Δ6- and Δ5-desaturase activities in the human fetal liver: kinetic aspects

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Abstract **Δ6- and Δ5-desaturase activities were studied in human fetal liver microsomes obtained after legally approved therapeutic abortion. Enzyme activities were measured by a radiochemical method using reverse-phase high performance liquid chromatography (HPLC). Free and phospholipid fatty acids were assessed in each liver sample by a combination of thin-layer chromatography (TLC) and gas–liquid chromatography (GLC) procedures. The kinetic measurements showed higher Δ6-desaturase activity for the n–3 series than for the n–6 series. Apparent K_m of 6.5, 3.9, and 24.5 μm and V_m of 7.5, 9.1, and 24.4 pmol·min^{-1}·mg^{-1} were obtained, respectively, for 18:2n–6 Δ6-, 20:3n–6 Δ5-, and 18:3n–3 Δ6-desaturases. Beyond 30, 20, and 60 μm of 18:2n–6, 20:3n–6, and 18:3n–3 concentration, respectively, the enzyme activity deviated from Michaelis-Menten kinetics, suggesting an inhibition by excess substrate which is unlikely to occur in vivo as endogenous substrate concentration is much lower. We observed a breakdown in linearity between desaturase activity and microsomal protein concentration beyond 4–5 mg microsomal protein, whatever the enzyme or substrate. Both this phenomenon and the inhibition due to excess substrate should be taken into account in the determination of Δ6- and Δ5-desaturase activities. Comparison of concentrations of the respective endogenous substrates and the kinetic constants of each enzyme suggested that the higher Δ6-desaturase activity observed for the n–3 series than for the n–6 series is not physiologically relevant in human fetal liver.—**Rodriguez, A., P. Sarda, C. Nessmann, P. Boulot, C. L. Leger, and B. Descomps. **Δ6- and Δ5-desaturase activities in the human fetal liver: kinetic aspects. *J. Lipid. Res.* 1998.** 39: **1825–1832.**

Supplementary key words kinetic • desaturase activity • human fetus • liver • microsomes • endogenous substrate

During gestation and just after birth, human development requires a large supply of polyunsaturated fatty acids (PUFAs) (1), especially for central nervous system, brain, and retina (2). During gestation, PUFAs are supplied to the fetus through the placenta or by fetal synthesis, essentially in the liver through the elongation/desaturation of the n–6 and n–3 series fatty acid (FA) precursors: linoleic (18:2n–6) and α-linolenic (18:3n–3) acids, respectively. Desaturases are key enzymes in the regulation of unsaturated FA biosynthesis and are mainly present in liver cell endoplasmic reticulum. The Δ6-desaturase is involved in the bioconversion of 18:2 into 18:3 and 24:4 into 24:5 in the n–6 series, and of 18:3 into 18:4 and 24:5 into 24:6 in the n–3 series (3). Though research about the regulation of biosynthesis and cell trafficking of C24 PUFAs are in progress both in n–3 (4) and n–6 series (5) very little is known about the kinetics of C24 substrate conversion. The Δ5-desaturase is involved in the last step of arachidonic acid (AA, 20:4n–6) biosynthesis from dihomo-γ-linolenic acid (20:3n–6), contributing to the balance between the prostanoids of the 1- and 2-series. Both Δ5- and Δ6-desaturases are highly dependent on numerous nutritional and hormonal factors (6).

Little is known about desaturase activities in human fetal liver. In newborns liver, a preliminary in vitro study showed that Δ5- and Δ6-desaturase activities could be detected, but were lower than in human adults or animals, especially rodents (7). An inhibition due to high substrate concentration was suspected (7), but was not detected by others (8). The relative efficiencies of bioconversion in the n–6 and n–3 series are still under discussion (9, 10).

Few if any kinetic studies of desaturases in humans have been carried out, but several studies have been conducted in rats. The aim of this work was to better characterize Δ5- and Δ6-desaturase activities in human fetal liver. Attention was particularly focused on *i)* the comparison of the bio-

Abbreviations: EFA, essential fatty acid; HPLC, high performance liquid chromatography; GLC, gas–liquid chromatography; TLC, thin-layer chromatography; FA, fatty acid; PUFA, polyunsaturated fatty acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FFA, free fatty acid; PL, phospholipid.

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conversion efficiency in the two series, *ii)* an inhibition of desaturase activities possibly due to excess substrate, and *iii)* the influence of microsomal protein variation on the desaturase activities, as was recently reported by Iraszu, Gonzalez-Rodriguez, and Brenner (11) in rat kidney. The activities of the n–6 and n–3 pathways were discussed in terms of the kinetic constants of Δ6- and Δ5-desaturases, and the respective substrate concentrations were estimated as the microsomal free fatty acid (FFA) fraction.

MATERIALS AND METHODS

Biological samples

Biological samples were obtained from the Fetal Pathology Centers of CHU Lapeyronnie-Arnaud de Villeneuve, Montpellier, and CHU Robert Debré, Paris, France after legally approved medical abortion, according to French law. These therapeutic abortions were obtained by oral dose of Myfegyne® (mifepristone, 600 mg) and/or Misoprostol® (cytotec, 200 mg/4 h) and Syntocinon® drip. The abortions were permitted for three reasons: fetal unviability, polymalformations, and 18 or 21 trisomy. The fetuses were 22 to 26 weeks old and the liver samples were obtained within 1–4 h of fetal death. Eight liver samples were used in the experiments reported in Tables 1 and 2 and three samples in Table 3.

Preparation of microsomes

The liver samples were washed in ice-cold 0.15 M NaCl, blotted on filter paper, weighed (4–5 g,) and then deep-frozen in liquid nitrogen. They were cut into thin slices, homogenized at 4°C in 6 volumes of 0.05 M phosphate buffer (pH 7.4) with 0.25 M sucrose solution. The crude homogenate was centrifuged at 13,000 *g* for 20 min; the pellet was discarded and the supernatant was centrifuged again at 105,000 *g* for 60 min in a Kontron model T 1075 ultracentrifuge (Kontron International CH-8048 Zurich), to obtain the microsomal pellet. The microsomal fraction was resuspended in 0.4 ml of supernatant and 0.8 ml of 0.05 M phosphate buffer (pH 7.4) with 0.25 M sucrose solution for determination of desaturase activities. These preparation steps were performed at 4°C. The microsomal protein concentrations were estimated by the method of Layne (12) with fatty acid-free crystalline bovine serum albumin as standard.

Enzymatic assays

Before enzyme activity measurement, each substrate: [1-^{14}C] linoleic acid ([1-^{14}C]18:2n–6), [1-^{14}C]α-linolenic acid ([1-^{14}C]18:3n–3), and [1-^{14}C]eicosatrienoic acid ([1-^{14}C]20:3n–6) (40–60 mCi/mmol, 99% pure, NEN Life Science Products-France S.A., Le Blanc Mesnil, France) was diluted in ethanol (Prolabo, Paris, France) to a specific activity of 10 mCi/mmol with the corresponding unlabeled fatty acid. The desaturation of fatty acids was measured by estimating the conversion percentage of [1-^{14}C] 18:2n–6 to [1-^{14}C] 18:3n–6 and of [1-^{14}C]18:3n–3 to [1-^{14}C]18:4n–3 by Δ6-desaturase; and of [1-^{14}C]20:3n–6 to [1-^{14}C]20:4n–6 by Δ5-desaturase.

The effect of substrate concentration on the Δ5- and Δ6-desaturase kinetics was investigated using increasing amounts of [1-^{14}C] 18:2n–6, [1-^{14}C]18:3n–3 and [1-^{14}C]20:3n–6 from 2.86 μm to 57.2 μm, 4.76 μm to 95.24 μm, and 2.86 μm, to 38 μm, respectively. All incubations were done with 3 mg of microsomal proteins.

The effect of microsomal protein concentration on Δ5- and Δ6-desaturase activities was determined as follows. Each precursor (28.6 μm) was incubated with increasing concentrations of microsomal proteins (2–6 mg) in the incubation conditions indicated below. In a subsequent experiment, three different substrate concentrations (28.6, 34.28, and 40 μm) of [1-^{14}C] 20:3n–6 were incubated with microsomal protein at different concentrations (2–10 mg).

For each enzyme measurement, the incubations were performed at 37°C for 15 min under agitation in a total volume of 2.1 ml incubation medium containing 72 mM phosphate buffer (pH 7.4), 4.8 mM $MgCl_2$, 4.8 mM ATP, 0.5 mM coenzyme A, and 1.2 mM NADPH (cofactors were purchased from Sigma, St. Louis, MO).

The reaction was stopped by addition of 15 ml $CHCl_3$–CH_3OH 1:1 (v/v) (Prolabo, Paris, France). After evaporation to dryness under nitrogen, the lipids were saponified in 1 ml of 0.5 N NaOH in CH_3OH at 90°C for 15 min. Transesterification was performed by addition of 2 ml of 14% boron trifluoride (Sigma) in CH_3OH and incubation at 90°C for 15 min. After addition of 2 ml of saturated aqueous NaCl, the fatty acid methyl esters were extracted three times with 1 ml of isooctane (Carlo-Erba, Val de Reuil, France) as reported by Slover and Lanza (13). The solvent was then evaporated and the samples were kept in 0.5 ml of acetonitrile (Carlo-Erba) at −20°C under N_2.

The distribution of radioactivity between substrate and product was determined by a reverse-phase HPLC procedure (14) using a Beckman model 110 A pump and a Nova-Pack C18 column (60 Å, 4 μm, 3.9 × 150 mm) (Waters, MA). Elution was carried out isocratically using acetonitrile–water (Carlo-Erba) 85:15 (v/v) as mobile phase at a flow rate of 1 ml·min^{-1}. Fatty acid methyl esters were collected and their radioactivity was measured in the solvent by liquid scintillation counting (Picofluor, Packard Instrument, Rungis, France) by means of a Packard Tri-Carb Model 2425. Activity was expressed as pmol substrate converted·min^{-1} and the specific activity was expressed as pmol converted·min^{-1}·mg^{-1} of microsomal protein. At least two measurements were done on each liver sample.

Fatty acid analysis

Free fatty acid (FFA) and phospholipid (PL) fatty acid composition of liver microsomes was performed as described by Sadou et al. (15). Briefly, lipids were extracted (16), then lipid classes were separated by TLC. After transesterification without gel desorption, the fatty acid methyl esters of free fatty acid and phospholipid fractions were analyzed with a 50 m C.P. Sil 88 capillary column (Chrompak, Les Ulis, France). The conditions were as follows: ionization detector, 250°C; injector, 230°C; oven program, 3.5°C/min from 115 to 230°C. Hydrogen was used as the carrier gas with a flow rate of 1 ml·min^{-1}. Quantitative analysis was achieved with reference to the internal standard (C21:0) by using a DELSI ENICA 10 integrator (Nermag, Argenteuil, France). The percentage of microsomal phospholipid fatty acids was given by weight for the fatty acids of main interest, and the concentration of free fatty acids introduced in the incubation medium with microsomal membranes was expressed in μm. In some experiments (three different livers), the microsomal free fatty acid and phospholipid fatty acid concentrations were expressed as a function of the weight of the liver sample.

Statistics

Results are expressed as means ± SEM. Linear regression analysis of fatty acid composition was performed by computer (excel 5.0) and plotted for fetus from 20 to 26 weeks of gestation age (n = 8). Sample correlation coefficients, significance levels, and linear equations are indicated in Results section.

RESULTS

Figures 1, 2 and 3 show the activity of n–6 Δ6- and Δ5-desaturases and n–3 Δ6-desaturase from human fetal liver

as a function of substrate concentration. The three enzyme activities deviated from Michaelis-Menten kinetics at high substrate concentration. Δ6-desaturase activity increased up to 6.5 pmol·min^{-1}·mg^{-1} within a linoleic acid concentration range of 20–30 μm (**Fig. 1**), then decreased for higher substrate concentrations (−35% at 57.2 μm). The apparent K_m and V_m values determined from the linear part of the Lineweaver-Burk plot were 6.5 μm and 7.5 pmol · min^{-1}·mg^{-1} protein, respectively. Between 2.86 and 14.3 μm of 20:3n–6 concentration, Δ5-desaturase activity increased up to 8.5 pmol·min^{-1}·mg^{-1} (**Fig. 2**). Beyond 14.3 μm, the enzymatic activity dramatically declined (−60% at 38 μm), suggesting an inhibition due to excess substrate. The apparent K_m and V_m values were 3.91 μm and 9.5 pmol·min^{-1}·mg^{-1}, respectively.

In the n–3 series, maximal Δ6-desaturase activity (about 17.5 pmol·min^{-1}·mg^{-1}) was observed for substrate concentration within 40–60 μm (**Fig. 3**), markedly higher than in the n–6 series. For concentrations higher than 60 μm, the enzyme activity weakly decreased, suggesting again an inhibition due to excess substrate (−20% at 95.2 μm). The apparent K_m and V_m values were 24.5 μm and 24.4 pmol·min^{-1}·mg^{-1} protein, respectively.

The effect of varying the microsomal protein concentration on enzyme activity was tested at 28.6 μm in both the n–3 and n–6 series. As expected, when the microsomal protein concentration increased, Δ6-desaturase activity increased linearly up to 3–4 mg in both series. However, beyond this protein concentration, the linear relationship was no longer observed (**Fig. 4** A, B). A similar pattern appeared (Fig. 4 C) for Δ5-desaturase activity. The linear relationship disappeared at protein concentrations higher than 5 mg. The influence of increasing microsomal protein concentration on Δ5-desaturase activity was evaluated at three different substrate concentrations: 28.6, 34.3, and 40 μm (**Fig. 5**). This confirmed that the linear relationship between enzyme activity and microsomal protein concentration did not hold beyond 5 mg. It is important to note that the same pattern was obtained with the three different substrate concentrations and with two different livers, suggesting that substrate concentration was not responsible for the breakdown in linearity.

Phospholipid fatty acid compositions of liver sample microsomes are reported in **Table 1**. In the n–6 series, arachidonic acid appeared to be relatively constant between the 20th and 26th gestation week, whereas linoleic acid significantly increased ($y = 0.48x - 6.6$; $r = 0.85$, $P < 0.01$). Oleic acid (18:1n–9) decreased significantly ($y = -0.69x + 27.99$; $r = 0.92$, $P < 0.01$), whereas its precursors (18:0) remained stable between the 17th and 26th gestation week. Mead acid (20:3n–9) and the palmitoleic acid (16:1n–7) remained below 1 and 2%, respectively, whatever the gestation age. 18:2n–6, 18:3n–3, and 20:3n–6 were present in incubation medium as compounds of the microsomal membranes. Their concentrations in the microsomal phospholipid and FFA fractions are reported in **Table 2**. The FFAs considered to be the potential endogenous substrates of desaturase were ranked as follows: 18:2n–6 > 20:3n–6 > 18:3n–3. The tissue concentrations were calculated from the microsomal concentrations of these acids (**Table 3**).

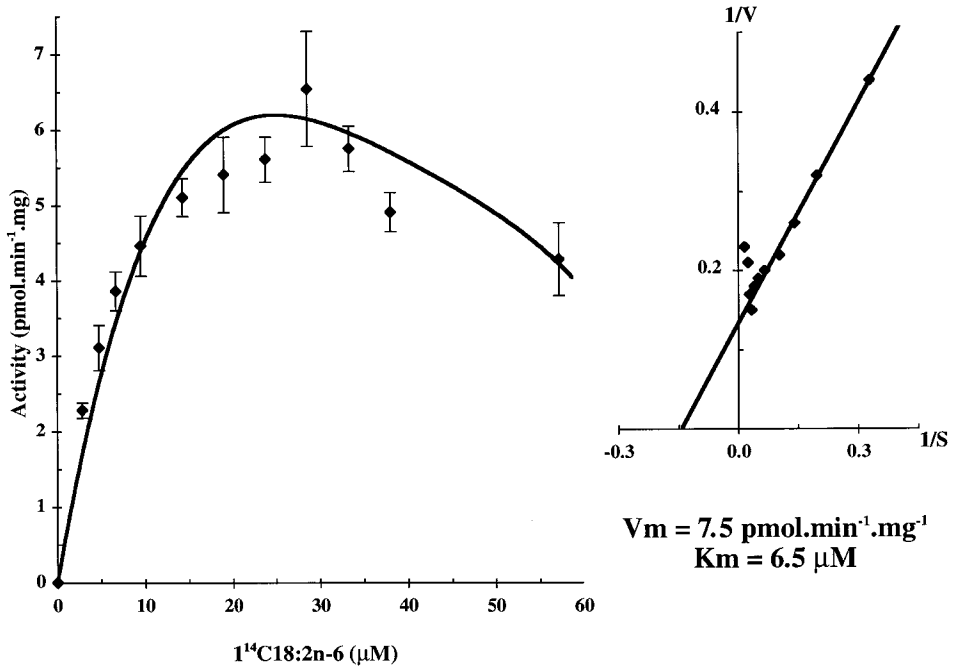

Fig. 1. Effects of [1-^{14}C]linoleic acid concentration on liver Δ6-desaturase activity. Microsomal proteins (3 mg) were incubated at 37°C for 15 min under the conditions described in Material and Methods. Figure insert is the double reciprocal plot for apparent K_m and V_m determination. Each point represents the mean ± SEM obtained from two samples from different livers (age = 24 and 25 weeks) each tested in duplicate.

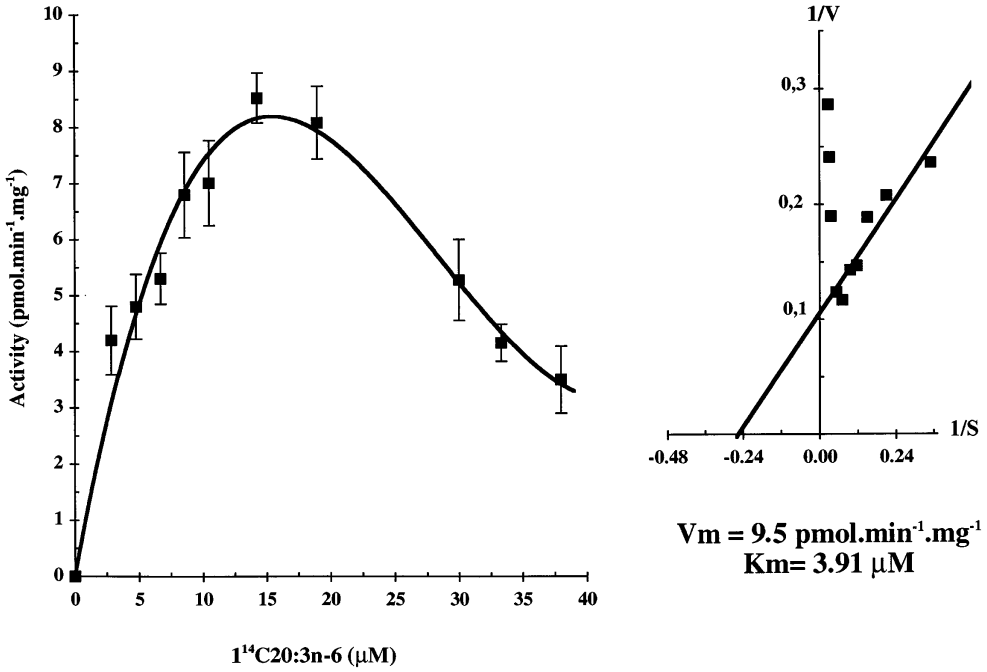

Fig. 2. Effects of [1-^{14}C]eicosa-8,11,14-trienoic acid concentration on liver Δ5-desaturase activity. Microsomal proteins (3 mg) were incubated at 37°C for 15 min under the conditions described in Material and Methods. Figure insert is the double reciprocal plot for apparent K_m and V_m determination. Each point represents the mean ± SEM obtained from two samples from different livers (age = 22.5 and 26 weeks) each tested in duplicate.

DISCUSSION

The present kinetic study was done on liver samples obtained from legally approved therapeutic abortions for chromosomal abnormalities. None of the mothers were affected with metabolic disorders or received therapeutic intervention except for the expulsion protocol. The liver samples were deep frozen in liquid nitrogen immediately after liver extraction and maintained there until processing. In these conditions, the difference in enzymatic activ-

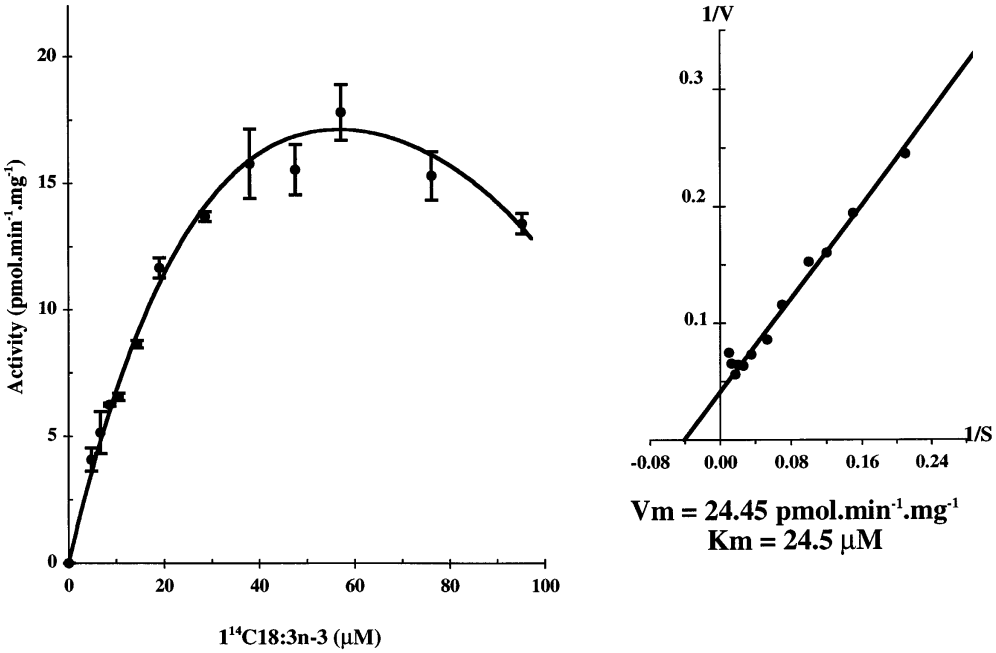

Fig. 3. Effects of [1-^{14}C]linolenic acid concentration on liver Δ6-desaturase activity. Microsomal proteins (3 mg) were incubated at 37°C for 15 min under the conditions described in Material and Methods. Figure insert is the double reciprocal plot for apparent K_m and V_m determination. Each point represents the mean ± SEM obtained from two samples from different livers (age = 24 and 26 weeks) each tested in duplicate.

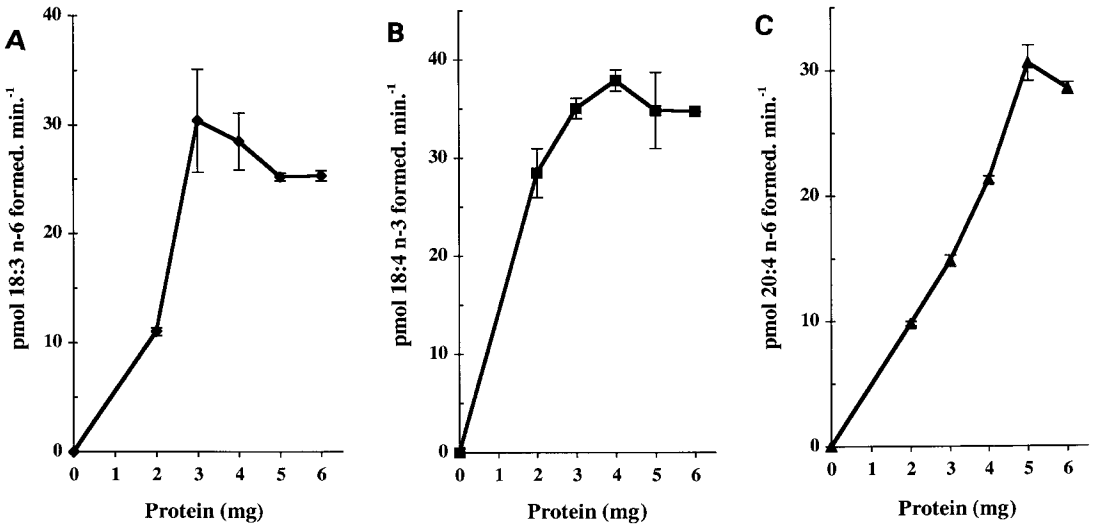

Fig. 4. Effects of microsomal protein concentration on liver Δ5- and Δ6-desaturase activities. Microsomal protein concentration varied from 2 to 6 mg. Incubations were carried out at 37°C for 15 min under the following conditions: A) 28.6 μm [1-^{14}C]linoleic acid, B) 28.6 μm [1-^{14}C]linolenic acid, and C) 28.6 μm [1-^{14}C]eicosatrienoic acid. The results are expressed as means ± SEM; determinations were done in duplicate using samples from the same liver (age = 23 weeks).

ities before and after deep-freezing remained within the range of the experimental variation (7).

In each of the liver samples, substantial amounts of the PUFAs of main interest, especially AA and DHA, were present within the range previously reported in infants born at term (7). The FA composition of liver microsomes did not show apparent abnormalities. Moreover, the levels of 20:3n–9 (Mead acid) and 16:1n–7, considered to be EFA deficiency indicators, were low and the 20:3n–9/20:4n–6 ratio (Table 1) was much lower (0.003–0.1) than the ratio (≤0.4) generally considered to be a marker of deficiency (17). It was also in the same range as that found recently in preterm infants (18). Thus, EFA deficiency or major dystrophy can be excluded in the studied fetuses.

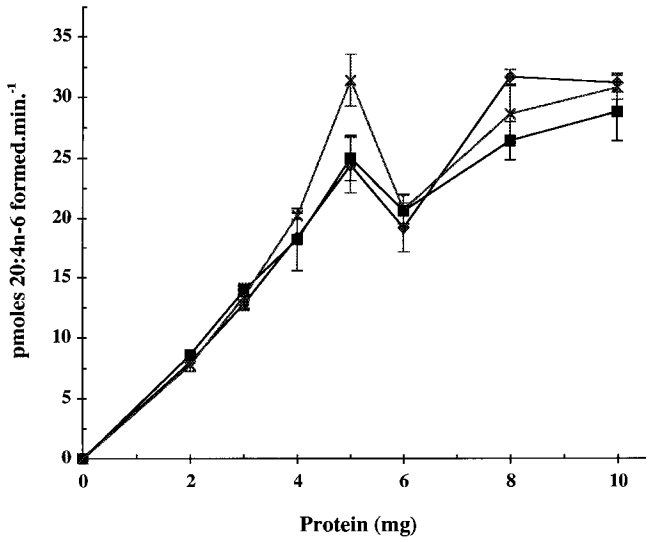

Fig. 5. Effects of microsomal protein concentration on liver Δ5-desaturase activity. Microsomal protein concentration varied from 2 to 10 mg. Incubations were carried out at 37°C for 15 min under the following conditions: 28.6 μm (◆—◆), and either 34.28 μm (■—■) or 40 μm eicosatrienoic acid (×—×). The results are expressed as means ± SEM, determinations were done in duplicate using samples from the same liver (age = 20 weeks).

Apparent V_m and K_m were determined from the michaelian domain of the kinetics. The present data demonstrate significant n–6 Δ5-, n–6 Δ6-, and n–3 Δ6-desaturase activities in human fetal liver that are nevertheless lower than in other species, especially rodents (19–22). The free fatty acid assessment allowed us to compare actual desaturation activities of n–6 and n–3 series in terms of actual substrate concentration.

It has recently been reported that lysophospholipid acyltransferase, acylCoA synthetase and endogenous linoleic acid could significantly affect the kinetic measurements of hepatic microsomal Δ6-desaturase (23). However, the substrate concentrations used in this study, were low (1.8–7.9 μm) and the enzyme activity assay was particularly exposed to interference by endogenous substrate. In our study, the highest desaturase activity was obtained with 29, 15, and 57 μm 18:2n–6, 20:3n–6 and 18:3n–3, respectively, and the endogenous substrate concentrations were measured in the incubation medium (Table 2). It is worth noting that the free acid fraction only accounted for 3.5%, 3.2%, and ≤17.3% of the PL fraction for 18:2n–6, 20:3n–6, and 18:3n–3, respectively and that actual endogenous substrate concentrations were only 0.73%, 0.61%, and ≤0.0075% of the exogenous labeled substrate concentrations. Thus, the presence of endogenous substrate cannot explain the lower measured Δ6-desaturase activity in the n–6 series than in the n–3 series. This conclusion is in accordance with data obtained in vitro in other species (24).

The maximal rates that we observed in human fetal

TABLE 1. Fatty acid composition (weight %) of liver microsomal phospholipids between 20 and 26 weeks of gestation age

	20 Wk	22.5 Wk	23 Wk	24 Wk[a]	25 Wk	26 Wk[a]
16:0	24.83	26.98	27.70	26.64 ± 0.05	24.66	25.54 ± 0.75
18:0	18.61	17.86	16.56	16.11 ± 0.34	18.26	18.08 ± 0.64
16:1n–7	1.52	1.45	1.75	1.81 ± 0.25	1.07	1.34 ± 0.03
18:1n–9	14.25	12.74	11.14	12.13 ± 0.53	10.48	10.03 ± 0.01
24:1n–9	2.49	3.29	1.82	2.29 ± 0.98	2.39	1.80 ± 0.05
18:2n–6	2.79	5.43	3.55	4.59 ± 0.06	5.31	5.95 ± 0.18
18:3n–6	0.08	0.11	0.12	0.15 ± 0.00	0.13	0.15 ± 0.00
20:3n–6	1.60	2.54	2.10	2.42 ± 0.15	2.08	3.00 ± 0.06
20:4n–6	18.40	16.67	16.82	18.42 ± 1.38	19.59	18.16 ± 0.45
22:4n–6	—	—	0.08	0.20 ± 0.04	0.02	0.30 ± 0.04
22:5n–6	—	—	0.92	0.36 ± 0.40	0.80	0.25 ± 0.35
20:5n–3	0.79	0.25	0.13	0.45 ± 0.43	0.08	0.32 ± 0.01
22:5n–3	0.29	0.21	0.13	0.16 ± 0.02	0.14	0.23 ± 0.01
22:6n–3	4.07	3.78	5.61	4.39 ± 0.76	4.06	6.50 ± 0.08
20:3n–9	0.83	0.45	0.17	0.70 ± 0.85	0.14	0.50 ± 0.57
20:3n–9/20:4n–6	0.05	0.03	0.01	0.04	0.01	0.03

[a] Mean ± SD for n = 2 livers. Eight liver samples were used in this experiment.

liver for Δ5- and Δ6-desaturases are lower than the values found in rat liver microsomes, indicating a lower desaturase capacity in the human fetus (24, 25). In several species, the potential Δ6-desaturase activity as measured at saturating substrate concentrations is clearly higher in the n–3 series than in the n–6 series, suggesting that n–3 PUFAs would be more rapidly synthesized than n–6 PUFAs. However, the situation should be quite different in the cell because, as shown in Table 3, the mean substrate concentrations (free acids) were almost equal to apparent K_m for 20:3n–6, lower than apparent K_m for 18:2n–6, and markedly lower for 18:3n–3 (3.09, 2.54, and ≤0.077, respectively, calculated as nmol/g tissue). In these conditions, the enzyme activity in cells is largely dependent on small substrate concentration variations. The free 18:3n–3 concentration was about 40-fold lower than that of 18:2n–6 and 20:3n–6, and the apparent affinity of n–3 Δ6-desaturase for its substrate was lower than that of the n–6 Δ6- and Δ5-desaturases (K_m = 24.5, 6.5 and 3.9 μm, for n–3 Δ6-, n–6 Δ6-, and Δ5-desaturases, respectively). Consequently, the high n–3 Δ6-desaturase apparent K_m value reduces the V_m/K_m ratio of this enzyme (about 1) to the range of that of the n–6 Δ6-desaturase. Thus, at least in human fetal liver, the difference between the conversion rates through n–3 and n–6 pathways in cells is certainly lower than suggested by in vitro measurements at saturating or subsaturating substrate concentrations. This conclusion is in agreement with recent reports of experiments using stable isotope-labeled FAs in newborns (9, 10).

The apparent K_m value for Δ5-desaturase (Fig. 2) was two-fold lower than the Δ6-desaturase value (Fig. 1). Such a difference was also observed in the rat liver (24, 25). The better apparent affinity of Δ5-desaturase may compensate for the lower concentration of its substrate, 20:3n–6, as compared to the substrate of Δ6-desaturase, 18:2n–6 (Table 1).

Beyond substrate concentrations of 30, 20, and 60 μm for n–6 Δ6-, n–6 Δ5-, and n–3 Δ6-desaturases, respectively, an inhibition by excess substrate appeared as hypothesized from data of a previous study, but in the liver of infants born at term (7). Another example of this inhibition has been described in rat liver microsomes (26). In any case, it is important to notice that the domain in which the kinetics of the three enzymes remained Michaelian largely overlapped the physiological substrate concentrations. In these conditions, it is unlikely that this inhibition by excess substrate would be operative in vivo.

Regarding the influence of the microsomal protein concentration on enzyme activities, the three substrates and the three enzymes showed the same behavior: a breakdown in linearity appeared beyond 4–5 mg microsomal protein (Fig. 4). Consequently, the n–6 and n–3 desaturase specific activities declined. A similar observation was reported in a study of n–6 Δ5-desaturase in rat kidney (11). This phenomenon may not be due to a lack of sub-

TABLE 2. Endogenous fatty acid concentrations of free fatty acid and phospholipid fractions introduced in the incubation medium as microsomal membranes

	18:2n–6	18:3n–3	20:3n–6
Free fatty acid	0.21 ± 0.07	≤0.0043 ± 0.0009	0.09 ± 0.03
phospholipid	6.04 ± 1.21	≤0.025 ± 0.005	2.73 ± 0.4
Free fatty acid/phospholipid	3.5%	≤17.3%	3.20%

Results are expressed as μmol/l of incubation medium (mean ± SEM, for n = 8).

TABLE 3. Tissue concentrations of microsomal membrane fatty acids in free fatty acid and phospholipid fractions

		18:2n–6		18:3n–3		20:3n–6	
Age	Total Liver	Free Fatty Acid	Phospholipid	Free Fatty Acid	Phospholipid	Free Fatty Acid	Phospholipid
(weeks)	*(g)*	*(nmol/g liver)*		*(nmol/g liver)*		*(nmol/g liver)*	
22.5	27	3.71	112.33	—	0.46	4.33	48.15
24	30	2.86	131.30	0.077	0.42	4.67	61.13
33	97.7	1.06	213.00	—	—	0.28	78.71

Three liver samples were used in this experiment.

strate, as the conversion yields were lower than 2%; thus, about 98% of initial substrate concentration remained available at the end of the incubation. Moreover, when increasing concentrations of 20:3n–6 were used (Fig. 5), the linear relationship between Δ5-desaturase activity and protein concentration was lost beyond 5 mg, whatever the substrate concentration. This phenomenon was not dependent on age because the two experiments were carried out on fetuses of different ages. This observation validates the choice of 3 mg for the enzyme assay in the present model.

When high microsomal protein amounts are present in the medium, incorporation of substrate FA into microsomal PLs could compete with the desaturation reactions, as hypothesized previously (27) and considered for Δ5-desaturase from rat kidney (11). Increasing concentrations of microsomal membranes in the incubation medium might also physically reduce substrate access to the enzyme active site, for instance, by increasing membrane–membrane interactions.

In conclusion, n–6 Δ5-, n–6 Δ6-, and n–3 Δ6-desaturases in human fetal liver exhibited inhibition due to excess substrate but at concentrations largely exceeding that of endogenous substrate. This finding should be taken into account for enzyme activity determination, at least in human liver. The kinetics demonstrated higher maximal activity in n–3 Δ6-desaturase than in the n–6 Δ5- and Δ6-desaturases, but the determination of the concentration of the respective endogenous substrate of each enzyme suggested that, in vivo, this difference might be corrected by the actual concentration of each substrate in human fetal liver.

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