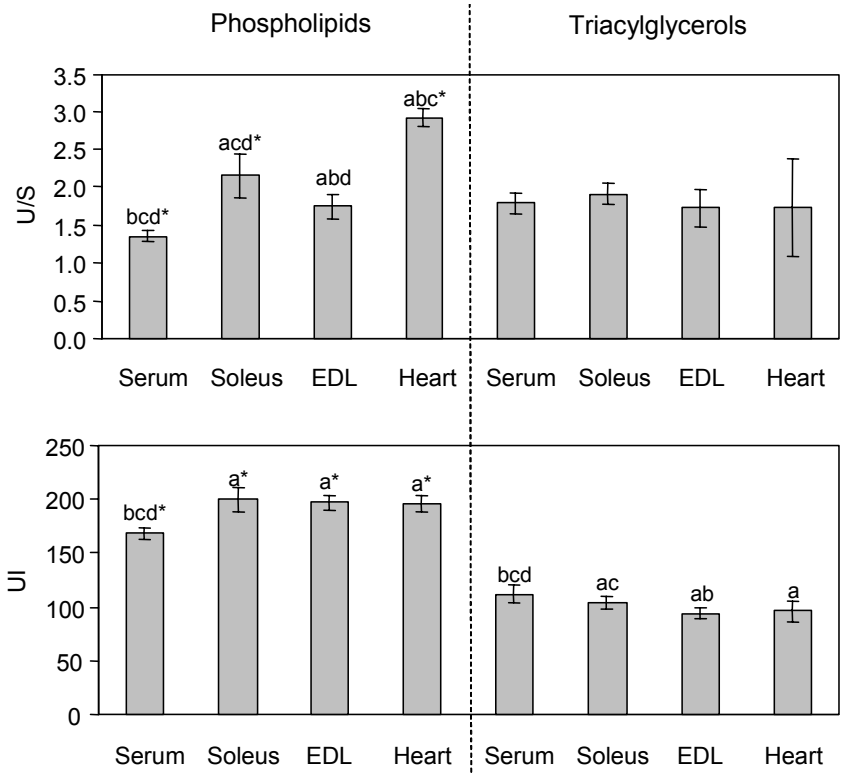

Fig. 1. Unsaturated-to-saturated fatty acid ratio (U/S) and unsaturation index (UI; the average number of double bonds per fatty acid multiplied by 100) in rat serum, skeletal muscles and heart lipids (means ± SD). a, b, c, d: Significantly different from serum, soleus, EDL, and heart, respectively in the same lipid class ($P<0.05$). * Significantly different from triacylglycerols in the same tissue ($P<0.05$).

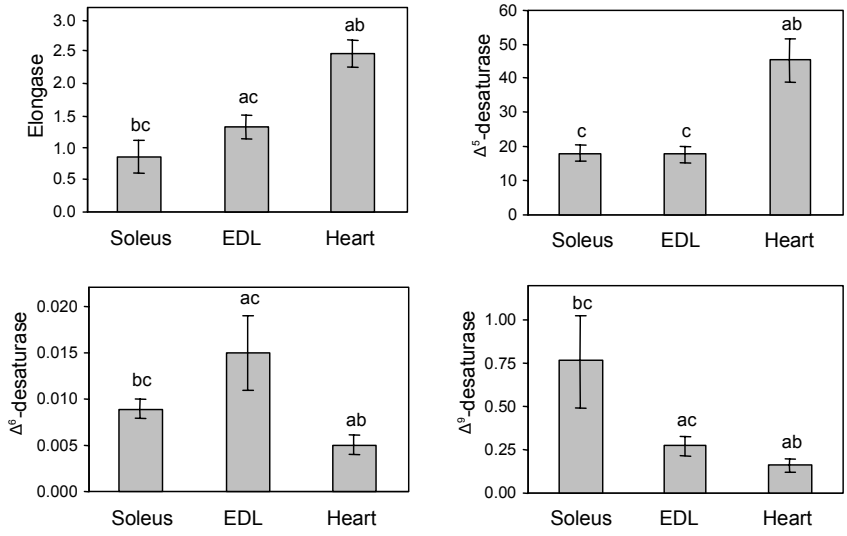

Fig. 2. Estimated fatty acid elongase and Δ^5-, Δ^6- as well as Δ^9-desaturase activities in the skeletal muscles and the heart (means ± SD). Enzyme activities were calculated through appropriate product-to-precursor ratios (18:0/16:0 for elongase, 20:4ω6/20:3ω6 for Δ^5-desaturase, 18:3ω6/18:2ω6 for Δ^6-desaturase, and 18:1ω9/18:0 for Δ^9-desaturase). The ratios were calculated from the sum of the concentrations of each fatty acid in PL and TG. a, b, c: Significantly different from soleus, EDL, and heart, respectively ($P<0.05$).

in the tissues studied. On the other hand, a more stringent control upon the fatty acid profile of PL seems to be exerted at the tissue level, as evidenced by the larger number of differences found across tissues.

Comparison of the fatty acid profile of PL and TG

Three highly unsaturated fatty acids (20:4ω6, 22:5ω3 and 22:6ω3) were characteristic for PL, amounting to 20-26 % of total fatty acids, as opposed to only 1-3 % in TG. On the other hand, the fatty acid pattern of TG was marked by two MUFA, 16:1ω7 and 18:1ω9, constituting 23-32 % of total fatty acids, as opposed to only 3-5 % in PL. A point that merits attention is the relatively high values of 16:1ω7 (a rather unappreciated fatty acid) in TG of both skeletal muscles, which renders its measurement necessary for a satisfactory assessment of their fatty acid profile. The much higher proportion of MUFA in TG compared to PL is consistently found across all tissues studied and is well conserved in various species, including hummingbird (Infante *et al.* 2001), rattlesnake (Infante *et al.* 2001), dog (van der Vusse and Roemen 1995), pig (Andrés *et al.*

2001) and man (Andersson *et al.* 1998).

Unsaturation state: a matter of the index chosen

We have used two measures of unsaturation, U/S and UI, the former considering all unsaturated fatty acids equally, the latter taking into account the degree of unsaturation of each. These two indices produced a different image of unsaturation in some cases. For example, while the UI of PL was very similar in the two skeletal muscles and the heart (ranging from 196 to 200), U/S was quite different (ranging from 1.74 to 2.93). Therefore, depending on the index chosen, it can be stated that the degree of unsaturation in the PL of these tissues is almost the same or that the heart has by far the most unsaturated profile.

Divergent results, depending on the measure of unsaturation selected, also emanate when one compares PL and TG in the skeletal muscles. The UI of PL was almost twofold higher than that of TG, in accordance with findings in red quadriceps rat muscles (Pan and Storlien 1993). However, the difference in U/S was much smaller. This is mainly because skeletal muscle PL are rich in highly unsaturated fatty acids (like 20:4ω6 and 22:6ω3), which make a great contribution to UI.

The two cases discussed above demonstrate the limitations of the formulas used to reveal the unsaturation state; they also demonstrate the need to calculate both indices in relevant studies. These discrepancies indicate that unsaturation is slightly higher in oxidative than glycolytic skeletal muscles; this has often been found by others (Ayre and Hulbert 1997, Kriketos *et al.* 1995). Again, we believe that this is, at least partly, due to the higher mitochondrial content of oxidative muscles.

What mechanisms cause the differences in fatty acid profile?

At present we have no indication as to the mechanism causing the observed differences in fatty acid profile among different tissues. Possibilities include altered rates and/or selectivities in 1) fatty acid transport across cellular membranes, 2) fatty acid release through TG and PL hydrolysis, 3) fatty acid oxidation, 4) lipid biosynthesis, and 5) deacylation-reacylation cycle. These alterations may be due to changes in the activity and/or expression of the proteins involved.

In summary, we have found many significant differences among the fatty acid profiles of phiospholipids and triglycerides in rat serum, soleus, extensor digitorum longus and heart. Although the two skeletal muscles examined had quite different muscle fiber type composition, they generally exhibited a similar fatty acid profile, denoting that fiber type is not a major determinant of the fatty acid composition of rat skeletal muscle. PL were rich in highly unsaturated fatty acids, whereas TG were rich in MUFA. Additionally, we showed that estimating the unsaturation state of a tissue through two of the most frequently used indices can produce variable results; this underlines the need to calculate both in similar experiments. Although the reasons for the differences in fatty acid profile among the tissues and between lipid classes examined are not known, it is likely that these differences have an influence upon a range of biological functions.

The present findings need verification and extension. In particular, the overlooked fatty acid profile of TG needs to be intensively studied under several experimental conditions, especially in view of recent studies relating it to insulin resistance (Houdali *et al.* 2003, Lessard *et al.* 2004).

Acknowledgements

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