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Hepatic microsomal membrane lipidic composition and growth hormone effect in adult male rat: evidence for a 'feminization' process of total phospholipid fatty acid pattern

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Abstract

Growth hormone (GH) effects on fatty acid composition and on Δ^5-, Δ^6-, Δ^9-desaturase and palmitic acid elongation activities were studied in male rat hepatic microsomes. Sham-operated and hypophysectomized animals were injected with two different dosages of GH, mimicking either the male or female GH secretion pattern. Half the hypophysectomized animals received thyroxine and cortisol in concentrations chosen to compensate for the lack of thyroid hormones and glucocorticoids. GH, administered to sham-operated or to cortisol/thyroxine-treated hypophysectomized rats resulted in an increase in stearic and arachidonic acid proportions, while palmitic acid percentage was decreased. Total monounsaturated fatty acids were dramatically reduced by this treatment. Δ^5-Desaturase and palmitic acid elongation activities were increased by GH treatment, while Δ^9-desaturase activity was decreased. These GH effects on desaturation and elongation activities could explain the modifications in microsomal fatty acid composition. Hypophysectomy markedly altered the fatty acid composition by reducing arachidonic and stearic acid proportions and increasing the linoleic acid proportion, while Δ^9-, Δ^5-desaturase and palmitic acid elongation activities were decreased. Restoration of most of the fatty acid proportions to control values was realized in hypophysectomized animals with a cortisol/thyroxine replacement administered alone or together with the low dosage of GH mimicking the male secretion pattern. High GH dosage produces essentially a 'feminization' process of the fatty acid composition of the hepatic microsomal membrane in male rats when compared to that of females. © 1997 Elsevier Science B.V.

Keywords: Growth hormone; Pulsatility; Liver microsome; Fatty acid composition; Desaturase; Elongase; Hypophysectomy

Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; BHT, 2,6-di-*tert*-butyl-4-methyl-phenol; CL, cholesterol; CL/PL, cholesterol to phospholipid ratio; CT, cortisol and thyroxine; DBI, double bond index ($\sum$ (% each unsaturated fatty acid $\times$ number of double bonds in the same fatty acid)); GH, growth hormone; MANOVA, multivariate ANOVA; MUFA, monounsaturated fatty acid; PL, phospholipid; PL/P, phospholipid to protein ratio; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; T_4, L-thyroxine; $\Sigma n-6$, sum of $n-6$ fatty acids; $\Sigma n-3$, sum of $n-3$ fatty acids

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1. Introduction

Microsomal membrane structure and functions seem to depend at least partly on its fatty acid composition, which is mainly regulated by dietary and hormonal factors [1–3]. This composition depends on dietary triacylglycerol supply, de novo synthesis of palmitic acid by fatty acid synthase and successive chain desaturation and/or elongation steps of dietary essential and non-essential fatty acids [4]. These enzymatic steps are involved in the biosynthesis of the saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). The rate-limiting steps of the desaturation reactions were reported to be the terminal enzymes, i.e. the desaturases (EC 1.14.99.−) [5,6]. Various desaturases introduce a double bond at the Δ^9-, Δ^6- or Δ^5-position in the fatty acid chain. The effect of nutritional (dietary fat and particularly essential fatty acids, dietary proteins or cholesterol), environmental (temperature), genetic and hormonal factors (especially thyroid hormones, glucocorticoids, insulin and glucagon) on desaturation activities has been well studied (for reviews, see [1,3,4,7]). However, among the various hormonal factors, the effect of growth hormone (GH) on these activities and on the fatty acid composition of microsomal membrane has been poorly investigated.

Besides its effects on general body growth, protein and carbohydrate metabolism, GH was reported to have important effects on lipid metabolism. This includes an increase in lipolysis and a stimulation of the production of ketone bodies in liver [8,9]. Moreover, GH was shown to decrease various lipogenic enzyme activities in vitro: malic enzyme (EC 1.1.1.40), fatty acid synthase complex, ATP-citrate lyase (EC 4.1.3.8) and glycerol-3-phosphate dehydrogenase (EC 1.1.99.5), while glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was reported to be increased by this hormone [10]. In addition, GH was reported to be particularly efficient in restoring fatty acid composition of mitochondrial membrane to normal levels after hypophysectomy in rats [11]. GH was also reported to 'feminize' fatty acid composition of total liver phosphatidylcholine in hypophysectomized female rats [12]. Yet, in microsomal membranes, it has been only suggested that a large concentration of GH increased membrane unsaturation and fatty acyl chain length [13]. Some authors have shown that some differences existed in the fatty acid composition of microsomal membrane between male and female rats [14]. These sex differences seem to be due to modifications in the various desaturase activities. As the sex-dependent activity of some hepatic enzymes (for example some cytochrome P-450 isoenzymes and some glutathione-*S*-transferase isoforms) has been shown to be regulated by the secretion pattern of GH [15–17], which is different between male and female rats, it is of interest to determine whether this hormone could also account for the sex differences that exist in the fatty acid composition of the hepatic microsomal membrane.

In order to estimate the effect of GH treatment on fatty acid composition of total phospholipids and on Δ^9-, Δ^6-, Δ^5-desaturation and palmitic acid elongation activities, sham-operated and hypophysectomized male rats were treated with two different concentrations of GH. The low dosage was chosen to mimic the male secretion pattern in hypophysectomized animals and the high dosage to mimic the female one in male rats, while female rats were used as controls. The conditions of restoration of the fatty acid composition, desaturation and elongation activities after hypophysectomy were examined using the same concentrations of GH with or without coadministration of physiological doses of thyroxine and glucocorticoid. Consequently, by using a factorial arrangement of physiological treatments of animals, we were able to quantify the deep sex-dependent implication of GH in the hepatic microsomal membrane fatty acid composition.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, hypophysectomized or sham-operated at 6 weeks of age, and female rats were purchased from Iffa-Credo (L'Arbresle, France). Animals were weighed every day from their arrival in the laboratory. They were individually housed under conditions of controlled temperature (20–23°C) and photoperiod (14:10 h light/dark) and were maintained ad libitum on a standard diet containing 64.8%

carbohydrates, 6% lipids (groundnut oil/rapeseed oil (3:2)), 22.2% proteins, vitamins and minerals. The fatty acid relative percentages of this diet were 12.9 SFA, 62.0 MUFA, 21.5 linoleic acid and 3.6 linolenic acid. After allowing them to recover for 2 weeks, hypophysectomized and sham-operated animals were randomly divided into 9 groups of 8. Age-matched females were used as a supplementary group. The effectiveness of hypophysectomy was verified by the absence of weight gain over this period. The animals were given (at 12-h intervals) a subcutaneous injection of recombinant human growth hormone (GH1, 0.25 IU/kg b.wt.; GH2, 2 IU/kg b.wt.) twice daily while GH controls (GH0) received only vehicle (25 mM sodium carbonate buffer, pH 9.5, 0.9% NaCl). Recombinant human growth hormone was a generous gift from Sanofi Recherches (Labège, France). Half the hypophysectomized animals were injected daily with subcutaneous cortisol (400 μg/kg b.wt.) and L-thyroxine (T_4) (50 μg/kg b.wt.) (Sigma, St-Quentin Fallavier, France) as described previously [15,18]. This T_4 concentration was found to render hypophysectomized rats euthyroid [18]. The remaining animals received only vehicle (0.9% NaCl). This cortisol/thyroxine treatment (CT) started 2 days prior to GH treatment and lasted throughout the experimental period. Weight gain during the treatment period showed that hormone replacement was effective. Control female rats only received the GH0 treatment.

After 16 days of treatment, animals were sacrificed between 09.00 and 11.00 h to avoid any circadian variation; blood was collected and the liver was immediately perfused with Ringer solution via the portal vein, excised, weighed and placed in liquid nitrogen before storage at −80°C. For microsomal preparations, livers were minced and homogenized at 4°C in 0.1 M sodium phosphate, pH 7.4. Homogenates were centrifuged sequentially at 11 000 *g* (15 min) and 105 000 *g* (60 min) at 4°C on a Centrikon T-1045 ultracentrifuge (Kontron Instruments, St-Quentin-en-Yvelines, France) in order to prepare the microsomal pellets. Microsomal membranes were resuspended in the homogenization buffer containing 20% glycerol and were stored at −80°C until use. The amount of microsomal protein was determined by the method of Lowry [19] using bovine albumin (Sigma) as the standard. By using an immunoradiometric assay (monoclonal antibodies) (hGH 'coatria', bioMérieux, Marcy-l'Etoile, France), plasmas from GH2-treated animals (sham-operated and hypophysectomized) were checked for the presence of a significant amount of recombinant human growth hormone at least 12 h after the last injection, which determined a continuous GH exposure in these animals.

2.2. Lipid extraction and fatty acid analysis

Total lipids were extracted according to the method of Bligh and Dyer [20] from liver microsomes in the presence of 0.02% 2,6-di-*tert*-butyl-4-methyl-phenol (BHT) (Aldrich, St-Quentin Fallavier, France) as an antioxidant. Quantitative estimation of phospholipids was performed by determination of inorganic phosphorus using the Bartlett procedure [21]. Cholesterol was assessed from the total lipid fraction by an enzymatic colorimetric test (Cholesterol CHOD-PAP method from Boehringer Mannheim, Mannheim, Germany). Neutral lipids and phospholipids were separated on Supelclean LC-Si SPE silica cartridges (Supelco, St-Germain-en-Laye, France) by successive elutions with chloroform and methanol as described previously [22]. The fatty acid composition of total phospholipids was determined after transmethylation in presence of BF_3 (10% in methanol) (Fluka, St-Quentin Fallavier, France) for 20 min at 90°C, followed by an extraction of the methyl esters with hexane. The fatty acid methyl esters were then analyzed by capillary gas–liquid chromatography on a Chrompak model 438A apparatus (Chrompak, Les Ulis, France), equipped with a Chrompak CP Wax 58 CB (50 m × 0.32 mm i.d. 0.2 μm film thickness). Peak areas were measured using a Hewlett-Packard model 3390A integrator (Hewlett-Packard, Les Ulis, France). Identification of peaks was assessed by comparison with the analysis of a reference fatty acid mixture (Nu-Chek-Prep Inc., Elysian, MN, USA) and confirmed by gas chromatography–mass spectrometry using a Delsi-Nermag GC apparatus coupled with a Nermag R10-10T mass spectrometer (Argenteuil, France).

2.3. Enzymatic assays

Unlabeled fatty acids, coenzymes and biochemicals were purchased from Sigma. [1-^{14}C]Linoleic

(1.48–2.22 GBq/mmol), [1-^{14}C]eicosa-8,11,14-trienoic (1.48–2.22 GBq/mmol) and [1-^{14}C]palmitic (1.48–2.22 GBq/mmol) acids were provided by DuPont NEN (Les Ulis, France). Each substrate was diluted into ethanol with the corresponding unlabeled fatty acid to obtain a specific activity of 130 kBq/mmol. Final ethanol concentration in the assay was 2%. The desaturation assays were carried out as described by Leikin and Brenner [23] with slight modifications. Δ^9-, Δ^6- and Δ^5-desaturase activities were determined for each liver microsomal preparation by measuring the conversion of [1-^{14}C]palmitic acid, [1-^{14}C]linoleic acid and [1-^{14}C]eicosatrienoic acid to the corresponding unsaturated acids at 37°C for 30 min in the presence of 1.3, 3 and 2 mg of microsomal proteins, respectively. The substrate concentration was 35 μM. The incubation medium contained, in a total volume of 1 ml: 72 mM phosphate buffer (pH 7.4), 5 mM $MgCl_2$, 2.5 mM ATP, 1 mM NADH, 0.33 mM niacinamide and 0.2 mM CoA.

The palmitic acid elongation assay was adapted from the method described by Mimouni et al. [24] for the elongation of arachidonic and eicosapentaenoic acids, with slight modifications. The palmitic acid elongation rate was determined in liver microsomes by measuring the conversion of [1-^{14}C]palmitic acid to [1-^{14}C]stearic acid at 37°C for 20 min in the presence of 1.3 mg of microsomal proteins. The substrate concentration was 35 μM and the incubation medium contained in a total volume of 1 ml: 72 mM phosphate buffer (pH 7.4), 5 mM $MgCl_2$, 2.5 mM ATP, 1 mM NADH, 1 mM NADPH, 3 mM glutathione and 0.1 mM malonyl-CoA. The incubations for elongation were carried out in anaerobic conditions (under nitrogen) in order to inhibit desaturation [25].

Under these conditions, the desaturation and elongation rates were found to be linear with respect to incubation time and microsomal protein amount. The reaction was stopped by adding 1 ml of 10% ethanolic KOH. Lipids were saponified by heating for 45 min at 80°C. Following acidification with 0.5 ml of 6 N-HCl, the fatty acids were extracted with hexane containing 0.02% (w/v) BHT. Each radiolabeled fatty acid and its corresponding desaturation or elongation product were analyzed separately by HPLC with a Philips model PU4100 apparatus (Argenteuil, France) equipped with an on-line radioisotope detector Radiomatic Flo-One/beta model A515 (Packard, Meriden, CT, USA) and a Hypersil C_8 column (150 × 4.6 mm i.d., 3 μm particle size from Interchim (Montluçon, France). [1-^{14}C]Linoleic acid, [1-^{14}C]eicosatrienoic acid and their corresponding desaturation products were separated isocratically with acetonitrile/methanol/25 mM ammonium acetate buffer pH 3.4 (57.5:17.5:25, v/v/v) at a flow rate of 1 ml/min. [1-^{14}C]Palmitic acid and its desaturation and elongation products were separated isocratically with acetonitrile/methanol/25 mM ammonium acetate buffer pH 3.4 (62.5:22.5:15, v/v/v) at the same flow rate. Peaks corresponding to elongation and desaturation products were identified, after methyl-coumarin derivatization according to the method of Jüngling and Kammermeier [26] by comparison of their retention time with those of authentic fluorescent standards. Conversion percentage in controls performed without cosubstrate (coenzyme A or malonyl-CoA), reflecting non-specific conversion, was removed from the experimental values.

2.4. Statistical analysis

All results are presented as means with their standard deviation (S.D.). Fatty acid composition data, expressed as percentage, were transformed into arcsine ($\sqrt{x}$) to correct for unequal variances. The effect of hormonal treatments on fatty acid composition and on desaturase and palmitic acid elongation activities was analyzed by analysis of variance (ANOVA) using the GLM procedure of SAS [27]. When using two-factor variance analyses on CT-untreated animals (additive model, Hypophysectomy * GH) or on hypophysectomized rats (additive model, GH * CT), first-order interactions between treatments most often proved to be highly significant. Thus, the effect of each treatment was also tested in the following reduced groups of animals: *GH treatment* in sham-operated rats, hypophysectomized CT-untreated rats and hypophysectomized CT-treated rats, separately; and *hypophysectomy* in GH-untreated rats. Inside these different groups, the statistical analysis of differences between the means was performed using Student–Newman–Keuls test. In order to further examine the conditions of hormonal restoration of control values after hypophysectomy, comparisons of means be-

tween the 9 groups of animals were performed using Student–Newman–Keuls test. In order to simultaneously assess the relative effects of the various physiological factors on the various fatty acid proportions, a multivariate variance analysis (MANOVA) was carried out, completed by a factorial discriminant analysis of the different groups of animals. This latter descriptive statistical methodology was first intended to give a hierarchy between the effect of hypophysectomy and the cortisol/thyroxine restitution treatment on the one hand and the GH treatment on the other, and second, to test a posteriori, simultaneously for the different fatty acid proportions, the hormonal conditions chosen to restitute control hormonal situation from hypophysectomized animals and the 'feminization' process with GH2 treatment. The discriminant analysis is equivalent to a principal component analysis performed on the barycenters of the different groups of animals which maximizes the distances between these groups and minimizes the distances between individuals inside these groups [28–30]. This analysis can reduce the total variance (or information) given by initial variables to some principal components (or discriminant axes) which summarize this information. This analysis gives an explanation of these linear discriminants by the corre-

Table 1
Sex-differences and effects of GH treatment, hypophysectomy and hormonal replacements on total phospholipid (PL) and cholesterol (CL) levels, phospholipid/protein (PL/P) and cholesterol/total phospholipid (CL/PL) ratios in microsomes from male rats

	Sham-operated		Hypophysectomized	
	Male rats	Female rats	CT −	CT +
PL(mg/g of liver)				
GH0	3.400 ± 0.58	3.060 ± 0.40 [a]	*2.100 ± 0.43* [c]	**3.000 ± 0.87** [d]
GH1	2.890 ± 0.80		**2.770 ± 0.93**	**2.400 ± 0.67**
GH2	2.940 ± 0.78		**2.290 ± 0.69**	**2.880 ± 0.72**
GH effect	n.s.		n.s.	n.s.
PL/P				
GH0	0.220 ± 0.06	0.200 ± 0.03	0.170 ± 0.03	0.180 ± 0.04
GH1	0.190 ± 0.06		0.180 ± 0.06	0.160 ± 0.03
GH2	0.190 ± 0.03		0.160 ± 0.02	0.170 ± 0.04
GH effect	n.s.		n.s.	n.s.
CL (mg/g of liver)				
GH0	0.230 ± 0.07	0.230 ± 0.04	0.160 ± 0.05	0.190 ± 0.04
GH1	0.190 ± 0.04		0.180 ± 0.07	0.170 ± 0.06
GH2	0.190 ± 0.05		0.180 ± 0.06	0.200 ± 0.06
GH effect	n.s.		n.s.	n.s.
CL/PL				
GH0	0.066 ± 0.006	0.082 ± 0.010 [b]	0.078 ± 0.019	0.065 ± 0.015
GH1	0.068 ± 0.011		0.071 ± 0.037	0.073 ± 0.012
GH2	0.063 ± 0.007		0.080 ± 0.021	0.074 ± 0.022
GH effect	n.s.		n.s.	n.s.

The results are the mean of eight individual determinations ± S.D. The measurements were done as described in Section 2. n.s., non-significant effect of GH treatment in the following groups of animals: sham-operated rats, hypophysectomized CT-untreated rats or hypophysectomized CT-treated rats. CT, cortisol/thyroxine treatment; GH, growth hormone treatment; GH0, no GH treatment; GH1, low-dosage treatment; GH2, high-dosage treatment.

[a] Significant difference between sexes at $P < 0.05$.

[b] Significant difference between sexes at $P < 0.001$.

[c] Italic value shows a significant effect of hypophysectomy at $P < 0.05$.

[d] Bold values show the necessary combination of hormonal treatments of hypophysectomized animals for a restitution of the control values (statistically not different from sham-operated untreated animals) with Student–Newman–Keuls test.

lations they have with the initial variables. The factorial discriminant analysis was performed using the DISCRIM procedure of SAS [27].

3. Results

3.1. Sex differences and effects of GH treatment, hypophysectomy and hormone replacements on phospholipid (PL) and cholesterol (CL) concentrations

In sham-operated rats, PL and CL concentrations (expressed as mg per g of liver), and PL/protein (PL/P) and CL/PL ratios were not affected by GH (Table 1). Hypophysectomy induced a significant reduction (by 38%) of PL concentration, though PL/P and CL/PL were not significantly altered. This modification of PL concentration due to hypophysectomy was reversed by all hormonal replacements. In females, PL concentration was 10% lower than in males and consequently, the CL/PL ratio was 24% higher.

3.2. Sex differences and effects of GH treatment on fatty acid composition of hepatic microsomal total phospholipids of male rats

In sham-operated animals, GH induced a significant increase in 18:0 (by 19 and 35% with GH1 and GH2 treatments, respectively) and an almost correlative significant decrease in 16:0 (by 15 and 30% with GH1 and GH2 treatments, respectively) (Table 2). Consequently, total SFAs were only slightly, but significantly, increased. Total MUFAs were very significantly decreased by GH (by 12 and 20% with GH1 and GH2 treatments, respectively) due to an

Table 2
Sex differences and effects of GH treatment on fatty acid composition of total phospholipids of sham-operated male animal liver microsomes

Fatty acids	Sham-operated male rats				Female rats	
	GH0	GH1	GH2	GH effect	GH0	sex effect
14:0	0.28 ± 0.06 [a]	0.25 ± 0.05 [a]	0.22 ± 0.02 [b]	$P < 0.05$	0.17 ± 0.02	$P < 0.001$
16:0	20.59 ± 0.86 [a]	17.58 ± 1.99 [b]	14.48 ± 0.39 [c]	$P < 0.001$	13.01 ± 0.51	$P < 0.001$
16:1$n-7$	1.77 ± 0.35 [a]	1.33 ± 0.44 [b]	0.67 ± 0.25 [c]	$P < 0.001$	0.61 ± 0.21	$P < 0.001$
18:0	21.06 ± 1.19 [a]	25.14 ± 1.90 [b]	28.36 ± 1.22 [c]	$P < 0.001$	33.66 ± 1.23	$P < 0.001$
18:1$n-9$	6.42 ± 0.91	6.02 ± 0.71	6.05 ± 0.75	n.s.	4.92 ± 0.46	$P < 0.001$
18:1$n-7$	4.10 ± 0.48 [a]	3.40 ± 0.35 [a,b]	2.94 ± 0.57 [b]	$P < 0.001$	1.32 ± 0.15	$P < 0.001$
18:2$n-6$	12.28 ± 1.40	11.36 ± 1.27	11.68 ± 0.94	n.s.	10.56 ± 0.93	$P < 0.01$
18:3$n-3$	0.09 ± 0.04	0.12 ± 0.04	0.11 ± 0.05	n.s.	0.08 ± 0.03	n.s.
20:3$n-6$	1.33 ± 0.17 [a]	1.23 ± 0.17 [a,b]	0.98 ± 0.15 [b]	$P < 0.01$	1.22 ± 0.20	n.s.
20:4$n-6$	21.99 ± 0.78 [a]	22.69 ± 1.57 [a,b]	24.20 ± 1.23 [b]	$P < 0.01$	23.54 ± 0.58	$P < 0.001$
20:5$n-3$	0.56 ± 0.08	0.68 ± 0.07	0.59 ± 0.06	n.s.	0.77 ± 0.21	$P < 0.01$
22:4$n-6$	0.39 ± 0.03	0.38 ± 0.05	0.32 ± 0.02	n.s.	0.41 ± 0.08	n.s.
22:5$n-6$	0.58 ± 0.11	0.74 ± 0.23	0.48 ± 0.06	n.s.	0.62 ± 0.25	n.s.
22:5$n-3$	0.89 ± 0.12 [a]	0.76 ± 0.21 [a]	0.59 ± 0.08 [b]	$P < 0.01$	0.75 ± 0.10	$P < 0.05$
22:6$n-3$	6.72 ± 0.88	7.04 ± 0.82	7.21 ± 0.72	n.s.	8.32 ± 0.85	$P < 0.001$
SFA	42.03 ± 0.67 [a]	43.06 ± 0.62 [b]	42.77 ± 0.82 [b]	$P < 0.05$	45.98 ± 0.81	$P < 0.001$
MUFA	12.68 ± 0.76 [a]	11.12 ± 0.92 [a,b]	10.16 ± 1.18 [b]	$P < 0.001$	7.31 ± 0.73	$P < 0.001$
PUFA	45.30 ± 0.78 [a]	45.83 ± 1.41 [a]	47.07 ± 0.89 [b]	$P < 0.01$	46.38 ± 0.19	$P < 0.001$
$\sum n-3$	8.26 ± 0.85	8.55 ± 0.83	8.56 ± 0.82	n.s.	9.87 ± 0.50	$P < 0.001$
$\sum n-6$	36.48 ± 0.87	36.61 ± 1.87	38.31 ± 0.93	n.s.	36.16 ± 0.66	n.s.
20:4/18:2	1.79 ± 0.28	2.02 ± 0.26	2.09 ± 0.22	$P < 0.08$	2.36 ± 0.21	$P < 0.001$
DBI	157.4 ± 8.4	162.4 ± 4.9	165.3 ± 6.0	$P < 0.08$	162.1 ± 2.7	n.s.

Data are expressed as mean of eight individual determinations ± S.D. Analysis of the samples was done as described in Section 2. n.s., non-significant effect of GH treatment or no sex difference. GH, growth hormone treatment; GH0, no GH treatment; GH1, low-dosage treatment; GH2, high-dosage treatment.

[a–c] Significant differences between GH treatments within each group at $P < 0.05$ (Student–Newman–Keuls test).

important reduction in the 16:1$n-7$ and 18:1$n-7$ proportions, while 18:1$n-9$ was unchanged. Total PUFAs were significantly increased by 4% by GH2 treatment, primarily due to an increase in 20:4$n-6$ (by 10%), while 18:2$n-6$ and 22:6$n-3$ remained unchanged and 20:3$n-6$ was decreased by 26% with GH2 treatment. In these GH2-treated animals, the double bond index (DBI) and the 20:4$n-6/18:2n-6$ ratio were only slightly increased ($P = 0.07$ for both variables) as compared with sham-operated untreated rats. Besides, total amounts of $n-3$ and $n-6$ fatty acids were not significantly altered by this treatment. In all cases, GH effect is dose-dependent (Table 2).

As compared to sham-operated untreated males, females showed very significant differences in the proportion of the major fatty acids (Table 2). In fact, 18:0 was dramatically increased by 64% in females and 16:0 was concomitantly decreased by 37%. Total SFAs were increased by 9%. Total MUFAs were decreased by 42% in females due to an important reduction in all the MUFA proportions (16:1$n-7$, 18:1$n-9$ and 18:1$n-7$ being decreased by 65, 23 and 68%, respectively). Total PUFAs were slightly, but significantly, increased by 2% in females as compared to males, primarily due to a 7% increase in 20:4$n-6$ and to a 24% increase in 22:6$n-3$, while 18:2$n-6$ was decreased by 14%. Consequently, the total amount of $n-3$ (but not $n-6$) fatty acids was enhanced by 19% in females as compared to males. The 20:4$n-6/18:2n-6$ ratio was increased by 32% in females, but the DBI showed no significant sex difference.

3.3. Effects of hypophysectomy and hormone replacements on fatty acid composition of hepatic microsomal total phospholipids of male rats

Hypophysectomy of male rats had the opposite effect to that observed after GH treatment. It significantly decreased the proportion of total SFAs (by 5%), especially 18:0 (by 11%), while the 16:0 proportion remained unchanged (Table 3). Total MUFAs were increased by 19% in hypophysectomized rats, with an important increase of the 18:1$n-9$ proportion (by 62%) while 16:1$n-7$ and 18:1$n-7$ were decreased (by 47 and 41%, respectively). Hypophysectomy significantly decreased both 20:4$n-6$ and 22:6$n-3$ by 35%, and increased the 18:2$n-6$, 18:3$n-3$ and 20:3$n-6$ proportions by 63, 155 and 92%, respectively. The 22:4$n-6$, 22:5$n-6$ and 22:5$n-3$ proportions were also significantly reduced (Table 3). Nevertheless, total PUFAs were unchanged. Hypophysectomy decreased dramatically the 20:4$n-6/18:2n-6$ ratio (by 60%) and the DBI by 24%. The $n-3$ PUFAs were also significantly reduced (by 27%) in hypophysectomized animals.

GH treatment of hypophysectomized animals tended to reverse the effects of hypophysectomy for most of the different fatty acid relative concentrations in a dosage-dependent manner and GH2 was sufficient to restore entirely the amounts of 18:0, 20:3$n-6$, 20:5$n-3$ and 22:6$n-3$, total SFAs, total MUFAs (but not each MUFA taken separately) and the $n-3$ fatty acid family. GH treatment alone was not sufficient to completely restore either the 18:2$n-6$ or the 20:4$n-6$ proportions, nor the 20:4$n-6/18:2n-6$ ratio and the DBI to the level of sham-operated rats.

Complete restoration of the 14:0, 18:2$n-6$, 18:3$n-3$ and 20:4$n-6$ proportions and of the 20:4$n-6/18:2n-6$ ratio to the level of sham-operated rats was achieved in hypophysectomized rats with CT treatment administered alone. However, concomitant treatment of hypophysectomized rats with CT and GH1 was necessary for a complete restoration of 22:4$n-6$ proportion. For 16:1$n-7$ and 18:1$n-7$, the restoration was far from complete, regardless of the treatment used. 18:1$n-9$ proportion and DBI were restored with concomitant CT and GH2 treatments in hypophysectomized rats.

3.4. Sex differences and effects of GH treatment, hypophysectomy and hormonal replacements on Δ^9-, Δ^5-, Δ^6-desaturase and palmitic acid elongation activities

In sham-operated animals, GH treatment increased palmitic acid elongation activity (by 40% with GH2 treatment) and Δ^5-desaturase activity (by 52% with GH2 treatment) in a dose-dependent manner (Fig. 1). In contrast, Δ^9-desaturase activity was significantly depressed (by 41%) with GH2 treatment only. Δ^6-Desaturase activity was not significantly changed, whatever the concentration of GH used.

As compared to untreated males, females showed a

Table 3
Effects of hypophysectomy and hormonal replacements on fatty acid composition of total phospholipids of liver microsomes in male rats

Fatty acids	Controls	Hypophysectomized							
	CT−	CT−				CT+			
	GH0	GH0	GH1	GH2	GH effect	GH0	GH1	GH2	GH effect
14:0	0.28 ± 0.06	*0.41 ± 0.09* [d]	0.47 ± 0.22	0.44 ± 0.14	n.s.	**0.28 ± 0.04** [e]	**0.24 ± 0.09**	**0.25 ± 0.03**	n.s.
16:0	20.59 ± 0.86	20.72 ± 0.75	22.22 ± 1.05	20.43 ± 1.15	n.s.	20.04 ± 0.80	18.47 ± 1.68	15.50 ± 1.97	n.s.
16:1*n* − 7	1.77 ± 0.35	*0.94 ± 0.20*	1.16 ± 0.53	0.98 ± 0.30	n.s.	0.81 ± 0.14 [a]	0.49 ± 0.22 [b]	0.35 ± 0.06 [b]	$P < 0.001$
18:0	21.06 ± 1.19	*18.66 ± 1.44* [a,b]	17.68 ± 2.13 [a]	**19.76 ± 0.90** [b]	$P < 0.05$	23.89 ± 0.73	24.70 ± 1.77	25.74 ± 2.51	n.s.
18:1*n* − 9	6.42 ± 0.91	*10.42 ± 0.57* [a]	9.79 ± 0.57 [a]	8.01 ± 0.69 [b]	$P < 0.001$	8.75 ± 1.01 [a]	8.52 ± 0.81 [a]	**7.19 ± 0.66** [b]	$P < 0.05$
18:1*n* − 7	4.10 ± 0.48	*2.42 ± 0.23* [a]	2.86 ± 0.21 [a,b]	3.31 ± 0.73 [b]	$P < 0.01$	2.67 ± 0.22	2.61 ± 0.33	2.43 ± 0.27	n.s.
18:2*n* − 6	12.28 ± 1.40	*20.01 ± 1.27* [a]	19.10 ± 1.07 [a]	15.61 ± 1.68 [b]	$P < 0.01$	**13.14 ± 0.46**	**13.01 ± 0.74**	**12.64 ± 0.65**	n.s.
18:3*n* − 3	0.09 ± 0.04	*0.23 ± 0.05*	0.20 ± 0.04	0.17 ± 0.06	n.s.	**0.08 ± 0.02**	**0.11 ± 0.04**	**0.09 ± 0.02**	n.s.
20:3*n* − 6	1.33 ± 0.17	*2.55 ± 0.34* [a]	2.35 ± 0.64 [a]	**1.41 ± 0.20** [b]	$P < 0.001$	**1.34 ± 0.12** [a]	**1.04 ± 0.17** [a]	0.66 ± 0.10 [b]	$P < 0.001$
20:4*n* − 6	21.99 ± 0.78	*14.23 ± 1.22* [a]	15.78 ± 1.89 [b]	19.39 ± 1.85 [c]	$P < 0.001$	**21.72 ± 1.17** [a]	**22.75 ± 1.77** [a,b]	**23.60 ± 1.45** [b]	$P < 0.08$
20:5*n* − 3	0.56 ± 0.08	*0.83 ± 0.26* [a]	**0.56 ± 0.17** [b]	**0.66 ± 0.11** [a,b]	$P < 0.05$	0.37 ± 0.04 [a,b]	**0.41 ± 0.04** [a]	0.26 ± 0.09 [a,b]	$P < 0.05$
22:4*n* − 6	0.39 ± 0.03	*0.19 ± 0.03*	0.24 ± 0.05	0.22 ± 0.03	n.s.	0.30 ± 0.04	**0.33 ± 0.02**	**0.33 ± 0.05**	n.s.
22:5*n* − 6	0.58 ± 0.11	*0.26 ± 0.13*	0.26 ± 0.08	0.36 ± 0.17	n.s.	0.34 ± 0.11	0.29 ± 0.06	0.32 ± 0.12	n.s.
22:5*n* − 3	0.89 ± 0.12	*0.60 ± 0.13*	0.65 ± 0.09	0.55 ± 0.14	n.s.	0.47 ± 0.09	0.60 ± 0.03	0.48 ± 0.08	n.s.
22:6*n* − 3	6.72 ± 0.88	*4.37 ± 0.67* [a]	5.44 ± 0.72 [b]	**6.45 ± 0.74** [c]	$P < 0.001$	4.72 ± 0.38 [a]	5.27 ± 0.34 [a,b]	**5.78 ± 0.52** [b]	$P < 0.001$
SFA	42.03 ± 0.67	*40.04 ± 0.98*	40.29 ± 1.84	**40.93 ± 1.80**	n.s.	44.26 ± 0.99	**43.47 ± 1.29**	44.93 ± 1.21	n.s.
MUFA	12.68 ± 0.76	*15.06 ± 1.56* [a]	14.47 ± 0.53 [a]	**13.26 ± 1.29** [b]	$P < 0.05$	**12.58 ± 0.88** [a]	**12.17 ± 1.03** [a]	10.52 ± 0.86 [b]	$P < 0.01$
PUFA	45.30 ± 0.78	45.47 ± 1.31	45.29 ± 1.75	45.81 ± 1.93	n.s.	43.15 ± 1.62	44.77 ± 1.92	44.96 ± 1.47	n.s.
$\sum n-3$	8.26 ± 0.85	*6.04 ± 0.91* [a]	6.80 ± 0.59 [a]	**7.82 ± 0.80** [b]	$P < 0.001$	5.62 ± 0.44 [a]	6.38 ± 0.39 [b]	6.61 ± 0.50 [b]	$P < 0.001$
$\sum n-6$	36.48 ± 0.87	37.80 ± 1.62	37.84 ± 1.38	37.97 ± 1.34	n.s.	37.05 ± 1.38	37.96 ± 1.78	37.43 ± 0.84	n.s.
20:4/18:2	1.79 ± 0.28	*0.71 ± 0.07* [a]	0.88 ± 0.19 [b]	1.26 ± 0.22 [c]	$P < 0.001$	**1.65 ± 0.08** [a]	**1.76 ± 0.19** [a,b]	**1.87 ± 0.15** [b]	$P < 0.05$
DBI	157.40 ± 8.4	*119.40 ± 4.4* [a]	132.60 ± 13.0 [b]	145.10 ± 10.1 [c]	$P < 0.001$	140.60 ± 5.6 [a]	148.00 ± 6.5 [a,b]	**150.20 ± 5.4** [b]	$P < 0.01$

Data are expressed as the mean of eight individual determinations ± S.D. Analysis of the samples was done as described in Section 2: Materials and methods. CT, cortisol and thyroxine treatment. CT−, CT-untreated animals. CT+, CT-treated animals. Controls are sham-operated untreated male rats. GH, growth hormone treatment; GH0, no GH treatment; GH1, low-dosage treatment; GH2, high-dosage treatment. n.s., non-significant effect of GH treatment in the following groups of animals: hypophysectomized CT-untreated rats or hypophysectomized CT-treated rats.

[a−c] Significant differences between GH treatments within each group at $P < 0.05$ (Student–Newman–Keuls test).

[d] Italic values show a significant effect of hypophysectomy at $P < 0.05$.

[e] Bold values show the necessary combination of hormonal treatments of hypophysectomized animals for a restitution of the control values (statistically not different from sham–operated untreated animals) with Student–Newman–Keuls tests.

reduced Δ^9-desaturase activity (by 21%). The other activities tested were not significantly different between male and female rats (Fig. 1).

All the activities tested, except for Δ^6-desaturase, were significantly depressed by hypophysectomy: by 81, 43 and 38% for Δ^9-, Δ^5-desaturase and palmitic acid elongation activities, respectively (Fig. 1).

In hypophysectomized rats, Δ^5-desaturase activity values of control animals were restored with GH2 or CT treatment. The other activities altered after hypophysectomy were not significantly modified by GH treatment administered alone at any concentration. The effect of hypophysectomy was completely reversed for Δ^9-desaturase activities with CT treatment, but CT and GH2 treatments administered together were necessary to reverse the depressing effect of hypophysectomy for palmitic acid elongation activity (Fig. 1).

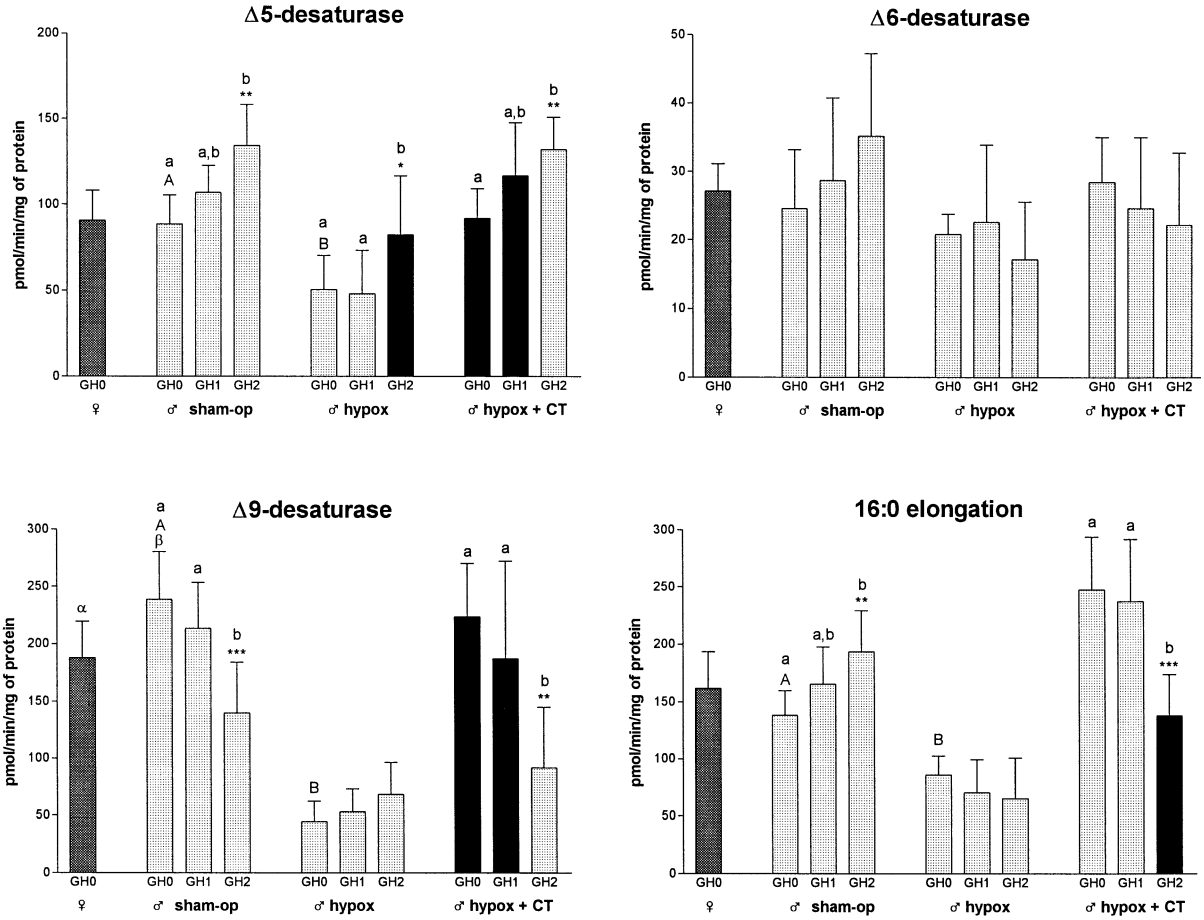

Fig. 1. Effect of GH treatment, sex, hypophysectomy and hormonal replacements on microsomal Δ^9-, Δ^5-, Δ^6-desaturase and palmitic acid elongation activities. Activities were measured as described in Section 2: Materials and methods. Values are the mean of eight individual determinations ± S.D. CT, cortisol/thyroxine treatment; GH, growth hormone treatment; GH0, no GH treatment; GH1, low-dosage treatment; GH2, high-dosage treatment; hypox, hypophysectomized rats; sham-op, sham-operated rats; ♂, male rats; ♀, female rats. Asterisks show a significant effect of GH in the following groups of animals: sham-operated male rats, hypophysectomized CT-untreated rats and hypophysectomized CT-treated rats with * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, respectively. a and b show significant differences between GH treatments within each group at $P < 0.05$ (Student–Newman–Keuls test). α and β show a significant effect of sex at $P < 0.05$. A and B show a significant effect of hypophysectomy at $P < 0.05$. Black bars represent the necessary combination of hormonal treatments of hypophysectomized rats for a restoration of the control values (statistically not different from sham-operated untreated rats) with Student–Newman–Keuls tests.

3.5. Relative importance of the different treatments used

MANOVA performed on the various fatty acid proportions was highly significant ($P < 0.0001$). The pseudo-*F*-value associated with this multivariate analysis was larger than any *F*-value associated with the univariate analysis of fatty acids, which enabled us to pursue a discriminant analysis on this set of variables. The first factorial map (Fig. 2) constructed from the first and the second linear discriminants accounted for 82.3% of the total variance (or information) (first component, 67.0%; and second component, 15.3%). The third, fourth and fifth linear discriminants represented 9.7, 3.7 and 2.4% of the total variance, respectively. The third to fifth linear discriminants were significant, but represented a low amount of information. Besides, they were explained by only minor fatty acids. For this reason and because they summarized an insufficient amount of information, they were excluded from the discriminant analysis interpretation. The first principal component was well explained by 20:4$n-6$ (correlation: −96%), 18:2$n-6$ (+95%), 14:0 (+92%), 18:3$n-3$ (+86%), 20:3$n-6$ (+86%), 18:0 (−79%), 22:4$n-6$ (+70%) and by 18:1$n-9$ (+69%). The second linear component was explained by 22:6$n-3$ (+64%), 20:5$n-3$ (+50%), 18:1$n-7$ (+48%), 22:5$n-3$ (+47%) and to a lesser extent by 16:1$n-7$ (+34%). Analysis of the projection of individuals on the first linear component gave a clear opposition between the group of hypophysectomized animals on the one hand, and the groups of sham-operated rats and hypophysectomized CT-treated rats on the other. Yet, there was no superimposition of the group of hypophysectomized CT-treated animals on the group of sham-operated animals when they were projected on the second factorial axis. In order to give a hierarchy between the effect of hypophysectomy, GH and CT, Mahalanobis distances (Euclidean distances corrected for correlations between initial variables) were calculated. Hypophysectomy appeared to have the major influence on the perturbation of microsomal fatty acid composition. CT effect was very sig-

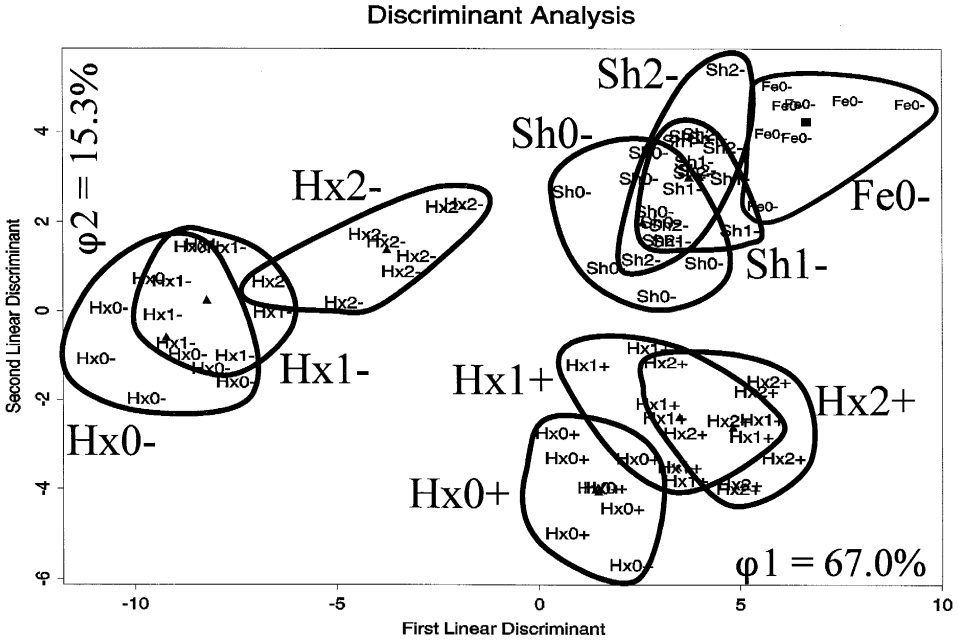

Fig. 2. Projection on the first factorial map built by the first two linear discriminant axes of the data of fatty acid compositions of individuals characterized by their belonging to the different treatment groups. The first and the second linear discriminants explain 67.0 and 15.3% of the total variance, respectively. Black triangles and square represent the location of group means for male and female rat groups, respectively. The female rat group is projected on the factorial map as a supplementary rat group. Sh, sham-operated rats; Hx, hypophysectomized rats; Fe, female rats; 0, control rats for GH treatment (GH0); 1, rats treated with the low GH dosage treatment (GH1); 2, rats treated with the high GH dosage treatment (GH2); +, rats treated with cortisol/thyroxine (CT) treatment; −, control rats for CT treatment.

nificant in hypophysectomized rats. In fact, for most of the major fatty acids (which explained the first linear component), CT treatment seemed to be sufficient in getting hypophysectomized rats superimposed to sham-operated controls. Nevertheless, this treatment was not sufficient to recover control values for 18:1$n-7$, 22:6$n-3$ and 16:1$n-7$ (explaining the second discriminant axis). GH1 and GH2 effects appeared to be also highly significant on microsomal fatty acid composition, the GH2 effect being more important in hypohysectomized CT-untreated rat than in hypophysectomized CT-treated or sham-operated rats (Fig. 2).

The female animals, projected as a supplementary group, tended to be superimposed on the GH2-treated sham-operated animal group. Moreover, when the attribution of the female individuals in the different male groups was tested, all the females were attributed to this sham-operated GH2-treated rat group. This clearly summarizes a 'feminization' process of the microsomal fatty acid pattern related to continuous GH exposure (GH2 treatment).

4. Discussion

4.1. GH-dependent 'feminization' of microsomal fatty acid composition

The fatty acid composition of microsomal phospholipids in sham-operated young adult Sprague–Dawley rats was comparable with that already described for the same strain and the same age animals [31]. In the present study, physiological dosages of GH were chosen in order to restore a male-like or a female-like secretion pattern of GH in hypophysectomized rats [32]. As such, the GH1 dosage chosen in our experiments is commonly used to mimic, in hypophysectomized rats, the pulsatile GH pattern present in male rats [33] and the GH2 dosage, which is 8 times higher than that of GH1, was chosen in order to determine, in male rats, a continuous GH exposure, as is the case in females. This GH2 dosage was sufficient to mimic female secretion profile in males, as shown by a female-predominant steroid metabolization activity (Δ^4-3-oxosteroid 5α-reductase) obtained at the same time in GH2-treated animals (unpublished data). Our results show that, in sham-operated animals, GH decreased total MUFAs by 12 and 20% with GH1 and GH2 treatments, respectively. Concomitantly, GH increased total PUFA amount with an increase in the 20:4$n-6$ proportion, but not in the 22:6$n-3$ one, while fatty acid chain length was increased in SFAs. Yet, the DBI, which reflects the membrane unsaturation state seemed to be increased ($P=0.07$) by this treatment. These results are in accordance with those of others authors [13], in which a larger dose of GH (10 IU/kg b.wt.) significantly increased the DBI, the 20:4$n-6$/18:2$n-6$ ratio and the 22:6$n-3$ proportion in the microsomal membrane. In our study, the more limited influence of GH on these latter parameters could be related to a dosage-dependent effect of GH on fatty acid composition of microsomal membrane. In fact, with the nearly physiological concentration of GH used, we have noted a dose-related effect for some other parameters: MUFAs, 20:4$n-6$, 18:1$n-7$, 16:0 and 18:0.

These results, together with the superimposition of the female group on the GH2-treated sham-operated male rat group observed in the discriminant analysis (Fig. 2), are in accordance with a 'feminization' process of the fatty acid composition of the microsomal membrane, since female rats show lower proportions of 16:0, 18:2$n-6$ and MUFAs, higher proportions of 18:0, 20:4$n-6$ and 22:6$n-3$ and a higher 20:4$n-6$/18:2$n-6$ ratio, as compared to male rats. This 'feminization' process appears to be dose-dependent in intact males. Such a dose-dependent 'feminization' process with GH was also reported by others [12] for the 'feminization' of fatty acid composition of liver total phosphatidylcholine in hypophysectomized female rats submitted to GH infusion.

Modifications of desaturation and/or elongation rates could account for the effects of GH treatment on the fatty acid composition of microsomal membranes, since GH caused important changes in Δ^9-, Δ^5-desaturase activities and in palmitic acid elongation rate. The increase in Δ^5-desaturase activity induced by GH could explain the enhanced 20:4$n-6$ proportion and the reduced 20:3$n-6$ one, this latter fatty acid being provided by a desaturation/elongation step involving Δ^6-desaturase which is not enhanced by GH treatment. The increase in palmitic acid elongation activity could account for the increase of the 18:0/16:0 ratio, although the decrease

in 16:0 could also be partially explained by an effect of GH treatment on fatty acid synthase activity, which was reported to be decreased by GH in cultured rat hepatocytes [10]. The decrease in Δ^9-desaturase activity (measured with 16:0 as substrate) could explain the reduced microsomal $16{:}1n-7$ proportion, but surprisingly, the $18{:}1n-9$ concentration, which should also depend on Δ^9-desaturase activity, is not modified by any GH treatment. Δ^9-desaturase activity, which was significantly reduced in females as compared to males, could explain the reduced MUFA proportions. Surprisingly, the other desaturation activities and the palmitic acid elongation activity presented no significant sex differences that could have accounted for the sex differences observed in major PUFAs and SFAs. Sex differences in microsomal fatty acid composition have been reported by others and were attributed to the effect of testosterone on the various desaturation activities [14]. In fact, these authors showed that testosterone had depressing effects on Δ^5- and Δ^6-desaturase activities and an activating effect on Δ^9-desaturase activity. For some other hepatic activities, the testosterone effect on some cytochrome P-450-mediated steroid hydroxylation activities is known to be exerted via its direct effect on the GH secretion pattern [16,34]. This GH secretion pattern shows, in male rats, few high-amplitude secretion peaks and complete and prolonged suppression of circulating GH levels during the trough period after a GH surge, while in females, it shows frequent low-amplitude secretion peaks that result in persistent GH circulating levels [15,32,35]. Administration of exogenous GH may interfere, in males, with testosterone regulation of GH secretion pattern mainly via hypothalamic somatostatin release [32,34,36] by removing these periods of suppression of GH circulating levels. In the same way, we can suggest that the continuous stimulation of GH in these animals (GH2 treatment), which results in a 'feminization' of the fatty acid composition of the microsomal membrane, parallels the modifications observed in the hepatic steroid hydroxylation and testosterone 5α-reductase patterns of these animals, particularly by increasing the testosterone 5α-reductase activity and lowering testosterone 2α- and 16α-hydroxylase activities (unpublished data). Such a 'feminization' of hepatic steroid metabolism in male rat with a continuous GH infusion has been well documented [34,36,37]. This may indicate that the modulation of the Δ^5-, Δ^9-desaturase and palmitic acid elongation activities is mediated by the secretion pattern of GH, as it has been shown for CYPIIC12 (responsible for the steroid 15β-hydroxylase activity) and CYPIIC11 (responsible for the testosterone 2α- and 16α-hydroxylase activities) [15,33]. In the same way, in hypophysectomized animals whose lack of thyroid hormones and glucocorticoids has been compensated by CT treatment, the GH2 treatment, that determined a continuous exposure to GH, tended to 'feminize' major SFA and PUFA proportions, when compared to the effects induced by GH1 treatment (Table 3 and Fig. 2). However, the complete assessment of the 'feminization' of the Δ^5-, Δ^9-desaturase and palmitic acid elongation activities depending on GH pulsatility should be achieved by the study of the conditions of 'masculinization' of hepatic microsomal fatty acid composition in hypophysectomized female rats.

4.2. GH coregulation with other pituitary-dependent factors of fatty acid composition and desaturase / elongase activities

Hypophysectomy dramatically decreased the $20{:}4n-6/18{:}2n-6$ ratio and the relative proportion of $22{:}6n-3$ in hepatic microsomal membrane. The same effects were described by others in rat hepatic mitochondrial membrane [11]. These modifications are in agreement with the results obtained on Δ^5-desaturase activity which is dramatically reduced (by 52%) and may become rate-limiting for the biosynthesis of $20{:}4n-6$. This modification could also account for the accumulation of $20{:}3n-6$ (increased by 92%) in hypophysectomized rats. The deeply reduced Δ^9-desaturase activity in these rats may explain the decrease in $16{:}1n-7$ proportion. Reduction of 18:0 (by 11%) following hypophysectomy is correlated with the reduced palmitic acid elongation rate. These modifications of the various microsomal desaturase activities due to hypophysectomy are consistent with the previous findings of Clejan and Maddaiah [11], but the effects observed in our study are more pronounced. Interestingly, these authors showed that microsomal total fatty acid elongation activity was enhanced by hypophysectomy, while our results show

a decrease in palmitic acid elongation rate. However, it has been shown that there are at least two distinct elongation systems: one for the saturated fatty acids and another for the unsaturated ones [38]. Clejan and Maddaiah [11] showed that bovine GH administration (1 IU/kg b.wt.) was sufficient to reverse the effects of hypophysectomy on fatty acid composition of mitochondrial membrane and on desaturase activities (except for Δ^9-desaturase activity when it is not induced by sucrose) [11]. Our results clearly show that GH treatment reversed the effects of hypophysectomy at least partly, while cortisol and/or thyroxine treatments were important for the recovery of the fatty acid composition of the microsomal membrane in hypophysectomized rats to the level of controls, as shown by the superimposition of the CT-treated hypophysectomized rat group on the sham-operated control one when projected on the first linear discriminant (Fig. 2). Moreover, CT treatment was a necessary physiological condition for the expression of the GH effect on Δ^9-desaturase and palmitic acid elongation activities in hypophysectomized animals. The observation that some MUFA proportions were not restored after hypophysectomy to the control values with CT and GH treatments could indicate that other pituitary factors are of importance in the regulation of microsomal membrane composition. Besides, Δ^9-desaturation activity may not be the only mechanism regulating the amount of MUFAs since this activity was restored by CT treatment after hypophysectomy. Thyroidectomy was shown to decrease dramatically Δ^9-, Δ^5- and Δ^6-desaturase activities, and consequently, to modify the fatty acid composition of microsomal membrane [39]. These effects, which are comparable to those observed after hypophysectomy, are reversed by thyroid hormone treatment and underline the importance of these hormones for the regulation of desaturation activities. This effect of thyroidectomy could be indirect by a great reduction of GH secretion, as it was demonstrated that thyroidectomy dramatically decreased GH secretion in rodents [40,41]. Nevertheless, our results clearly show that thyroxine and/or cortisol are also essential endocrine conditions involved in fatty acid desaturation regulation. The effect of hypophysectomy is the result of the deprivation of several pituitary factors. Some of these, as adrenocorticotropic hormone (ACTH) and glucocorticoids, have a depressing effect on Δ^5- and Δ^6-desaturase activities [42,43] and some others, as GH and thyroid hormone, have an enhancing effect on these activities, as demonstrated by our results. In the case of the Δ^5-desaturase activity, the effect of the enhancing hormones seems to be the most important, since the overall effect of hypophysectomy induces a marked decrease in this activity. In the case of the Δ^6-desaturase activity, the respective effects of depressive and enhancing hormones seem to be more balanced since no overall effect of hypophysectomy can be shown in this study.

Finally, the regulation of fatty acid composition of microsomal membrane involves, besides dietary factors, several hormonal systems which are overlapped and not additive. GH and thyroid hormones play an important role in this regulation. The exact mechanism by which GH modifies the desaturation/elongation systems and consequently the fatty acid composition of microsomal membrane is still unknown. Therefore, Waxman et al. [44] and Ram et al. [45] have very recently shown that intermittent GH pulsation (male GH secretion pattern) could trigger tyrosine phosphorylation and the nuclear translocation of a liver-expressed DNA-binding protein. Such a nuclear regulation factor could be invoked for the transcription regulation of the genes of these desaturation/elongation systems. The fact that GH treatment can 'feminize' the hepatic fatty acid composition of microsomal membrane via the modulation of the desaturation/elongation activities, as it 'feminizes' the hepatic steroid metabolism, could indicate that the secretion pattern of GH is implicated in the regulation of these desaturation/elongation activities.

In conclusion, the 'feminization' process of the microsomal fatty acid composition in male rats treated with GH only slightly affects overall unsaturation in fatty acids, but it increases their carbon chain length. The consequences of such global modifications on the structure and the functions of microsomal membrane, particularly on some membrane-bound enzymes and on membrane-bound receptors or molecule carriers, which are known to depend on environmental factors, such as membrane integrity and fluidity [46], remain to be established. The modifications in the proportion of special fatty acids, such as $20{:}4n-6$, which is the precursor of numerous compounds of biological importance such as eicosanoids and of reactive lipoperoxidation products, such as 4-hy-

droxynonenal, could also have important consequences that have to be determined.

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