Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 791 (2003) 235–244
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Multidimensional gas chromatography–mass spectrometry for tracer studies of fatty acid metabolism via stable isotopes in cultured human trophoblast cells

Alexandra Muth[a], Armin Mosandl[a,*], Adelheid Bursen[b], Rolf Marschalek[b], Adrian C. Sewell[c], Hans Böhles[c]

[a]*Department of Food Chemistry, J.W. Goethe-University, Biocenter, Marie-Curie-Strasse 9, D-60439 Frankfurt/Main, Germany*
[b]*Institute for Pharmaceutical Biology, J.W. Goethe-University, Frankfurt/Main, Germany*
[c]*Department of Paediatrics, J.W. Goethe-University, Frankfurt/Main, Germany*

Received 16 December 2002; received in revised form 11 February 2003; accepted 11 March 2003

Abstract

The determination of placental fatty acid metabolism using stable isotope-labeled tracers was investigated in the human placental choriocarcinoma (JAR) cell line. Stable isotope incorporation was measured by MDGC–MS. The cultured trophoblast cells incorporated and metabolized the essential fatty acids to long-chain polyunsaturated fatty acids. The described method enables the detection of a low Δ^6-desaturase activity in this human placental cell line. The developed MDGC–MS method allows the assessment of long-chain polyunsaturated fatty acid biosynthesis in cultured cells with high sensitivity and selectivity. In this respect, tracer studies with MDGC–MS will be a powerful tool to clarify the significance of placental fatty acid metabolism.

Keywords: Stable isotopes; Trophoblast cells; Fatty acids

1. Introduction

Long-chain polyunsaturated fatty acids (LCPUFAs) are of crucial importance in fetal and infant development [1,2,4]. These fatty acids play a major role as precursors of biologically active eicosanoids (as precursors for prostacyclins, prostaglandins, thromboxanes and leukotrienes) and as constituents of membrane lipids in order to maintain membrane fluidity and their physiochemical functions [3,4]. Arachidonic acid (AA) and docosahexaenoic acid (DHA) are the two major ω-6 and ω-3 fatty acids found in brain and retina membrane lipids [1]. Consequently, a sufficient supply of these fatty acids during pregnancy is necessary and critical for optimal brain and visual development in the fetus. An inadequacy of LCPUFAs as critical membrane lipids may result in an irrevocable damage [1].

In mammals, LCPUFAs are biosynthesised by sequential desaturation and elongation from the essential fatty acids (EFAs), linoleic acid (LA) and α-linolenic acid (α-LNA) [1,2]. Several reports have shown that the levels of LCPUFAs in comparison with the EFAs are higher in the fetal than in the

*Corresponding author. Tel.: +49-69-798-29202; fax: +49-69-798-29207.
E-mail address: mosandl@em.uni-frankfurt.de (A. Mosandl).

1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00220-4

maternal circulation – termed ''biomagnification'' [2,3,5].

The placenta is primarily responsible for the transport of maternal EFAs and LCPUFAs to the fetus [6–8]. So far, the question is whether human placental trophoblast cells are dependent upon exogenous LCPUFAs or whether they are capable of synthesising LCPUFAs from the precursor EFAs.

The aim of this study was to develop an advanced method for tracer studies of the metabolic conversion of the EFAs in human trophoblasts using stable isotopes. Stable isotope labels are suitable for both the in vivo and in vitro elucidation of metabolic pathways since they avoid the disadvantages of radioactive tracers [9,10]. For this investigation of placental fatty acid metabolism, the human placental choriocarcinoma (JAR) cell line – a monolayer-forming trophoblast cell line – was used as a model for human trophoblasts. Human cytotrophoblasts have the ability to differentiate spontaneously to syncytiotrophoblasts, which are unable to form a confluent, consistent monolayer in culture [11]. In order to examine the biosynthesis of LCPUFAs, these cells were incubated with the uniformly ^{13}C-labeled precursors 18:2 ω-6 (LA) and 18:3 ω-3 (α-LNA), the cell lipids extracted and the individual fatty acids analyzed as methyl esters (FAMEs).

Here we were able to show for the first time the general use of multidimensional gas chromatography coupled with mass selective detection (MDGC–MS) for tracer studies of fatty acid metabolism.

(The fatty acid notation is of the form ''*N*:*B* ω-*x*''. *N* is the number of carbons, *B* is the number of all-*cis*, methylene-interrupted double bounds, and *x* is the position of the first double bound counting from the methyl end.)

2. Experimental

2.1. Chemicals

14% BF_3 in methanol (w/v), sodium hydroxide, chloroform and silica gel (0.063–0.200 mm) were obtained from Merck (Darmstadt, Germany); *n*-hexane, diethyl ether and butylated hydroxytoluene (BHT) from Fluka (Buchs, Switzerland). Methanol, sodium chloride and sodium sulfate were from Riedel-de Haën (Seelze, Germany). NH_3 (25%) (aq) was from Grüssing (Filsum, Germany) and ethanol from Roth (Karlsruhe, Germany). Bovine serum albumin BSA (essential fatty acid free) and octadecatetraenoic acid (for analysis methylated with 14% BF_3 in methanol) were obtained from Sigma (St. Louis, MO, USA). The fatty acid methyl ester (FAME) GLC-Standard (No. 462) were from Nu-ChekPrep (Elysian, MN, USA). [U-^{13}C]LA and [U-^{13}C]α-LNA with an isotopic enrichment of >98% were obtained from Martek Biosciences (Columbia, MD, USA). All tissue culture reagents and RPMI 1640 medium with 25 m*M* Hepes buffer were from Gibco (Gaithersburg, MD, USA).

2.2. Cell culture

JAR cells (ACC 462) were obtained from DMSZ (Braunschweig, Germany) and were grown in RPMI 1640 medium containing 15% fetal bovine serum.

Cells were maintained as monolayers in 75-cm^2 tissue culture flasks at 37 °C with 5% CO_2 in a humidified air atmosphere.

2.3. Incubation with [U-^{13}C]LA and [U-^{13}C]α-LNA

After reaching 85–90% confluency (~1×10^7 cells per flask), the culture medium was removed, the cells were rinsed with 30 ml phosphate-buffered saline, and 20 ml of freshly prepared culture medium containing the label were added to each flask.

2.3.1. [U-^{13}C]LA- and [U-^{13}C]α-LNA-labeled medium

From a 14 m*M* stock solution of uniformly ^{13}C-labeled fatty acids in absolute ethanol an aliquot was pipetted into a vial and evaporated under a stream of nitrogen until almost dry. Appropriate quantities of BSA (5% in serum-free medium) were pipetted into the tube and incubated at 37 °C for 60 min, in order to promote the binding of the fatty acid to the albumin. Final concentration of each fatty acid in complete medium (20 ml) was 40 μ*M*, complexed to BSA in a molar ratio of 2:1 (fatty acid to protein).

The culture medium was removed and freshly prepared labeled medium (sterilized by filtration through a 0.22-μm filter) was added to each flask.

The cells were incubated with the incubation medium for 24, 44 and 48 h. As a negative ''control'', cells were incubated with culture medium without the labeled fatty acids. In addition, incubations for 44 and 48 h were performed with a final concentration of 10 μ*M* labeled fatty acid in the culture medium.

The incubations were terminated by removing the medium, followed by washing the monolayer three times with 30 ml of phosphate-buffered saline. The cells were detached using trypsin-EDTA and transferred to a centrifuge tube. The flask was washed with 10 ml phosphate-buffered saline to ensure complete collection of all cells, combined with the cell suspension and centrifuged (1000 *g*) for 5 min. The cell pellet was resuspended in 1 ml distilled water.

2.4. Lipid extraction and methylation

Cell lipids were extracted according to the method described previously [12]. The lipid extract was hydrolyzed by adding 160 μl 0.5 N methanolic sodium hydroxide and heated at 60 °C for 20 min. Adding 200 μl BF_3 in methanol (14%, w/v) and heating at 100 °C for 3 min yielded FAMEs [13]. The FAMEs were extracted with hexane containing butylated hydroxytoluene (BHT) as antioxidant, neutralized with 25% NH_3 (aq) and washed with saturated sodium chloride solution. The organic phase was dried over sodium sulfate and chromatographed over silica gel (1 cm in a standard glass pasteur pipette) with the eluent hexane–diethylether (95:5, v/v). Finally, the solvent was evaporated to dryness and the residue resuspended in hexane with added BHT. The FAMEs were finally analyzed by GC and MDGC.

2.5. Instrumentations

2.5.1. GC conditions

FAMEs were analyzed using a Carlo Erba GC6000 Vega Series 2 instrument equipped with a flame ionisation detector (FID) at 250 °C and a split-splitless injector at 250 °C. The column was a DB-Wax fused-silica capillary column (30-m×0.32-mm I.D.; 0.25 μm d_f (J&W, Folsom, USA)) and H_2 was employed as carrier gas (35 kPa). A protection column was installed prior to the pre-column (1-m× 0.32-mm I.D. deactivated high-temperature fused-silica capillary column). The samples were injected in the split mode (split 1:30); the injection volume was 1 μl. The initial column temperature was 100 °C, increased by 4 °C/min to 250 °C and then held for 30 min.

2.5.2. MDGC–MS conditions

The MDGC–MS system consisted of a Siemens SiChromat 2 double oven system with two independent temperature controls. The injections were in the split mode for the reference compounds and in the splitless mode (0.5 min) for the tracer studies of the JAR cells at 250 °C. The injection volume was 1 μl. The pre column was equipped with a flame ionisation detector at 250 °C, whereas the main column was connected with an ITD-transfer line (250 °C) and an open split interface (250 °C) to an ITD 800 mass spectrometer (Finnigan MAT, Bremen, Germany), with detection in the electron ionisation mode. Sweep flow (helium) was 1 ml/min, ion trap manifold 230 °C and electron energy was 70 eV.

The pre and main column were connected with a live-switching coupling piece (live-T-piece).

The pre column was a 30-m×0.25-mm I.D. SolGel-Wax (SGE, Austin, TX), coated with a 0.25-μm film. A protection column was installed prior to the pre column (1-m×0.25-mm I.D. deactivated high-temperature fused-silica capillary column).

A fused-silica capillary column (30-m×0.25-mm I.D.), coated with a 0.2-μm film of SP-2560 (Supelco, Bellefonte, PA) was used as the main column.

For the pre column the carrier gas was helium, pressure 175 kPa; split 1:30; the initial temperature was 130 °C, ramped 4 °C/min to 250 °C and held for 30 min.

For the main column the carrier gas was helium, pressure 140 kPa.

2.5.3. Temperature program A, used for the separation of the ω-6 FAMEs

The oven temperature program was set to start at 100 °C, increased at 4 °C to 180 °C and held for 20 min, finally ramped at 4 °C to 230 °C and held for 30 min.

Table 1
Cut-times for FAMEs of the ω-6 family, using temperature program A for the main column separation

FAMEs	Cut-times pre column (min)
18:2	32.7–33.3
18:3	33.6–34.2
20:2	38.5–39.2
20:3	39.6–40.3
20:4	40.5–41.2

2.5.4. Temperature program B, used for the separation of the ω-3 FAMEs

The oven temperature program was set to start at 100 °C, increased at 4 °C to 180 °C and held for 40 min, finally ramped at 4 °C to 230 °C and held for 30 min.

The cut-times of the analyzed FAMEs are shown in Tables 1 and 2.

3. Results and discussion

In this study, the ability of human placental cells to elongate and desaturate EFAs has been examined, using for the first time a MDGC–MS system for the detection of stable isotopically labeled fatty acid metabolites incorporated into cellular lipids. MDGC–MS is a highly selective and sensitive method [14,15]. Preseparated components are selectively transferred from the pre column online via a ''live-T-piece'' onto the main column with different polarity and are identified with mass selective detection. Therefore, a minimum of background and an efficient sample cleanup are obtained which is necessary for an optimal efficiency of mass selective detection [16]. Consequently, MDGC–MS systems are the method of choice for the direct analysis of minor components from complex matrices.

Table 2
Cut-times for FAMEs of the ω-3 family, using temperature program B for the main column separation

FAMEs	Cut-times pre column (min)
18:3	34.0–34.6
18:4	34.9–35.6
20:3	41.0–41.7
20:5	43.3–44.0
22:5	54.8–55.8
22:6	56.9–57.9

In this regard, we used the described MDGC–MS system for the investigation of the LCPUFA biosynthetic pathways using stable isotope tracers. Fig. 1 shows the main chromatograms of the investigated FAMEs (reference compounds). The comparison with two distinct retention times and mass spectra provides an accurate identification of the analysed compounds. Moreover, the combination of two different columns (SolGel-Wax and biscyanopropylpolysiloxane (100%)) and the performing of two different temperature programs provide the optimal separation of the investigated FAMEs.

The relevant FAMEs of the JAR cells are separated from the whole lipid extract on the pre column

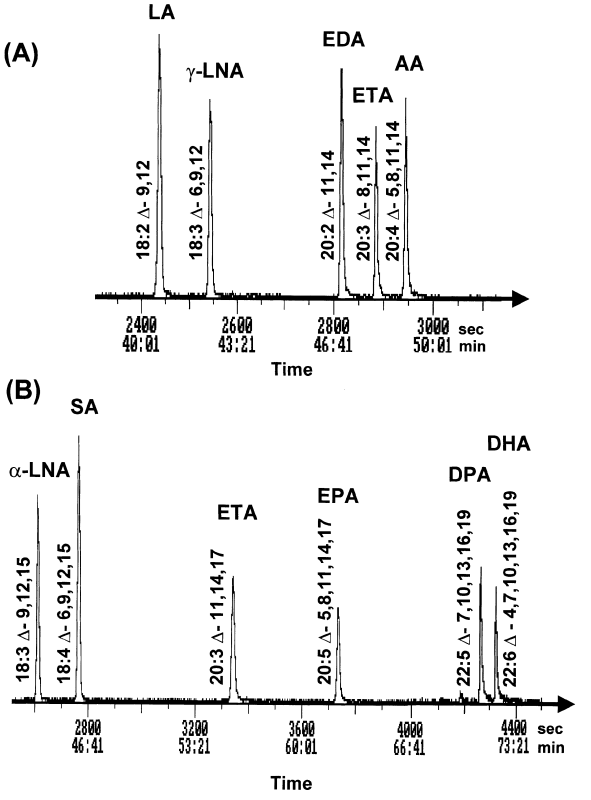

Fig. 1. Main column separation of the analyzed FAMEs (reference compounds) at $m/z = 67$. (A) FAMEs of the ω-6 family, using temperature program A (see Section 2.5). (B) FAMEs of the ω-3 family, using temperature program B (see Section 2.5).

(sample cleanup). Thus, even the identification of minor components of the total cell lipid extract was achieved.

3.1. Incubation experiments

Cultured human trophoblasts (JAR) were incubated with 40 μ*M* [U-^{13}C]LA and [U-^{13}C]α-LNA for 24 h and over 40 h. The extracted and saponified cell lipids were analyzed as methyl ester derivatives. The derivatisation was monitored by gas chromatography. Furthermore, a typical profile of the total fatty acid composition (as methylesters) from JAR cell lipids was obtained.

In comparison with retention times of a FAME standard mixture the relevant PUFAs were identified as minor and trace components.

Therefore, for a reliable measurement of an isotope incorporation into these fatty acid metabolites by the trophoblasts, the lipid extracts were analysed with MDGC–MS.

The highly enriched tracers were used to distinguish between labeled and genuine metabolites. Detection of isotopic incorporation could be clearly measured by selected ion monitoring (SIM). Measurement in the SIM mode improves the peak-noise ratio, which is necessary for analyzing trace compounds.

Mass spectra of the analyzed FAMEs, detected by EI-MS, show prominent peaks due to fragmentations along the alkyl chain [17]. A typical mass spectrum of methyl α-LNA is shown in Fig. 2A.

The most prominent peaks at $m/z=67$, 79 (base peak) and 93 are due to alkyl chain fragments. Mass spectra of unsaturated FAMEs—especially of highly unsaturated compounds—show a typical peak at $m/z=91$, which may be due to tropylium ions formed by cyclisation and rearrangement [17]. For the precise identification of an isotope incorporation in FAME metabolites the JAR lipid extracts were analyzed by SIM (mass range m/z 67–102 for LA metabolites and m/z 67–98 for α-LNA metabolites). Labeled metabolites were measured at the peak maxima on mass lane $m/z=72$ and 98, for LA and eicosadienoic acid (EDA) on mass lane $m/z=72$ and 102, respectively, without interference of natural ^{13}C abundance.

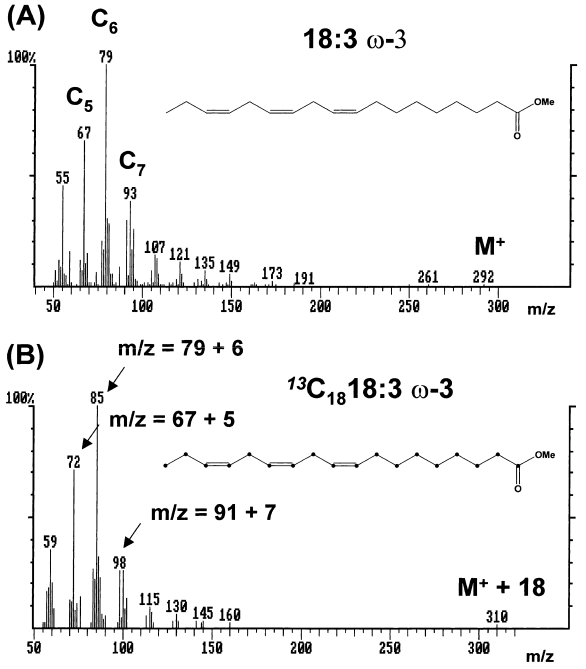

Fig. 2. MS spectra of methyl 18:3 ω-3 (α-linolenic acid, α-LNA). (A) MS-spectrum of unlabeled methyl 18:3 ω-3 (α-linolenic acid, α-LNA) (reference compound). (B) MS-spectrum of ^{13}C-labeled methyl 18:3 ω-3 (α-linolenic acid, α-LNA) after incubation of trophoblast (JAR) cells with 40 μ*M* [U-^{13}C]α-LNA; ^{13}C positions (●).

Fig. 2 shows the mass spectra of unlabeled and labeled methyl α-LNA. On the mass lane $m/z=72$ and 98, which are shifted by five and seven mass units compared with the unlabeled FAME (Fig. 2A), the labeled methyl α-LNA (Fig. 2B) is clearly detectable.

3.1.1. [U-^{13}C]LA utilization

The cultured trophoblasts incorporated exogenous [U-^{13}C]LA from the cell medium. [U-^{13}C]LA was identified as methyl ester at $m/z=72$, 87, 102 and 312 ($M^+ + 18$) compared to endogenous LA ($m/z=$ 67, 81, 95 and 294 (M^+)). After 24 h, labeled EDA (20:2 ω-6), the chain elongation product of LA, was predominant. Fig. 3 shows the main column chromatogram of labeled methyl EDA. The isotope incorporation was identified on mass lane $m/z=72$ and 102.

After an incubation period of about 40 h, on mass

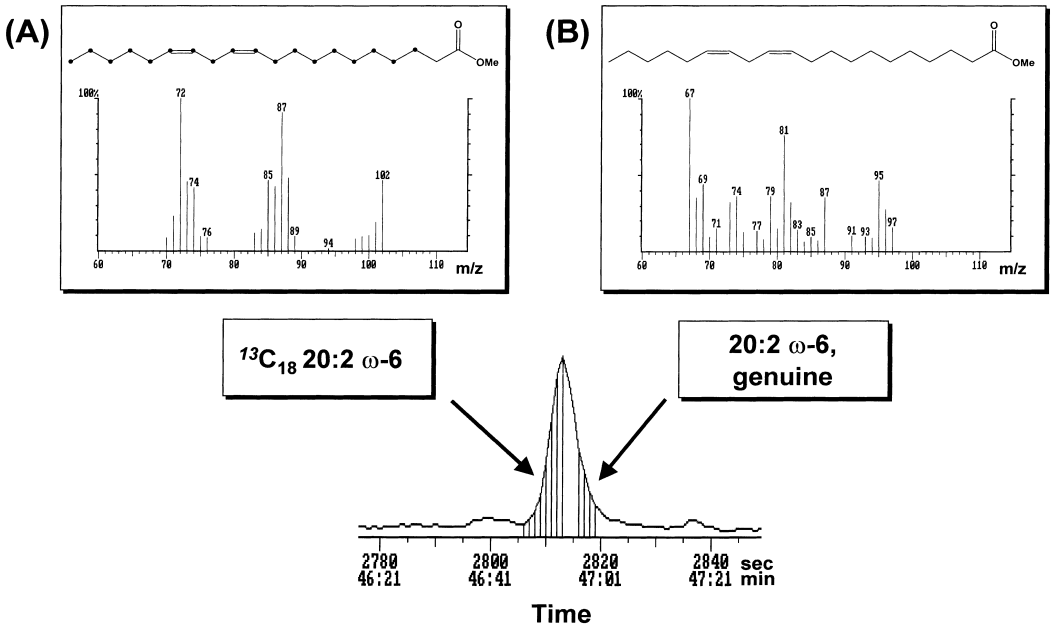

Fig. 3. Selected ion chromatogram (mass range m/z from 67 to 102) of methyl 20:2 ω-6 (eicosadienoic acid, EDA) after incubation of trophoblast (JAR) cells with 40 μ*M* [U-^{13}C]18:2 ω-6 (linoleic acid, LA) for more than 40 h. (A) MS spectrum of ^{13}C-labeled methyl 20:2 ω-6 (eicosadienoic acid, EDA) with the characteristic ions $m/z=72$, 87 and 102. (B) MS spectrum of genuine, unlabeled methyl 20:2 ω-6 (eicosadienoic acid, EDA) ($m/z=67$, 81 and 95); ^{13}C positions (●).

lane $m/z=72$ and 98 labeled γ-LNA (18:3 ω-6, methyl ester) – the Δ^6-desaturation product of the precursor LA – was clearly identified (Fig. 4).

As a result of the so-called inverse isotope effect in gas chromatography [18], the ^{13}C-labeled isotopomers show a shift of their peak maxima of approximately 2 s relative to the unlabeled peak maxima (Figs. 3 and 4).

After 24 h, traces of labeled γ-LNA were detected (Fig. 5B) and in the ''control'' cells only genuine γ-LNA occurred (Fig. 5A).

Fig. 6 illustrates the relative distribution of ^{13}C-label in the analyzed FAMEs. These results prove a large uptake of [U-^{13}C]LA by the trophoblast cells. However, these cells predominantly converted exogenous [U-^{13}C]LA to labeled EDA, indicating that the Δ^6-desaturation activity is decreased in this cell line.

3.1.2. [U-^{13}C]α-LNA utilization

A similar result was obtained when cells were incubated with 40 μ*M* [U-^{13}C]α-LNA. When the trophoblast cultures were incubated with labeled α-LNA for 24 h, a significant amount of labeled α-LNA accumulated in the cell lipids (Fig. 2). Only traces of genuine α-LNA were identified as methyl ester ($m/z=67$). There was a significant elongation of α-LNA to 20:3 ω-3 (eicosatrienoic acid, ETA), detectable at $m/z=72$ and 98. After an incubation period of about 40 h, trace amounts of ^{13}C-labeled methyl 18:4 ω-3 (stearidonic acid, SA) were detected (Fig. 7). In these cultured cells only traces of endogenous ETA and SA were identified as methyl esters at $m/z=67$.

Studies also were done with a lower concentration of EFAs (10 μ*M* [U-^{13}C]LA and [U-^{13}C]α-LNA) to avoid the possibility of an inhibitory effect of high concentrations of the precursors on Δ^6-desaturase. Under these conditions, only labeled chain-elongation products – EDA and ETA, respectively – were identified. These results suggested that the failure of these trophoblast cells to convert large amounts of LA and α-LNA to LCPUFAs (AA and DHA) was not due to substrate inhibition of the Δ^6-desaturase.

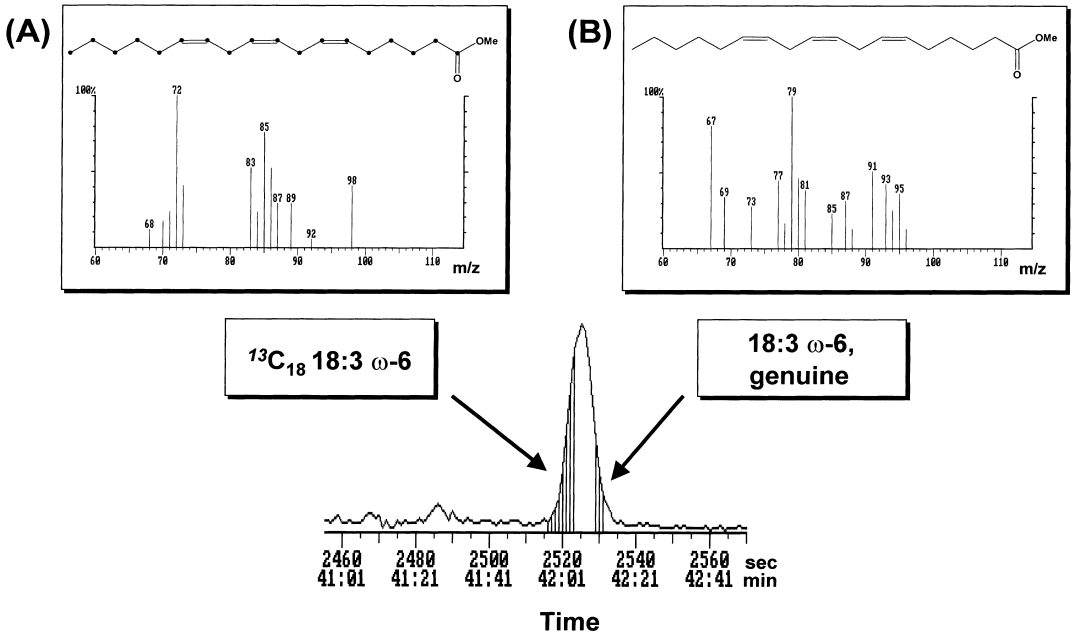

Fig. 4. Selected ion chromatogram (mass range m/z from 67 to 98) of methyl 18:3 ω-6 (γ-linolenic acid, γ-LNA) after incubation of trophoblast (JAR) cells with 40 μ*M* [U-^{13}C]18:2 ω-6 (linoleic acid, LA) for more than 40 h. (A) MS spectrum of ^{13}C-labeled methyl 18:3 ω-6 (γ-linolenic acid, γ-LNA) with the characteristic ions $m/z=72$, 85 and 98. (B) MS spectrum of genuine, unlabeled methyl 18:3 ω-6 (γ-linolenic acid, γ-LNA) ($m/z=67$, 79 and 91); ^{13}C positions (●).

3.2. Conclusions on the LCPUFA biosynthesis in JAR cells

To date, there has been little information available on the metabolism of EFAs—the precursors of LCPUFAs—within the placenta itself. Studies by Noble et al. [19] with sheep placenta indicated that the placenta is involved in the provision of LCPUFAs to the fetus, derived from the C_{18} fatty acid precursors. Several authors have reported that human placental tissue lacks Δ^6- and Δ^5-desaturase activity [3,20]. Such studies have typically used radioactive tracers. Using the highly sensitive and selective MDGC–MS system, even a low Δ^6-desaturase activity could be ascertained in the human trophoblast (JAR) cell line.

The first Δ^6-desaturase reaction is known as the rate-limiting step in the conversion of LA and α-LNA to LCPUFAs of the ω-6 and ω-3 families [2]. JAR cells have only a limited capacity for PUFA biosynthesis, as concluded from their inability to effectively synthesize AA and DHA from the precursors LA and α-LNA. Another probable explanation for our findings could be the fact that the incorporation of AA and DHA into cell lipids might be hindered as high concentrations of the precursor EFAs (or their chain elongation products) are available. Similar observations were reported by Spector et al. [21] in human umbilical vein endothelial cells, who presumed a limited capacity of AA biosynthesis or a competitive inhibitory effect of high amounts of LA on AA incorporation into cellular lipids. Moreover, these authors had previously suggested that LA enrichment of cultured human umbilical vein endothelial cells causes a decreased prostacyclin synthesis. Consequently, these cells as well as the investigated trophoblast (JAR) cells, are dependent on an uptake of preformed AA and DHA, respectively, in order to maintain adequate stores of these important LCPUFAs in cell lipids.

Recently, Crawford [5] reported that in experiments with isolated placental microsomes of guinea pigs at midterm, the Δ^6-desaturation of ^{14}C-labeled LA was barely detected in comparison with fetal and

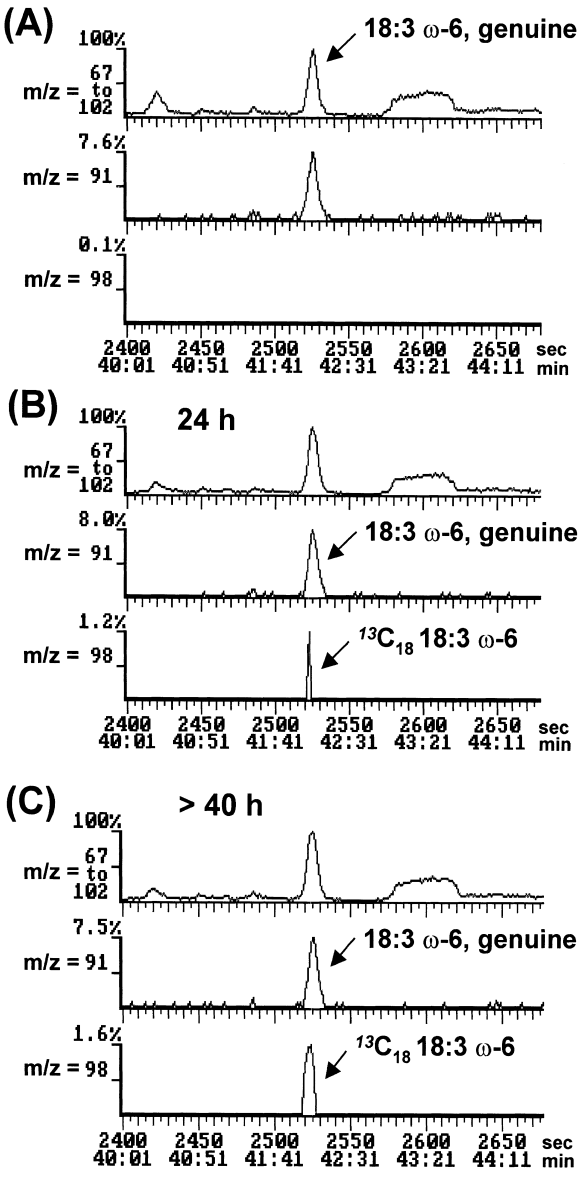

Fig. 5. Selected ion chromatograms (main column) of methyl 18:3 ω-6 (γ-linolenic acid, γ-LNA) formed by trophoblast (JAR) cells. (A) Control: analysis of γ-LNA formed by trophoblast (JAR) cells, not exposed to labeled EFAs. (B) Analysis of γ-LNA formed by trophoblast (JAR) cells after incubation with 40 μ*M* [U-^{13}C]18:2 ω-6 (linoleic acid, LA) for 24 h. (C) Analysis of γ-LNA formed by trophoblast (JAR) cells after incubation with 40 μ*M* [U-^{13}C]18:2 ω-6 (linoleic acid, LA) for more than 40 h.

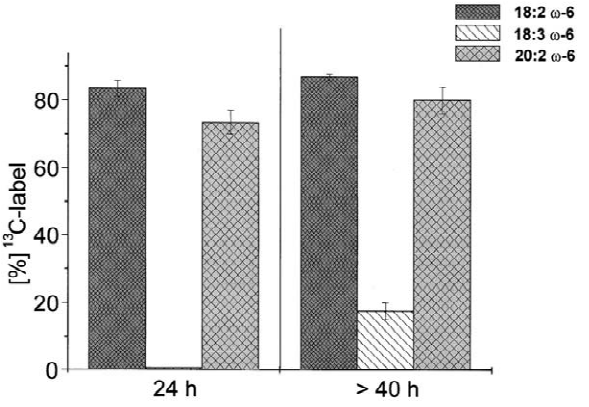

Fig. 6. Relative distribution of ^{13}C-label in the analyzed FAMEs, obtained after incubation of trophoblast (JAR) cells with 40 μ*M* [U-^{13}C]18:2 ω-6 (linoleic acid, LA). Each bar is the mean±SE of two separate cultures. The relative isotope distribution was obtained from the ratio of ion intensity on mass lane $m/z=67$ (genuine) compared to the resultant labeled species on mass lane $m/z=72$. For γ-LNA, the ratio of ion intensity on mass lane $m/z=91$ (genuine) compared to the labeled one on mass lane $m/z=98$ was used.

maternal liver microsomes. Therefore, it was suggested that the result of this study could not explain the biomagnification process in terms of placental fatty acid anabolism.

A further consideration is whether this limited conversion of EFAs by Δ^6-desaturase is unique to transformed cell lines or whether it is also true for primary cell cultures. Previous studies reported that deficiencies in Δ^6-desaturation have been observed in fatty acid metabolic studies with radiolabeled EFAs in transformed cell lines [22]. Spector et al. [23] reported that a number of malignant cell lines, e.g., Y29 human retinoblastoma cells [24], contain a Δ^6-desaturase activity, indicating that a deficiency in Δ^6-desaturation cannot be generalized to transformed cells.

4. Conclusions

In summary, this paper describes a novel, efficient and sensitive MDGC–MS method for the inves-

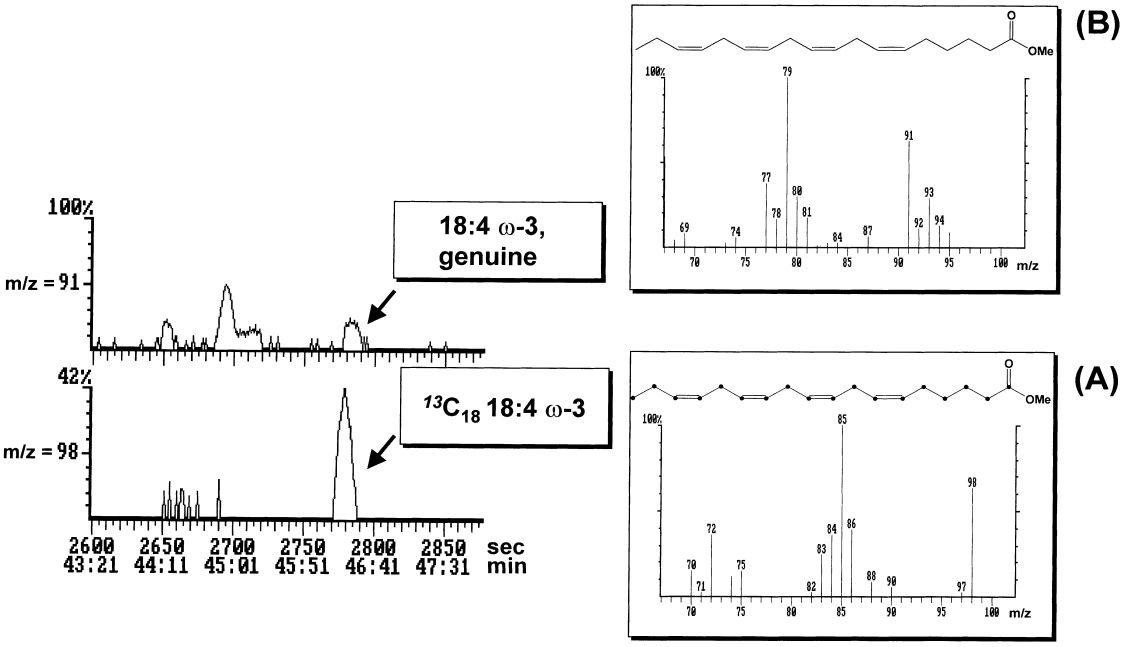

Fig. 7. Selected ion chromatograms (main column) of methyl 18:4 ω-3 (stearidonic acid, SA) after incubation of trophoblast (JAR) cells with 40 μ*M* [U-^{13}C]18:3 ω-3 (α-linolenic acid, α-LNA) for more than 40 h. (A) MS spectrum of ^{13}C-labeled methyl 18:4 ω-3 (stearidonic acid, SA) with the characteristic ions $m/z = 72$, 85 and 98. (B) MS spectrum of genuine, unlabeled methyl 18:4 ω-3 (stearidonic acid, SA) ($m/z = 79$ and 91); ^{13}C positions (●).

tigation of the PUFA biosynthesis in cultured cells. The MDGC–MS system combines the separation of selected compounds by MDGC with the sensitivity of detection offered by MS. Consequently, this system is capable of detecting very low concentrations of biologically relevant metabolites in a complex sample. Moreover, this study has demonstrated that MDGC–MS is a very powerful tool for stable isotope tracer studies of fatty acid metabolism in cells. Therefore, the data presented above provide important new information about fatty acid metabolism in cultured trophoblast cells. Thus, many questions regarding placental fatty acid uptake and metabolism remain to be answered. Further activities will be required to understand the role of the placenta in the metabolism of LCPUFAs, which are important as critical structural components of developing fetal membranes as well as precursors of bioactive mediators regulating membrane signal pathways.

Acknowledgements

The authors thank the Deutsche Forschungsgemeinschaft (DFG) and the Fonds der Chemischen Industrie for financial support.

References

[1] S.M. Innis, Prog. Lipid Res. 30 (1991) 39.
[2] M.A. Crawford, A.G. Hassam, P.A. Stevens, Prog. Lipid Res. 20 (1981) 31.
[3] D.C. Kuhn, M. Crawford, Prog. Lipid Res. 25 (1986) 345.
[4] R. Uauy, P. Mena, C. Rojas, Proc. Nutr. Soc. 59 (2000) 3.
[5] M.A. Crawford, Am. J. Nutr. 71 (2000) 275S.
[6] A.K. Dutta-Roy, Am. J. Clin. Nutr. 71 (2000) 315S.
[7] P. Haggarty, J. Ashton, M. Joynson, D.R. Abramovich, K. Page, Biol. Neonate 75 (1999) 350.
[8] F. Liu, M.J. Soares, K.L. Audus, Am. J. Physiol. 273 (1997) C1596.
[9] K.J. Goodman, J.T. Brenna, Anal. Chem. 64 (1992) 1088.

[10] H. Demmelmair, T. Sauerwald, B. Koletzko, T. Richter, Eur. J. Pediatr. 156 (1997) S70.
[11] R.A. Coleman, E.B. Haynes, J. Lipid Res. 28 (1987) 1335.
[12] M. Kates, in: R.H. Burdon, P.H. Van Knippenberg (Eds.), Techniques of Lipidology: Isolation, Analysis and Identification of Lipids, Elsevier, Amsterdam, New York, Oxford, 1986, p. 100.
[13] AOAC, in: S. Williams (Ed.), Official Methods of Analysis of AOAC, 14th edition, Assoc. Official Anal. Chem, Arlington, VA, 1984, p. 513.
[14] M. Heil, F. Podebrad, T. Beck, A. Mosandl, A.C. Sewell, H. Böhles, J. Chromatogr. B 714 (1998) 119.
[15] A. Muth, J. Jung, S. Bilke, A. Scharrer, A. Mosandl, A.C. Sewell, H. Böhles, J. Chromatogr. B (2003) in press.
[16] A. Mosandl, Food Rev. Int. 11 (1995) 597.
[17] B. Hallgren, R. Ryhage, E. Stenhagen, Acta Chem. Scand. 13 (1959) 845.
[18] M. Matucha, W. Jockisch, P. Verner, G. Anders, J. Chromatogr. 588 (1991) 251.
[19] R.C. Noble, J.H. Shand, W.W. Christie, Biol. Neonate 47 (1985) 333.
[20] J. Chambaz, D. Ravel, M. Manier, D. Pepin, N. Mulliez, G. Bereziat, Biol. Neonate 47 (1985) 136.
[21] A.A. Spector, T.L. Kaduce, J.C. Hoak, G.L. Fry, J. Clin. Invest. 68 (1981) 1003.
[22] L.M. Dunbar, J.M. Bailey, J. Biol. Chem. 250 (1975) 1152.
[23] A.A. Spector, S.N. Mathur, T.L. Kaduce, B.T. Hyman, Prog. Lipid Res. 19 (1981) 155.
[24] M. Huang, S. Muddana, E.N. Horowitz, C.C. McCormick, J.P. Infante, J.T. Brenna, Anal. Biochem. 287 (2000) 80.