

Impact of a Standard Rodent Chow Diet on Tissue n-6 Fatty Acids, $\Delta 9$ -Desaturation Index, and Plasmalogen Mass in Rats Fed for One Year

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Abstract Although many studies focus on senescence mechanisms, few habitually consider age as a biological parameter. Considering the effect of interactions between food and age on metabolism, here we depict the lipid framework of 12 tissues isolated from Sprague–Dawley rats fed standard rodent chow over 1 year, an age below which animals are commonly studied. The aim is to define relevant markers of lipid metabolism influenced by age in performing a fatty acid (FA) and dimethylacetal profile from total lipids. First, our results confirm impregnation of adipose and muscular tissues with medium-chain FA derived from maternal milk during early infancy. Secondly, when animals were switched to standard croquettes, tissues were remarkably enriched in n-6 FA and especially 18:2n-6. This impregnation over time was coupled with a decrease of the desaturation index and correlated with lower activities of hepatic $\Delta 5$ - and $\Delta 6$ -desaturases. In parallel, we emphasize the singular status of testis, where 22:5n-6, 24:4n-6, and 24:5n-6 were exceptionally accumulated with growth. Thirdly, 18:1n-7, usually found as a discrete FA, greatly

accrued over the course of time, mostly in liver and coupled with $\Delta 9$ -desaturase expression. Fourthly, skeletal muscle was characterized by a surprising enrichment of 22:6n-3 in adults, which tended to decline in older rats. Finally, plasmalogen-derived dimethylacetals were specifically abundant in brain, erythrocytes, lung, and heart. Most notably, a shift in the fatty aldehyde moiety was observed, especially in brain and erythrocytes, implying that red blood cell analysis could be a good indicator of brain plasmalogens.

Keywords Age · Desaturase · Medium-chain fatty acid · 18:2n-6 (linoleic acid) · 18:1n-7 (*cis*-vaccenic acid) · Plasmalogen · Liver · Testis · Skeletal muscle · Rat

Abbreviations

FA	Fatty acid
8d	8 days old
3w	3 weeks old
4m	4 months old
1y	1 year old
RBC	Red blood cells
WAT	White adipose tissue
BAT	Brown adipose tissue
MD	Methylated derivative
FAME	Fatty acid methyl ester
DMA	Dimethylacetal
GC-MS	Gas chromatography–mass spectrometry
MCFA	Medium-chain fatty acid
MUFA	Monounsaturated fatty acid

Introduction

Ageing is characterized by a progressive decline in organism functions at cellular, tissue, and organ level, which

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over time can generate multiple diseases [1–4]. Molecular mechanisms are established gradually over the course of time and are not taken into account in research projects when ageing effects are not included in the aim of the work. In nutritional studies, particularly when experiments are focused on lipid metabolism, work is usually performed on animals whose age varies depending on the study. Comparison between nutritional trials is already difficult, as experimental conditions differ in terms of time, control diet, animal model, etc. The impact of age on basal metabolism may also be considered as conclusive as far as nutrient effects are concerned, in relation with the nutritional context of the lifespan of animals.

Fatty acids (FA), and particularly polyunsaturated fatty acids (PUFA), are bioactive lipids that display pleiotropic functions themselves [5, 6] or as derivatives such as eicosanoids [7–9], docosanoids [10–12], linotriins [13], isoprostanes or phytyloprostanols [14]. They can be either generated by *de novo* lipogenesis or supplied from food, so FA profiles reflect the balance between diet and biosynthesis potential, in predefined conditions of nutrition and physiology. Nutritional imbalance between saturated and unsaturated FA or between n-6 and n-3 FA has been subject to various studies, mainly based on customized diets. The results are particularly important because nutritional recommendations include data obtained from such animal experiments in addition to epidemiological trials. Considering the effect of the interaction between food and age on metabolism, study of the lipid profile of rat tissues is appropriate, since little is known about the basic life of rat models commonly used in experiments. In the present study, the lipid background of the Sprague–Dawley strain, fed successively with maternal milk and standard rodent chows, was characterized over 1 year. The results focus on the expression and activity of hepatic enzymes coupled with the FA profiles of 12 tissues selected to provide an overview of metabolism at organism scale. In addition, plasmalogen-derived dimethylacetals were analyzed, as plasmalogens represent potential markers of pathologies such as neurological diseases or inflammatory disorders [15]. These particular etherphospholipids are also known as endogenous antioxidants and should be of research interest, considering that they are active components of membrane dynamics and reservoirs of PUFA, lipid mediators, and second messengers [16].

Materials and Methods

Materials

Chemicals were provided by Sigma–Aldrich (Saint-Quentin Fallavier, France), and solvents were purchased from Fisher Scientific (Elancourt, France).

Animals and Diets

All protocols complied with the European Union Guideline for animal care and use (2010/63/CEE). Male Sprague–Dawley rats were purchased from Janvier Labs breeding center (Le Genest-Saint-Isle, France). They were studied at different ages: 8 days after birth (8d), after weaning corresponding to 3 weeks old (3w), 4 months old (4m), and 1 year old (1y). Animals had access to water and food *ad libitum*. Mothers were fed with a breeding diet (Ssniff[®], Bio Services BV, Uden, The Netherlands), while adult rats were fed with a maintenance diet [Special Diet Services (SDS), Augy, France]. The breeding diet from Ssniff[®] was composed of 5.1 % fat, 18.0 % protein, 53.3 % carbohydrate, and 4.3 % fiber. The maintenance diet from SDS consisted of 3.2 % fat, 17.9 % protein, 57.5 % carbohydrate, and 3.9 % fiber. Both diets were supplemented with a similar mix of minerals and vitamins. After an overnight fast (except for 8d), rats were anesthetized by intraperitoneal injection of pentobarbital (90 mg/kg of Euthasol Vet, France). Animals were weighed (17.2 ± 1.4 g for 8d, 68.3 ± 0.6 g for 3w, 566.7 ± 16.8 g for 4m, 777.7 ± 97.4 g for 1y), and blood was collected in heparin tubes by cardiac puncture. Plasma was separated from red blood cells (RBC) after centrifugation (15 min, 15 °C). The other organs were sampled, snap-frozen, and stored at -80 °C until analysis, except for the liver, which was partly used fresh for enzymatic assays. White adipose tissue (WAT) was subcutaneous; skeletal muscle was removed from the hip muscles of hind legs. Maternal milk was extracted from the mammary glands at 8 days and 3 weeks by the breeding center (Janvier Labs).

Lipid Analyses

Lipids from tissues, rodent chow, and milk were extracted according to the Folch method. They were saponified with 0.5 M NaOH in methanol at 70 °C for 20 min and methylated with BF₃ (14 % in methanol) at 70 °C for 15 min. Fatty acid methyl esters (FAME) and dimethylacetals (DMA) were extracted with pentane and separated by 7890 N Agilent GC equipped with a BPX70 capillary column (60 m × 0.25 mm; 0.2 μm film thickness, SGE, Milton Keynes, UK) and coupled to a 5975C MS (Agilent Technologies, Les Ulis, France). Helium was used as carrier gas at constant velocity of 24 cm/s. The column temperature ramped from 150 to 200 °C at 2 °C/min. The mass spectrometer was operated under electron ionization at 70 eV and 230 °C source temperature. Analyses were performed in scan mode over the *m/z* range of 50–550. Agilent MSD ChemStation software was used for data acquisition. Components were identified according to the retention time of authentic FAME standards and by using the National

Institute of Standards and Technology (NIST) mass spectral library (version 2.01). FAME results are expressed in mole % of total identified FA. Plasmalogen-derived fatty aldehyde dimethylacetals (DMA) were quantified in mmol/mg of tissue and in mass % of total identified methylated derivatives (MD, i.e., DMA and FAME). FA and DMA concentrations were calculated by using a 17:0 methyl ester as standard.

Triacylglycerols (TAG) and total cholesterol were quantified in duplicates from rat plasma and liver by using a Biomérieux kit (Craponne, France). The livers were homogenized in phosphate-buffered saline, and the protein concentration was determined by Bradford assay (Bio-Rad, Marnes La Coquette, France).

mRNA Relative Quantification

Total RNA was extracted from the liver with Trizol[®] (Life Technologies, Saint-Aubin, France) and retrotranscribed by using the high-capacity cDNA RT kit (Applied, Fisher, Illkirch, France). Real-time polymerase chain reaction (PCR) of *Fads* (Taqman[®]) was performed as described elsewhere [17]. *Scd1* was amplified by using primers and FAM/TAMRA probe as mentioned in Table 1. Amplification of *PPAR α* , *Fas*, and *SREBP-1c* was performed with 300 nM primers (Table 1) and run with SsoFast EvaGreen supermix (Biorad) as follows: 3 min at 95 °C, 40 cycles of 10 s at 95 °C, and 30 s at 55 °C. mRNA expression was evaluated as delta Cycle threshold ($\Delta\text{Ct} = \text{Ct}_{\text{gene}} - \text{Ct}_{18\text{S}}$) with a 100 % efficacy checked by using a standard curve. Results obtained from triplicates per animal are presented in arbitrary units from $2^{\Delta\text{Ct}}$ calculation.

Desaturase Expression

Desaturase expression was estimated by Western blot after sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) on 40 μg ($\Delta 5/\Delta 6$) or 30 μg ($\Delta 9$)

tissue extracts. Rat $\Delta 5$ -desaturase was visualized with a polyclonal antibody (anti- $\Delta 5\text{D}$) targeting the specific ¹⁰⁰QSSFEPKTKNKALTDE peptide and produced in a rabbit respecting a 28-day immunization protocol (Eurogentec, Angers, France). Rat $\Delta 6$ -desaturase was detected with a polyclonal antibody (anti- $\Delta 6\text{D}$) which recognizes the N terminal sequence of the protein (³AGGNQGEGL-TEL) and produced in 10-week immunized rabbits (DB-BioRun, Nantes, France). Both antibodies were purified by affinity chromatography (Eurogentec) and incubated overnight at 4 °C at 1 $\mu\text{g}/\text{mL}$ in Tris buffer saline (20 mM Tris-HCl, 150 mM NaCl, pH 7.4) containing 0.05 % Tween-20 and 5 % bovine serum albumin. The SCD1 antibody produced against the $\Delta 9$ -desaturase was from Santa Cruz Biotechnology (Heidelberg, Germany) and was used in the same conditions. Primary antibodies were coupled to horseradish peroxidase-conjugated anti-IgG, and the peroxidase activity was determined by chemiluminescent detection using Immobilon reagents (Millipore, Molsheim, France). The apparent molecular mass of proteins was determined using a standard curve constructed with the Kaleidoscope marker (Bio-Rad, Marnes La Coquette, France) in 10 % SDS-PAGE. The relative expression of proteins was evaluated by measuring the intensity of bands using ImageJ (nih.gov).

$\Delta 5$ - and $\Delta 6$ -Desaturase Activity Assays

The liver was fractioned in a phosphate buffer containing 0.25 M sucrose to recover the post-mitochondrial supernatant as previously described [18]. Enzymatic activities were assayed in duplicates with different substrates: [1-¹⁴C]20:3n-6 for $\Delta 5$ -desaturase and [1-¹⁴C]18:1n-9, [1-¹⁴C]18:2n-6, and [1-¹⁴C]18:3n-3 for $\Delta 6$ -desaturase (60 $\mu\text{mol}/\text{L}$, 10 mCi/mmol). Fatty acids were separated by high-performance liquid chromatography (HPLC) and quantified after scintillation counting.

Statistics

Results are expressed as mean \pm standard deviation (SD) as mentioned in the legends. At least three animals per group were compared by nonparametric tests for lipid profiles, using one-way analysis of variance (Kruskal–Wallis) followed by multiple comparisons with Dunn's test. For gene expressions, desaturase activities, and lipid dosages, a Kolmogorov–Smirnov test of normality was performed and the effect of age was assessed by analysis of variance (ANOVA) with a post hoc Tukey's test. Differences were considered significant at $p < 0.05$. Statistical analysis was performed by using PRISM (GraphPad 6, San Diego, CA).

Table 1 RT-PCR primers

	Gene	Forward primer	Reverse primer
Taqman [®]	<i>Scd1</i>	caaagagacgggag-gaacgc (5'FAM/TAMRA3' probe ctggcctctgctcatgtgcttca)	aaagttagacc-cagcagta
SYBRGreen [®]	<i>PPARα</i>	ttcacgatgctgctcctt	catccagttc-gagggcattg
	<i>Fas</i>	tggtgatagccggtatgctc	tcagctttccagaccgctta
	<i>SREBP-1c</i>	cacttccagctagaccceaa	ggtgagagccttgagacagt
	<i>18S</i>	cgcggttctattttgtggt	agtcggcatcgttatgctc

Primers and probe were designed for quantification of *Scd1*, *PPAR α* , *Fas*, and *SREBP-1c* mRNA levels determined by real-time PCR

Results

Diet Impregnation in Early Infancy

During infancy, animals were fed with maternal milk that we analyzed after extraction from the mammary glands of females 8 days and 3 weeks after birth (Table 2a). We observed a comparable profile between the two milks. They both contained around 60 $\mu\text{g}/\mu\text{L}$ of FA and were particularly enriched in 12:0, 14:0, and medium-chain FA (MCFA). Impregnation of these specific FA was visualized in white adipose tissue (WAT) but also in brown adipose tissue (BAT) and to a lesser extent in skeletal muscle (supplemental Tables 2 and 5). As illustrated for WAT in Fig. 1, we observed a shift in the chromatogram according to the age of animals, with a specific switch from medium- to longer-chain FA (mainly C18). Thus, our results confirm that milk influenced the FA composition in early infancy, but after switching to standard rodent chow, the diet remained the same until the age of 1 year. In that context, our study then focused on the impact of age on the FA profile in various tissues (supplemental Tables 1–6).

Standard Rodent Chows and Tissue Enrichment in Linoleic Acid

First, the FA profile of the breeding and maintenance standard chows was determined. Indeed, 8d and 3w rats were suckled with maternal milk from mothers fed with breeding croquettes, whereas older animals were then fed with the maintenance diet. Our results showed a comparable profile in the FA composition between the two regimens (Table 2b). Linoleic acid was the most abundant FA, reaching 50 % of total FA. On the contrary, α -linolenic acid differed by regimen, representing 9.4 % in the breeding diet against 4.6 % in the maintenance diet, affecting the n-6/n-3 ratio (5.3 versus 10.8, respectively).

Secondly, in rats, an increase in n-6 FA was observed in all tissues, except brain, retina, and lung (supplemental Tables 3 and 4). If the n-6 family was quantitatively characterized by the 18:2 precursor and the 20:4 derivative, age had little effect on 20:4n-6. The major impact of age was observed on 18:2n-6 in liver, heart, and WAT (Fig. 2a). Such a rise led to a consequent proportion of linoleic acid reaching 25.5, 25.6, and 36.6 % of total FA, respectively.

Finally, the most significant effect of age was perceived in n-6 FA of testis (supplemental Table 5 and Fig. 2b). Indeed, this tissue was characterized by C22 and C24 n-6 FA with remarkable enrichment over the course of time, while 20:4n-6, as well as 22:4n-6, decreased in 1 year. The most prominent result was the enhancement of 22:5n-6, which was almost absent at 8d and represented 16 % at 4m

and 1y. Furthermore, testis was the only tissue where C24 intermediates were detected, reaching up to 10.1 % of total FA at 1y.

Controversial Effects on n-3 FA

On the whole, n-3 PUFA decreased or remained stable over time, except in skeletal muscle, lung, and testis (supplemental Tables 4 and 5). Muscle was unexpectedly the richest tissue in n-3 FA (14.1 % at 4m), where 22:6n-3 increased by 7 in one year (Fig. 3), to the detriment in particular of 12:0, 14:0, and MCFA for newborns and 18:1n-9 for older animals (supplemental Table 5). This rise was specific to skeletal muscle, as no such enrichment was observed in heart (supplemental Table 1). In lung and testis, newborns showed a large fraction of C22 n-3 (5.8 and 4.5 %, respectively), the level of which decreased severely over the year. Brain, used here as a reference, was the richest tissue in 22:6n-3, and its proportion remained relatively stable over time, but in liver, which was particularly affected by age, the proportion of 22:6n-3 weakened by 63 % in 1 year.

A One-Year Diet with Standard Rodent Chow Affects the PUFA Desaturation Index

Our results on n-6 and n-3 FA profiles underline an impoverishment of long-chain PUFA in tissues, although the same diet was administered after weaning. To understand this observation, we further analyzed the desaturase profile in liver.

First, the expression of $\Delta 5$ - and $\Delta 6$ -desaturases was evaluated by mRNA quantification of *Fads1* and *Fads2*, respectively (Fig. 4a). We observed an important increase for both genes in early infancy ($\times 1.6$ between 8d and 3w), and then an upturn until 1y. These results show that the two *Fads* genes were more expressed in young rats than in older animals, especially at 1y.

Secondly, we estimated the protein level by Western blot (Fig. 4b). Six protein isoforms were detected for the $\Delta 5$ -desaturase, whereas only three were observed for the $\Delta 6$ -desaturase. For the latter desaturase, two major proteins were visualized (53 and 66 kDa) at an equivalent level throughout the animal's life. A low level of the minor, 45 kDa isoform was observed 8 days after birth, but it became more abundant with age. For the $\Delta 5$ -desaturase, a similar level of the three protein isoforms (36, 41, and 75 kDa) was obtained independently of age. The other three isoforms (55, 59, and 68 kDa) appeared only at 3 weeks old, with a particular abundance of the 55-kDa protein from 4m to 1y. These results show that the $\Delta 6$ -desaturase level was weakly influenced by age, in contrast to that of the $\Delta 5$ -desaturase.

Table 2 Animal diets

a		
FA	Maternal milk	
	8d	3w
8:0	1.4	1.6
10:0	8.0	9.7
11:0	<i>tr</i>	0.0
12:0	7.9	8.5
13:0	0.0	0.0
14:0	8.5	7.7
15:0	0.2	0.2
16:0	20.5	17.3
18:0	3.5	3.6
20:0	<i>tr</i>	1.0
22:0	<i>tr</i>	0.0
24:0	<i>tr</i>	0.0
∑ saturates	49.9	49.7
16:1n-7	2.0	0.9
18:1n-7	1.9	1.4
∑ n-7	3.9	2.3
16:1n-9	0.3	0.3
18:1n-9	18.1	15.6
20:1n-9	0.2	0.1
20:3n-9	<i>tr</i>	0.0
∑ n-9	18.6	16.1
10:1n-6	0.1	0.0
18:2n-6	21.6	26.3
18:3n-6	0.5	0.3
20:2n-6	0.4	0.4
20:4n-6	0.5	0.2
20:4n-6	1.0	0.8
22:4n-6	0.2	2.0
∑ n-6	24.2	28.1
18:3n-3	2.3	2.8
18:4n-3	0.1	0.1
20:3n-3	0.0	0.0
20:4n-3	<i>tr</i>	0.0
20:5n-3	0.3	0.2
22:5n-3	0.2	0.2
22:6n-3	0.2	0.2
∑ n-3	3.1	3.5
14:1n-5	<i>tr</i>	0.0
20:1n-11	0.3	0.2
5, 11, 14–20:3	<i>tr</i>	0.1
∑ others	0.3	0.3
μg/μL	66.9	55.6
b		
FA	Standard chow	
	Breeding diet	Maintenance diet
14:0	0.2	0.2

Table 2 continued

FA	b	
	Standard chow	
	Breeding diet	Maintenance diet
16:0	14.3	20.8
18:0	4.2	3.5
18:1n-9	21.4	20.1
18:1n-7	1.2	1.4
18:2n-6	49.3	49.4
18:3n-3	9.4	4.6
n-6/n-3	5.3	10.8

Lipids from maternal milk (a) and rodent chows (b) were extracted according to the Folch method. After saponification, FA were derived by methylation and analyzed by GC-MS. FA content is expressed in mole %. *tr* trace (<0.1 %)

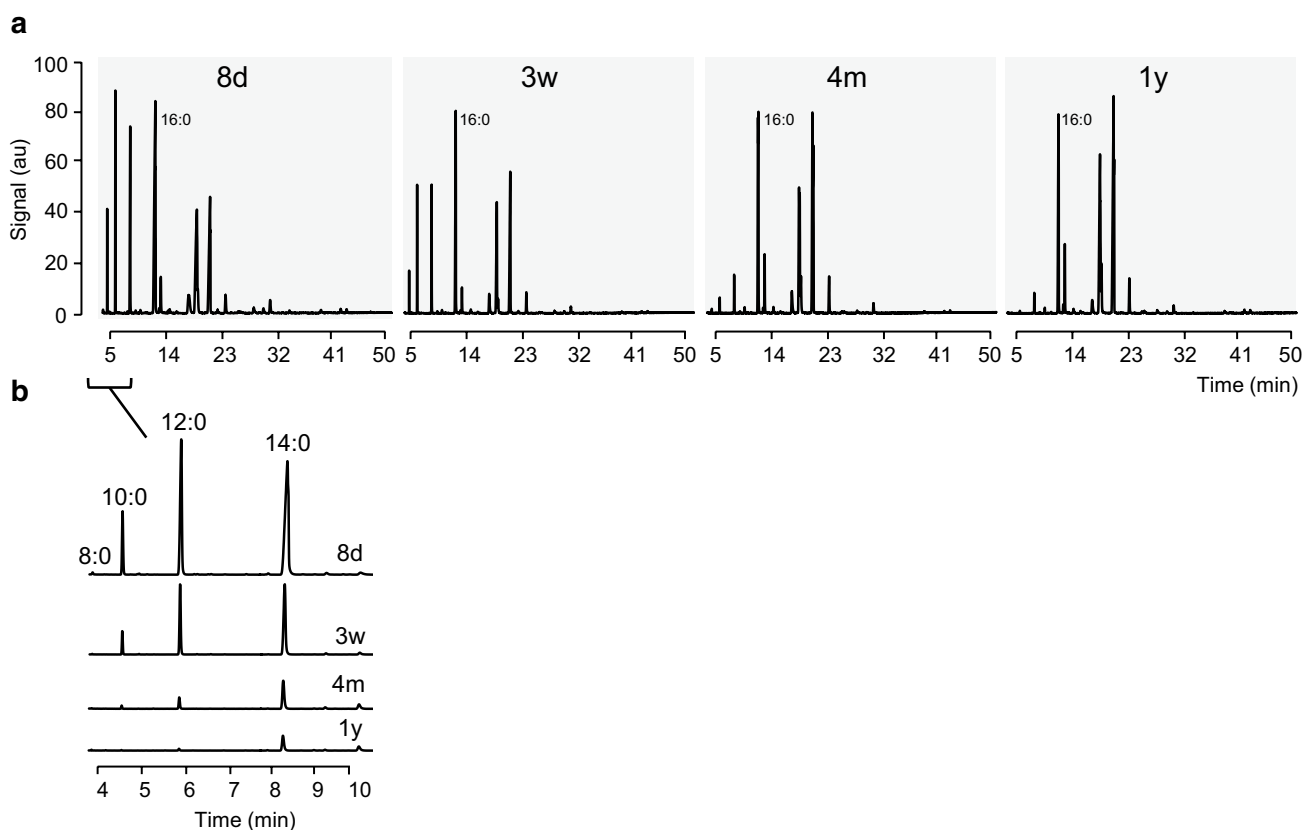


Fig. 1 Diet impregnation of white adipose tissue in infancy. GC-MS chromatograms of WAT averaged by age; FA are represented as % of total signal, shown in arbitrary units (au), as a function of time of elu-

tion, with 16:0 indicated as reference (a). A focus on the first elutions showed 12:0, 14:0, and medium-chain FA (b)

As the protein level did not coincide with the gene expression, we finally assessed enzymatic activities by using specific substrates for the $\Delta 5$ - and $\Delta 6$ -desaturases (Fig. 4c). Our results showed the same profile for the different enzymes and substrates. First, the activity of neonates was very low as compared with the other ages, representing from 9 % (FADS1 on 20:3n-6) to 20 % (FADS2

on 18:3n-3) of the maximum desaturase activity. Secondly, the highest activity was measured at the age of 3 weeks in all experimental conditions. Thirdly, desaturases were less active in adult rats. To conclude, these results were related to the gene expression rather than the protein level and were in concordance with the decreased desaturation index measured in the oldest tissues.

Fig. 2 One year on standard rodent chow impacts the n-6 FA level. Enrichment of 18:2n-6 was described in liver, heart, and WAT (a). The singular case of testis revealed very long-chain PUFA (b). Results are expressed in mole % of total identified FA as mean \pm SD

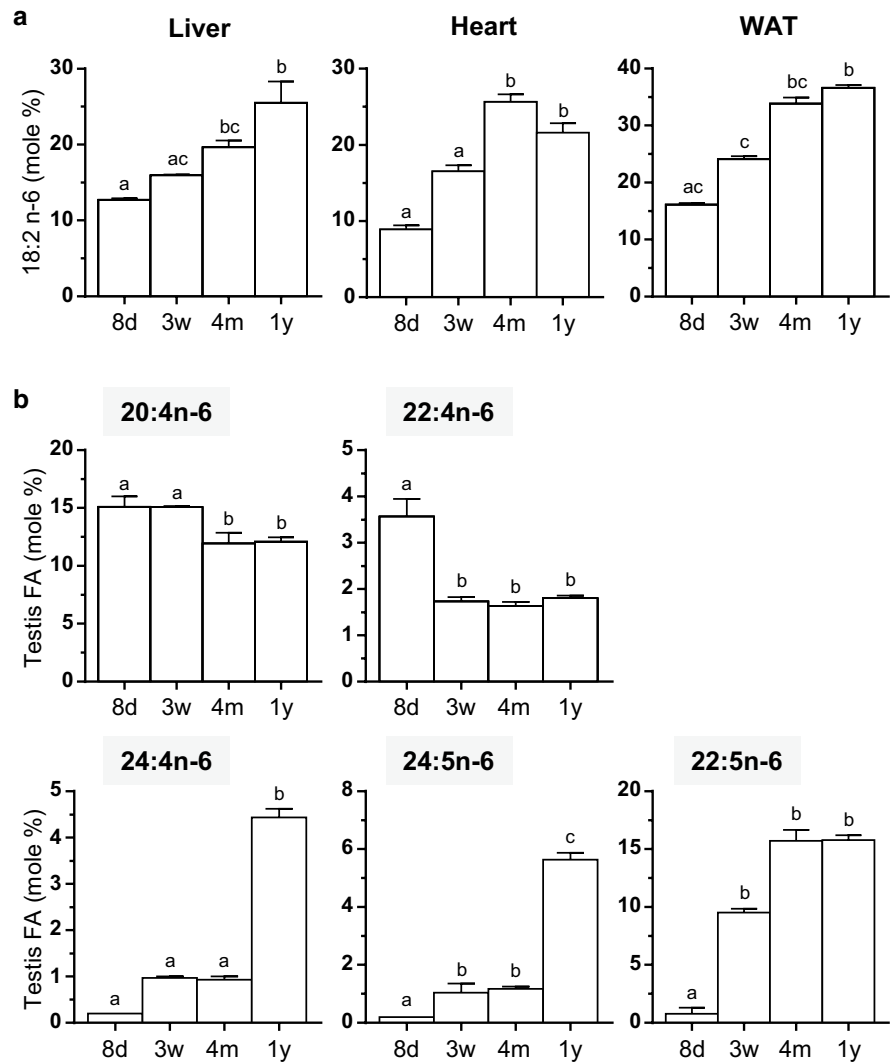
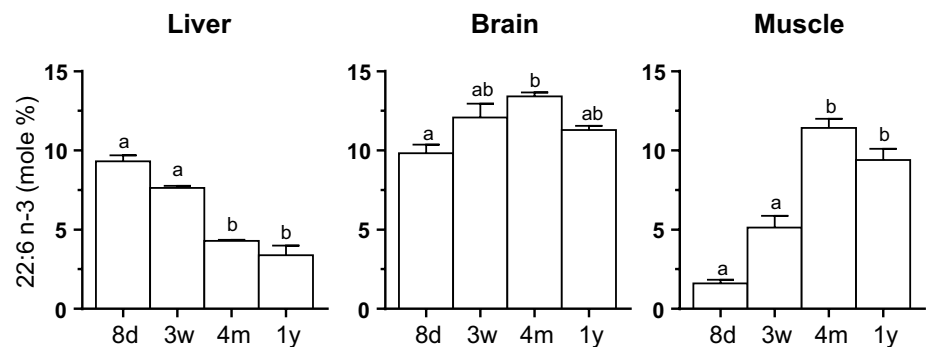


Fig. 3 DHA status in tissues as a function of age. The progression of 22:6n-3 is shown in liver, brain, and skeletal muscle. Results are expressed in mole % of total identified FA as mean \pm SD



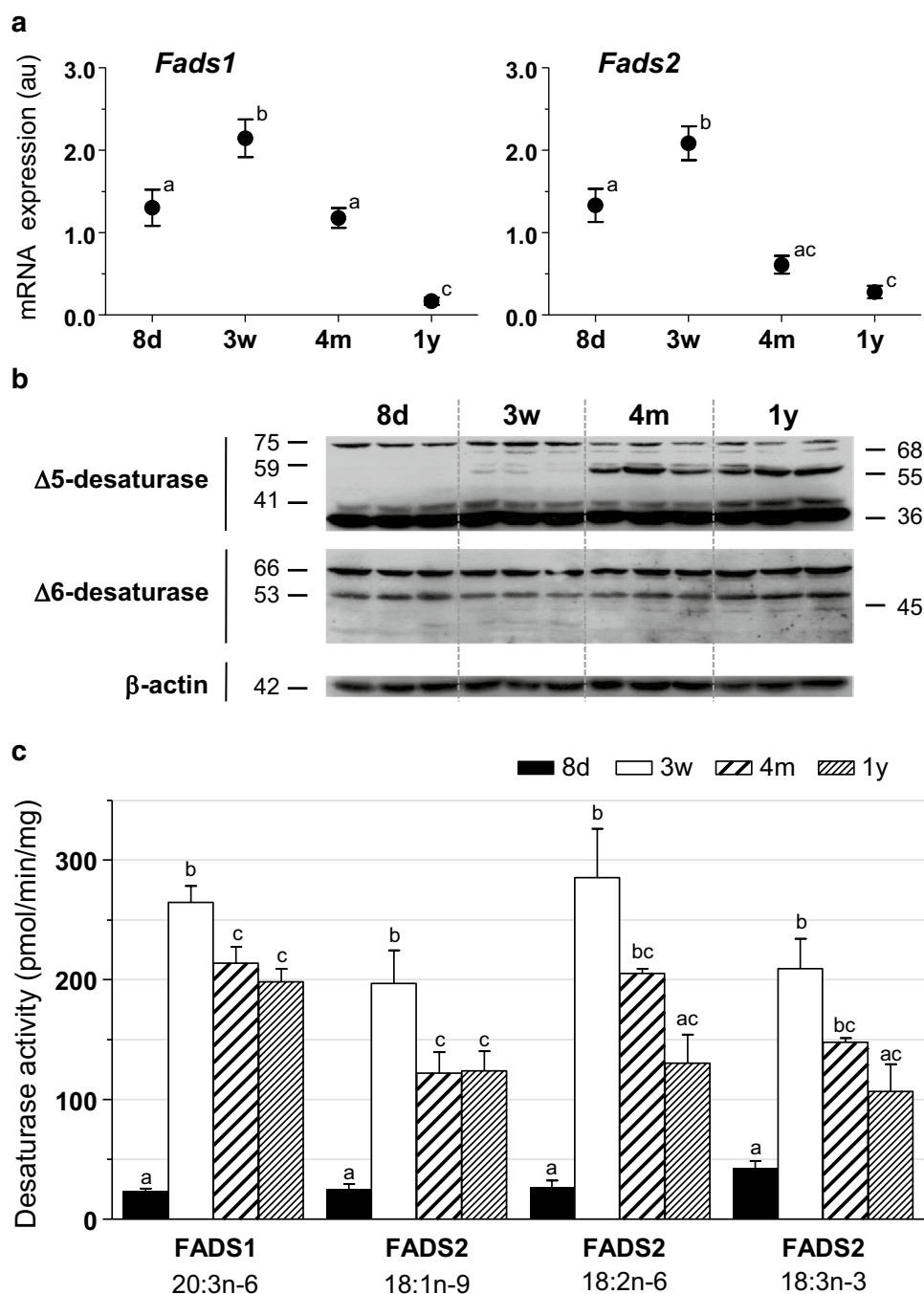
Is n-7 Monoene Accretion a Sign of Age?

In all tissues, n-7 monounsaturated FA (MUFA) increased significantly according to the age of animals, except in retina and testis, where a slight decrease was measured (supplemental Tables 3 and 5). The proportion of n-7 FA was usually very low (less than 5 % of total FA) but could

reach around 10 %, e.g., in WAT and to a lesser extent in BAT (supplemental Table 2). In most tissues, 18:1n-7 was the preponderant n-7 FA, although it was measured equally with 16:1n-7 in the adipose tissues.

When we compared the $\Delta 9$ -desaturase activity on 16:0 and 18:0, we observed a significant effect of age on both indexes in liver, WAT, and brain (Fig. 5a). Brain was also

Fig. 4 Desaturase profile in liver. Relative expression of *Fads1* and *Fads2* was determined in liver by real-time PCR (Taqman[®]) and expressed as mean \pm SEM (a). Hepatic expression of $\Delta 5$ - and $\Delta 6$ -desaturase was visualized by Western blot with anti- $\Delta 5D$ and anti- $\Delta 6D$ antibodies, respectively. One animal was represented per well (b). Desaturase activity was assayed on specific radiolabeled substrates and performed on post-mitochondrial supernatant of liver homogenates for 20 min at 37 °C. Results are expressed as mean \pm SEM (c)



unique in exhibiting a strong decrease in 16:1 after 8d, coupled with a remarkable rise of 18:1 (Fig. 5b). A significant proportion of 20:1 was found in the oldest animals, which constitutes another unique feature of n-7 and n-9 MUFA in that tissue.

Accumulation of monoenes was correlated to a decrease of saturated FA observed in most tissues, and was compared with hepatic biomarkers of FA synthesis or β -oxidation (Fig. 6a). In liver, we observed an impact of age on gene expression of *PPAR α* , *Fas*, and *SREBP-1c*, which decreased

after 8d. A rise was then perceived in old animals for *Fas* and *SREBP-1c* but not *PPAR α* . The opposite profile was obtained for *Scd1*: a low level was found for breastfed neonates and greater but stable expression was then measured in fasted animals. At the tissue scale, a ponderal decline of total RNA after 4 months old has to be mentioned, suggesting a particular low level of *Scd1* at the age of 1 year (Fig. 6b). On the contrary, SCD1 protein expression increased with age (2 \times between 8d and 1y) and was coherent with the MUFA accumulation in the tissue (Fig. 6c).

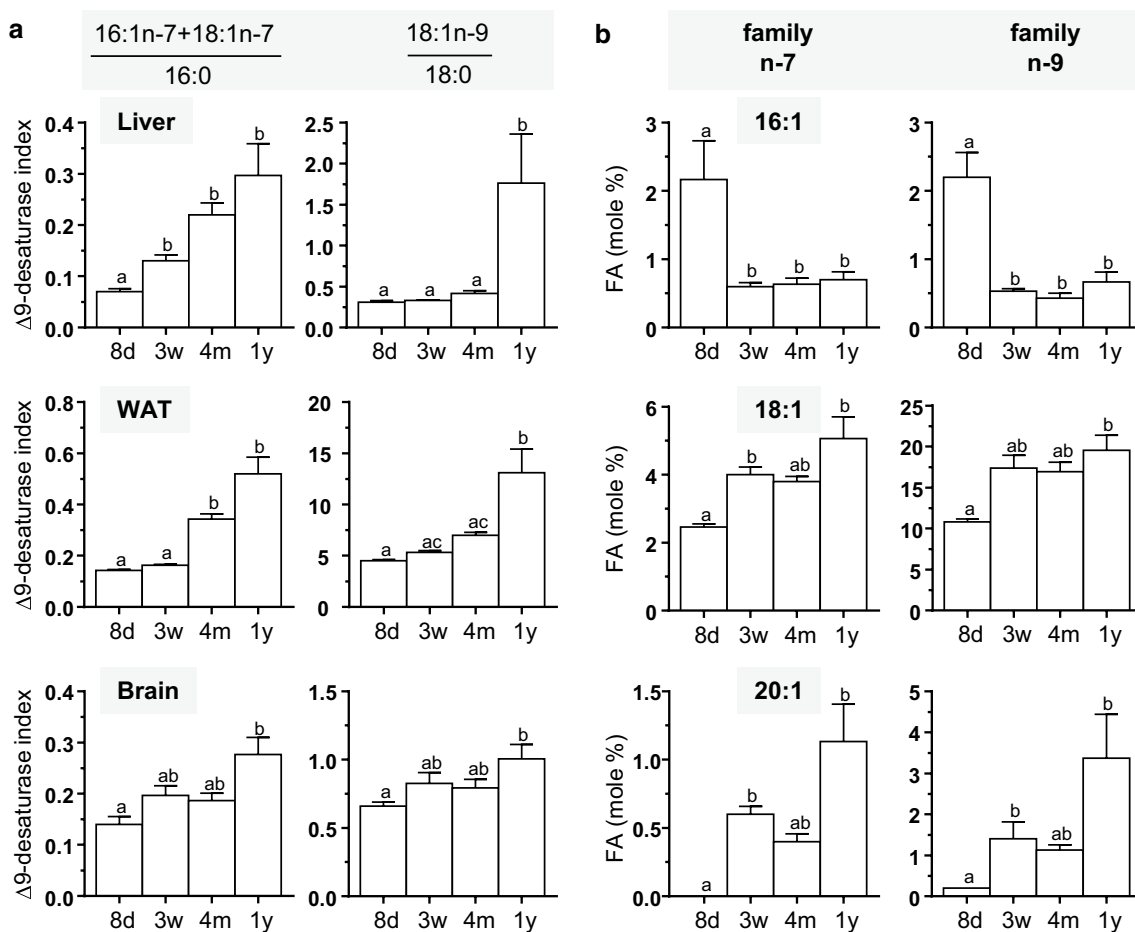


Fig. 5 $\Delta 9$ -desaturase index and monoene accretion. The $\Delta 9$ -desaturase index was evaluated in liver, WAT, and brain as the ratio of $\Delta 9$ -desaturase products and corresponding substrates, quanti-

fied initially in mole % (a). The monoene status of n-7 and n-9 families was considered in brain as a function of chain length and age (b). Results are expressed in mole % of total identified FA as mean \pm SD

Following the monoene accretion, we observed an important increase of triacylglycerols in the liver, as well as in cholesterol but in contrast with plasma (Fig. 6d). This enrichment in lipids was quantified as well by the FA accumulation, which doubled in liver over 1 year and particularly after 4m (supplemental Fig. 1).

Fluctuation of Plasmalogen-Derived Dimethylacetals

FA rank among the key components of lipids, but fatty aldehydes derived from plasmalogens were also profiled. Sparsely quantified in tissues, plasmalogens represent a major class of phospholipids involved in various physiological functions, for instance storage of specific PUFA or second messengers. In this way, DMA, i.e., the chemical structure of GC-detected fatty aldehydes, were analyzed to evaluate the plasmalogen level according to tissue and animal age (Table 3). Our results showed that brain was the most concentrated in DMA, especially in the oldest rats (1.37 mmol/mg at 1y). Lung and heart were also rich in DMA, but with no significant impact of age.

Besides, DMA concentrations were usually in compliance with the DMA proportions calculated in mass % over total methylated derivatives, the maximum of which was reached in brain with 13.8 % after the age of 1 year. Finally, although C16 and C18 were the main carbon chain structures detected in the tissues, plasma and retina displayed a particular profile, as DMA were poorly quantified with 12:0 as the only identified species. Kidney was also remarkable as the only tissue with 14:0-DMA and only at 8d (not shown).

The impact of age on DMA from brain, heart, and RBC was further analyzed (Fig. 7). Although heart is well known for its plasmalogen content, no significant effect of age on the DMA distribution was obtained. Conversely, a similar profile was observed between brain and RBC, where the desaturation index increased with time. Indeed, the 16:0-DMA was reduced over a year during the growth process, in favor of 18:0-DMA and particularly of 18:1n-9-DMA and 18:1n-7-DMA. Moreover, RBC was enriched in 18:2n-6-DMA, the species of which remained specific to this tissue (although weakly detected in lung; result not shown).

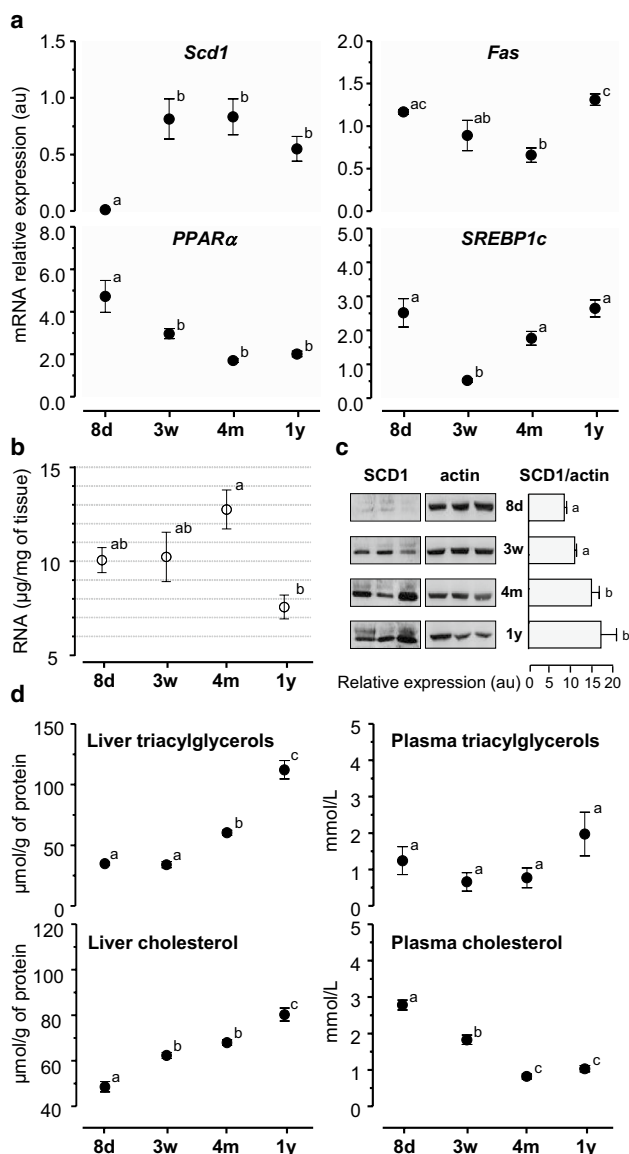


Fig. 6 Biomarkers of FA synthesis in liver as a function of age. The gene expression in liver was evaluated by real-time PCR by using Taqman[®] for *Scd1* and Sybr[®]Green for *Fas*, *PPARα*, and *SREBP1c*. Results are expressed as mean ± SEM (a). Total RNA was also quantified and is expressed as mean ± SD (b). Protein expression of SCD1 was estimated in post-mitochondrial supernatant of liver by Western blot and normalized with actin. Relative expression was evaluated by measuring the intensity of bands and is expressed as the ratio between SCD1 and actin (c). Triacylglycerols and cholesterol were quantified by using a Biomérieux assay on liver and compared with plasma. Results are expressed as mean ± SEM (d)

Discussion

At the beginning of life, specificity in the FA profiles was observed in lactating rats, where 12:0, 14:0, and MCFA were recovered in high quantities, especially in adipose

tissues and skeletal muscle. These FA coming from maternal milk would be rapidly absorbed by neonatal rats and preferentially oxidized rather than glucose as an energy source [19–22]. Such tissue accretion then declined in weaning animals. Indeed, the animal diet was switched from maternal milk to maintenance rodent chow, whose FA composition changed with a remarkable enrichment of 18:2n-6, representing 50 % of total FA in this regimen. As a consequence, accumulation of n-6 FA, and especially 18:2n-6, was visualized in tissues. This insidious increase, which was observed in this study, could parallel the situation in humans as the Western diet is also characterized by the use of n-6-enriched oils. More than an accretion inherent to the n-6 enrichment of the diet, the present work showed that age modified the n-6 status of tissues in terms of impregnation. Among prominent observations, we confirmed the exceptional singularity of gonads, where high unsaturated n-6 FA were detected in testis, whereas the desaturation index was lowered in the other tissues. Docosapentaenoic acid (22:5n-6) was the most abundant FA and remains well known to naturally accumulate in mammalian testis during sexual maturation [23–25]. In our study, testis maturation was past at the age of 4 months, but we still showed a remarkable rise of C22–C24 polyenes from young to adult animals. Such accretion in testes was not in agreement with the desaturase pattern described by Saether, who showed a decline of $\Delta 5$ - and $\Delta 6$ -desaturase expression in rat after 1 month old [26]. However, different works have emphasized the involvement of these long-chain n-6 PUFA in reproductive functions, since peroxisomal deficiency or loss of $\Delta 6$ -desaturase generates sterility [27–29]. Specifically, n-6 docosapentaenoic acid is presumed to be involved in testicular function, since its decrease in phosphatidylcholine was correlated with shorter sperm tail length and abnormal spermatozoa [30]. Zanetti et al. particularly emphasized its implication during capacitation and acrosomal reaction [31]. More surprisingly, our results show accumulation of 24:4n-6 and 24:5n-6 at 1 year old, as if the carbon chain-shortening to 22:5n-6 became defective. Moreover, these C24 FA are known to especially accumulate in 1-alkyl-2,3-diacylglycerols, which are ether-triglycerides widely present in gonads [32]. No effect of age has been described concerning the metabolism of etherlipids, and the specific role of C24 polyenes, other than on membrane fluidity, has to be further investigated, as the ratio between 22:5n-6 and 24:5n-6 could be a potent marker of growth or age in testes. If 1 year old is not considered as old or senescent, it remains too old in animal facilities such as the Janvier Labs breeding center, since Sprague–Dawley rats are usually used as reproducers until 8 months old, after which their reproductive capacity is considered insufficient.

Table 3 Dimethylacetal content in tissues

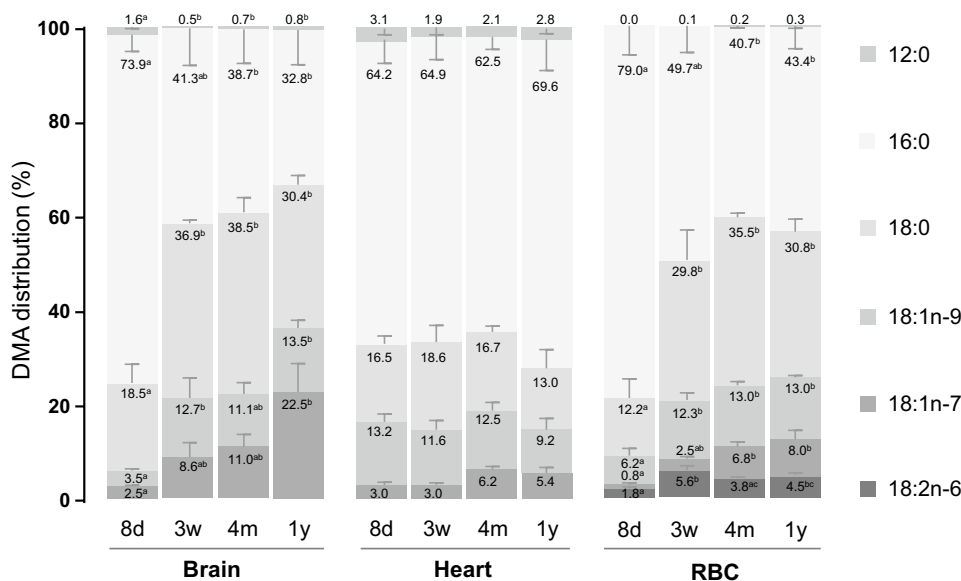
Tissue	DMA	8d	3w	4m	1y
Liver	Mass % of MD mmol/mg	1.5 ± 0.7	1.4 ± 0.9	1.4 ± 0.8	0.6 ± 0.4
		0.11 ± 0.05	0.11 ± 0.07	0.13 ± 0.08	0.08 ± 0.02
Heart	Mass % of MD mmol/mg	5.0 ± 0.5 ^{a, b}	4.0 ± 0.3 ^a	4.5 ± 0.9 ^a	7.1 ± 1.7 ^b
		0.25 ± 0.04 ^a	0.23 ± 0.05 ^a	0.26 ± 0.01 ^a	0.44 ± 0.16 ^b
WAT	Mass % of MD mmol/mg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
BAT	Mass % of MD mmol/mg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Brain	Mass % of MD mmol/mg	11.3 ± 4.2	10.7 ± 1.7	12.0 ± 1.2	13.8 ± 0.4
		0.68 ± 0.18 ^a	0.94 ± 0.11 ^a	1.15 ± 0.20 ^b	1.37 ± 0.29 ^b
Retina	Mass % of MD* mmol/mg	0.3 ± 0.3	0.5 ± 0.3	0.2 ± 0.0	0.1 ± 0.1
		0.01 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Kidney	Mass % of MD mmol/mg	5.0 ± 0.1 ^a	3.4 ± 0.1 ^b	4.2 ± 0.0 ^{a, c}	4.1 ± 0.1 ^{b, c}
		0.19 ± 0.00 ^a	0.18 ± 0.03 ^a	0.22 ± 0.01 ^{b, c}	0.21 ± 0.01 ^{a, c}
Lung	Mass % of MD mmol/mg	4.5 ± 0.5 ^a	9.8 ± 1.4 ^b	10.6 ± 2.5 ^b	7.1 ± 3.3 ^{a, b}
		0.34 ± 0.02 ^a	0.53 ± 0.11 ^{b, c}	0.54 ± 0.10 ^b	0.37 ± 0.08 ^{a, c}
Muscle	Mass % of MD mmol/mg	2.1 ± 1.0 ^a	2.4 ± 0.4 ^a	4.5 ± 0.5 ^b	4.3 ± 1.1 ^b
		0.13 ± 0.01 ^{a, b}	0.11 ± 0.01 ^a	0.14 ± 0.02 ^b	0.14 ± 0.02 ^b
Testis	Mass % of MD mmol/mg	4.9 ± 0.2 ^a	4.7 ± 0.3 ^a	4.0 ± 1.0 ^{a, c}	3.2 ± 0.2 ^c
		0.15 ± 0.01 ^a	0.16 ± 0.02 ^a	0.15 ± 0.04 ^{a, c}	0.00 ± 0.00 ^{b, c}
Plasma	Mass % of MD* mmol/mg	0.4 ± 0.1 ^a	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b
		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RBC	Mass % of MD mmol/mg	14.6 ± 2.9 ^a	8.5 ± 0.4 ^{a, c}	8.0 ± 0.1 ^{b, c}	8.2 ± 0.6 ^b
		0.15 ± 0.03 ^a	0.06 ± 0.01 ^{a, c}	0.05 ± 0.00 ^{b, c}	0.06 ± 0.00 ^b

Lipids were extracted with the Folch method and saponified. Fatty aldehydes derived from plasmalogens were methylated in DMA and analyzed by GC-MS. Results are expressed as mean ± SD in mass % of total methylated derivatives (MD: DMA + FAME) and in mmole/mg of tissue

* 12:0-DMA as unique DMA

Different letters indicate significant differences between ages at $p < 0.05$

Fig. 7 Distribution of dimethylacetal species over a year. The distribution of plasmalogen-derived dimethylacetal species is presented for brain, heart, and RBC. Results were calculated from mole % of total methylated derivatives and are expressed as mean ± SD



Ageing is also related to declining mobility and may compromise the contractile function by impacting on structural and functional features of skeletal muscle. Indeed, growing older is characterized by muscular atrophy involving mitochondrial dysfunction and modification of glycolytic enzyme activity coupled with oxidative capacity [33–36]. Furthermore, the PUFA pattern of skeletal muscle influences exercise performance in modulating muscle damage, inflammation, and metabolism during exercise [37, 38]. In this regard, we analyzed the FA profile of skeletal muscle from growing animals and found a surprising enrichment in docosahexaenoic acid (DHA, 22:6n-3) over 1 year, whereas the n-3 precursor supply was lowered in the diet over time. This accumulation may be inherent to the muscular incorporation of long-chain PUFA, since 18:3n-3 would not be converted into derivatives such as DHA in such tissue [39, 40]. In addition, different studies have shown the beneficial effects of dietary DHA on muscle physiology. For example, Peoples et al. demonstrated that DHA improves oxygen consumption and contractile function [41]. DHA upregulates expression of uncoupling protein 3, thereby reducing production of reactive oxygen species and enhancing transport and oxidation of FA in skeletal muscle [42, 43]. Finally, it increases the TAG concentration and ameliorates mitochondrial metabolism [44]. Now if we consider DHA as a marker of growth or age in muscle, then its preservation would suggest full capacity of contractile function. In our work, while DHA was highly present in adult animals, a slow decline was perceived between 4 months and 1 year old, suggesting implementation of muscular atrophy before 1 year old.

Finally, the most relevant FA considering growth or age markers could be *cis*-vaccenic acid (18:1n-7), the level of which usually remains discrete but evolves positively with age, whose occurrence was coupled with the Δ 9-desaturase index and expression. Recent studies have shown that this FA could represent a potent marker of various diseases such as chronic kidney disease [45], hypertension [46] or heart failure [47]. Tripathy et al. also recently demonstrated that 18:1n-7 was inversely associated with hepatic enzymes involved in gluconeogenesis, glucose intolerance, and hyperglycemia [48]. In our case, 18:1n-7 accretion in tissues may be considered as a sign of age before potential symptoms of pathologies associated with ageing appear in our rat model. Further investigations should be done to evaluate to what extent 18:1n-7 accumulation modulates the basal physiology of the tissue.

Altogether, our results underline the impact of the growth process on lipid metabolism, promoting FA as potential markers of age, as well as fatty aldehydes derived from plasmalogens. This minor class of phospholipids is particularly known to be involved in oxidative stress [49, 50] and can be found as

plasmenylethanolamine, for example in brain [51] and RBC [52], or plasmenylcholine more specifically in heart [53]. Our study showed that DMA were dispersed throughout the organism except adipose tissues. Age impacted on the DMA proportions, concentrations or distributions, depending on the tissue. In humans, a negative correlation with age was observed with 16:0-DMA or 18:0-DMA from plasma and erythrocyte volunteers [52, 53]. No such correlation was obtained in our study. However, when the plasmalogen content was evaluated by the DMA/FAME ratio, age impacted positively on brain and RBC regarding the 18:0 and 18:1 chain lengths (supplemental Fig. 2), as also observed in humans by Labadaridis between neonates and children [54]. Moreover, our study underlined that circulating DMA would reflect the plasmalogen content in brain. In early infancy, the lipid composition in myelin changes during brain maturation [55] when plasmalogen synthesis is particularly active [56]. In adults, the plasmalogen level decreases in humans [57], the decrease of which was demonstrated to be potentially correlated with pathological situations and predicted before clinical symptoms [58]. In the case of our healthy rats, at the age of 1 year, the plasmalogen level in the tissues tended to increase, whereas Norton et al. showed no major impact of age [55]. Thus, it seems the plasmalogen status does not indicate any defective function in our rat model, while plasmalogens are crucial, for instance, for Schwann cell development and differentiation [59] or as neuroprotective against apoptosis [60], although the role of the alkenyl species remains poorly documented and requires detailed study. Yamazaki showed a structural evolution in plasmenylcholine and plasmenylethanolamine in metabolic syndrome [61]. Kaddurah-Daouk also demonstrated a modulation in the fatty aldehyde structure during the psychotic episode of schizophrenia [62]. If plasmalogens are quantitatively correlated with health and disease [63, 64], no effect based on the fatty aldehyde entity has been characterized yet. Such investigations could focus in particular on heart, lung, and testis, where the plasmalogen content was affected at 1 year old and could be related to the PUFA content determined in tissues.

In conclusion, we describe the lipid profile of Sprague–Dawley rats fed with standard rodent chow. This study was not carried out on senescent animals but underlines the basic pattern of healthy animals over 1 year, which is a lifespan commonly found in the literature. 18:2n-6, 22:6n-3, and DMA are likely to be relevant markers of growth or age associated with diet and tissues, whilst accretion of 18:1n-7 may be an independent and inclusive sign of age.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

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