DOI 10.1002/mnfr.200500054 Mol. Nutr. Food Res. 2005, *49*, 756–762

Distribution of conjugated linoleic acid in total and subcellular fractions from normal and cancerous parts of human testes

Kristina Hoffmann[1], Jörg Blaudszun[2], Claus Brunken[3], Roland Tauber[3], Wilhelm-Wolfgang Höpker[2] and Hans Steinhart[1]

[1]Institute of Biochemistry and Food Chemistry, University of Hamburg, Hamburg, Germany
[2]Department of Pathology, General Hospital Barmbek, Hamburg, Germany
[3]Department of Urology, General Hospital Barmbek, Hamburg, Germany

The objective of the present study was to examine differences in the fatty acid composition of subcellular fractions from normal and cancerous parts of human testes. The conjugated linoleic acid (CLA) content was significantly higher in total testicular carcinoma (TC), but significantly lower in the mitochondrial fraction of TC in comparison to normal testicular tissue. The subcellular distribution pattern of CLA was similar to that of monounsaturated fatty acids, but different to that of 18:2n-6 (linoleic acid), underlining the different physiological properties of CLA and 18 : 2*n*-6. Because polyunsaturated fatty acids (PUFAs) have been suggested to have an effect on cancer risk and previous research has found that CLA inhibits the metabolism of 18 : 2*n*-6 into 20 : 4*n*-6, the contents of *n*-6 and *n*-3 PUFAs were determined. Significant differences were observed for 18 : 2*n*-6, 18 : 3*n*-3, 20 : 5*n*-3, and 22 : 6*n*-3, with 18 : 2*n*-6, 18 : 3*n*-3, and 20 : 5*n*-3 contents being higher and 22 : 6*n*-3 content being lower in TC than in normal testicular tissue. These results indicate a changed availability of substrates for the cyclooxygenase (COX) or lipooxygenase (LOX) pathways generating eicosanoids. Although not statistically significant, the reduced content of 20 : 4*n*-6 shown in this study might be due to an increased metabolism of this fatty acid into eicosanoids.

Keywords: Conjugated linoleic acid / Human tissue / Polyunsaturated fatty acids / Subcellular fractionation / Testicular carcinoma

Received: April 19, 2005; revised: June 2, 2005; accepted: June 2, 2005

1 Introduction

Conjugated linoleic acid (CLA) is the collective term for a number of positional and geometric isomers of 18 : 2*n*-6 (linoleic acid) with conjugated double bonds. The major sources of CLA in the human diet are meat and dairy products, particularly cheese [1]. Since the end of the 1980s, CLA was shown to possess anticarcinogenic properties in several *in vivo* and *in vitro* studies [2, 3]. Due to such a potential, the feasibility of increasing CLA content of food is already being considered to increase CLA intake in humans for cancer prevention [4]. As yet, however, only little data dealing with the association of CLA with cancer in humans is available and only breast cancer was examined [5–8]. Therefore, the objective of this study was to examine the correlation between CLA and testicular cancer (TC). TC accounts for only 1% of all malignancies in males, but it is the most common cancer type in males between 15 and 35 years, with an annual incidence of 2–8 per 100 000. The trend of increasing incidence is the same worldwide for the white male population. In the United States and in the Western Societies the incidence doubles every 30 years [9]. Despite such an increasing incidence only little is known about the causes of TC. There is some evidence for genetic factors and trauma affecting the testis, as well as for some environmental exposures to heavy metals and benzene and its derivates [9]. Furthermore, nutritional factors have been associated with the etiology of TC [10, 11].

Several studies have shown that diets containing CLA are associated with altered polyunsaturated fatty acid (PUFA) metabolism and eicosanoid formation [12, 13]. In order to provide valuable clues to potential biochemical target sites of CLA, the contents of total CLA and of single CLA iso-

Correspondence: Prof. Dr. Dr. Hans Steinhart, Institute of Biochemistry and Food Chemistry, University of Hamburg, Grindelallee 117, D-20146 Hamburg, Germany
E-mail: hans.steinhart@chemie.uni-hamburg.de
Fax: +49-40-42838-4342

Abbreviations: CLA, conjugated linoleic acid; **COX**, cyclooxygenase; **FAME**, fatty aid methyl ester; **LOX**, lipooxygenase; **MUFA**, monounsaturated fatty acid; **PUFA**, polyunsaturated fatty acid; **TC**, testicular carcinoma/cancer

mers in total and subcellular fractions from normal and cancerous parts of human testes were determined and compared. Furthermore, the contents of the long-chain PUFA, which are involved in eicosanoid biosynthesis, were determined. Several studies have already focused on the incorporation of CLA into individual lipid classes [14, 15]. Only a few studies, however, have dealt with the distribution of CLA in cell organelles [16, 17] and no study reports on the distribution of CLA in cell organelles in human tissues.

2 Material and methods

2.1 Tissue procurement

A total of 20 patients with a clinical diagnosis of TC were included in the study. They all gave written consent to a protocol approved by the ethics commission of the Hamburg medical association. After radical surgery of the testes at the Hospital of the Federal Armed Forces, Hamburg, histological diagnosis of the tumors was done at the Department of Pathology, General Hospital Barmbek, Hamburg. A piece of cancerous tissue and a piece of normal testicular tissue was removed from the organ for study. Samples were snap-frozen and stored at −80°C until analysis.

2.2 Cell fractionation

About 500 mg of tissue specimens were fractionated into nuclear, mitochondrial, plasma membrane, and cytosolic fractions as described previously [18]. Briefly, tissue specimens were suspended in a tenfold volume of homogenization medium (0.25 M sucrose, 0.01 M Tris-HCl, 1 mM EDTA, pH 7.5) and homogenized, first in an Ultra-Turrax homogenizer for 5 s and second in a Potter-Elvehjem homogenizer with six up-and-down strokes of a Teflon pestle. The homogenate was filtered through a coarse metal sieve and was centrifuged at $1000 \times g$ for 20 min to sediment a crude nuclear fraction. The supernatant fluid was removed for further separation and the crude nuclear fraction was resuspended in 1.38 M sucrose and purified by centrifugation at $40\,000 \times g$ for 80 min. The supernatant fluid of the first centrifugation step ($1000 \times g$ for 20 min) was further centrifuged at $39\,000 \times g$ for 45 min to sediment mitochondria, plasma membranes, and the main parts of the lysosomes and peroxisomes. The $39\,000 \times g$ supernatant was regarded as the cytosolic fraction which also contains the microsomes. In order to separate the plasma membranes from the mitochondria, the pellet was resuspended in 1.38 M sucrose and homogenized in a Potter-Elvehjem homogenizer with three up-and-down strokes. 0.25 M sucrose was loaded on top of the suspension and the resulting discontinuous gradient was centrifuged at $57\,000 \times g$ for 90 min. The plasma membranes together with the lysosomes appear as a band at the sucrose interface and the mitochondria together with the peroxisomes are in the bottom pellet. The identity and purity of the subcellular fractions was determined by measurement of the activity of marker enzymes [18].

2.3 Lipid analysis

Lipids were extracted from whole-tissue homogenates as well as from cell fractions by extracting them twice with dichloromethane/methanol (2 : 1 v/v) and once with hexane. Subsequently, fatty acid methyl esters (FAMEs) were generated by transesterification of the lipid extract with 5% potassium methoxide in methanol (Merck-Schuchardt, Hohenbrunn, Germany) at 60°C for 15 min according to Pfalzgraf *et al.* [19]. The total FAME pattern was analyzed by GC (Hewlett Packard GC system 6890 with flame ionization detector; Waldbronn, Germany) using a CP-Sil 88 capillary column (100 m × 0.25 mm id with 0.2 µm film thickness; Chrompack, Middelburg, The Netherlands). The inlet temperature was 200°C and the detector temperature 260°C. The oven temperature was initially 70°C, then ramped 8°C/min to 180°C, held for 10 min, ramped again 3°C/min to 235°C and held another 10 min. A volume of 2 µL was injected at a split ratio of 10 : 1. Hydrogen was used as the carrier gas at 1.5 mL/min. CLA isomer distribution was determined by HPLC analysis (Fig. 1). Separation was carried out with a Waters 515 HPLC system (Waters, Milford, MA, USA) equipped with a photodiode array detector 996. Two silver-loaded Chromspher 5 Lipids columns (Chrompack), 5 µm particle size, 250 × 4.6 mm, were used in tandem with a mobile phase of hexane/acetonitrile (100 : 0.5 v/v) at a flow rate of 1 mL/min. Because of their conjugated double bonds, CLA isomers could be detected at 234 nm, whereas unconjugated unsaturated fatty acids absorbed at 200 nm. Commercial FAME standards (Sigma Chemical, Deisenhofen, Germany), including a mixture of CLA isomers, were used to identify and quantify sample FAMEs in area-% of total FAMEs and total CLA, respectively.

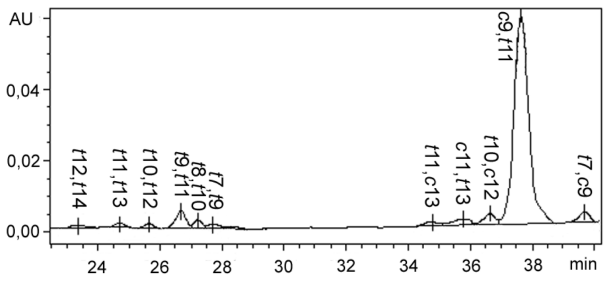

Figure 1. HPLC-diode array detector (DAD) chromatogram of CLA isomers from human testicular carcinoma.

2.4 Statistical analysis

Data were analyzed with SPSS Statistical Software, Version 11.5.2.1 (SPSS, Chicago, IL, USA). All data are expressed as mean ± SD. Statistical comparisons between normal and cancerous testicular tissue were made using Student's two-tailed *t*-test for paired values ($P < 0.05$). One-way analysis of variance (ANOVA) with Scheffé, Student-Newman-Keuls, and Tukey-B as post-hoc-tests was used to determine significant differences in fatty acid distribution between the subcellular fractions ($P < 0.05$).

3 Results

The 20 TC patients studied were born between 1948 and 1982 (mean year of birth 1967 ± 9.9). The tumor histopathologies were collected at the Department of Pathology, General Hospital Barmbek, Hamburg, and are shown in Table 1. Comparing normal with cancerous testicular tissue, the CLA content was significantly higher in total TC (normal: 0.25 ± 0.12%, cancerous: 0.35 ± 0.15%), but significantly lower in the mitochondrial fraction of TC (normal: 0.36 ± 0.04%, cancerous: 0.26 ± 0.09%). The CLA content of nuclei (normal: 0.27 ± 0.10%, cancerous: 0.36 ± 0.21%), cytosol (normal: 0.28 ± 0.18%, cancerous: 0.38 ± 0.12%), and plasma membranes (normal: 0.21 ± 0.12%, cancerous: 0.28 ± 0.15%) tended to be higher in TC than in normal testicular tissue, although no significant differences were detected (Fig. 2).

Regarding the distribution pattern of CLA within the subcellular fractions of each of the two types of tissue studied, a great similarity to the distribution pattern of the monounsaturated fatty acids (MUFAs) 14 : 1*cis*9, 16 : 1*cis*9, 18 : 1*cis*9, 20 : 1*cis*11, 22 : 1*cis*13, and 24 : 1*cis*15 in sum was observed for normal as well as for cancerous testicular tissue (Fig. 3). Although hardly any significant differences in CLA and MUFA content, respectively, between the subcellular fractions within one kind of tissue studied were detectable, the same tendencies for CLA and MUFAs were obvious. Comparison of the distribution pattern of CLA with the distribution pattern of 18 : 2*n*-6 revealed a different pattern of these two fatty acids for normal as well as for cancerous testicular tissue (Fig. 4). 18 : 2*n*-6 is more evenly distributed than CLA.

Table 1. Tumor classifications (WHO/UICC) of the 20 testicular carcinomas studied

Histopathology	Tumor classification (WHO/UICC)[a]	
	pT1[b]	pT2[c]
Seminoma	–	11
Seminomatous mixed germ cell tumor	–	5
Nonseminomatous mixed germ cell tumor	1	2
Teratoma	1	–

a) WHO, World Health Organisation; UICC, Unio Internationale Contra Cancrum
b) pT1, tumor in testis or rete testis
c) pT2, tumor spreads beyond tunica albuginea or into epididymis.

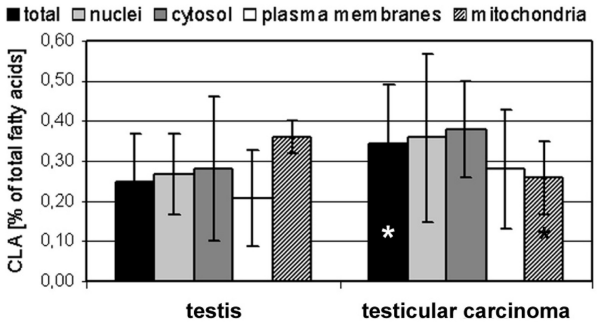

Figure 2. CLA content in tissue samples of human testis and testicular carcinoma as well as in their subcellular fractions. Values are means ± SD ($n = 20$). No significant differences between the subcellular fractions within one kind of tissue were detected ($P < 0.05$). *Corresponding normal and cancerous fractions were significantly different at $P < 0.05$.

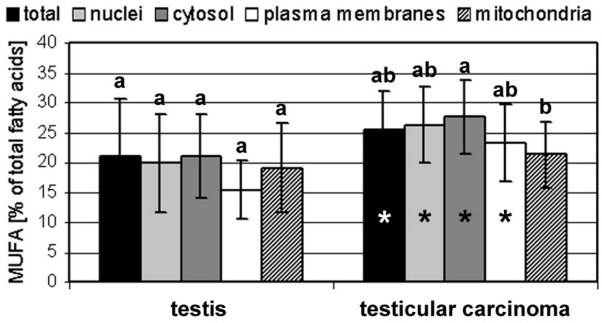

Figure 3. Contents of the MUFAs 14 : 1*cis*9, 16 : 1*cis*9, 18 : 1*cis*9, 20 : 1*cis*11, 22 : 1*cis*13, and 24 : 1*cis*15 in sum in tissue samples of human testis and testicular carcinoma as well as in their subcellular fractions. Values are means ± SD ($n = 20$). Columns marked with different letters within one kind of tissue are significantly different ($P < 0.05$). *Corresponding normal and cancerous fractions were significantly different at $P < 0.05$.

Furthermore, significant differences in MUFA and 18 : 2*n*-6 content, respectively, between normal and cancerous testicular tissue were detected (Figs. 3 and 4). The MUFA content was significantly higher in total lipid extract of TC than of normal testicular tissue (normal: 21.02 ± 9.49%, cancerous: 25.47 ± 6.30%). The same was found for the nuclear (normal: 19.92 ± 8.31%, cancerous: 26.34 ± 6.35%), cytosolic (normal: 21.03 ± 6.96%, cancerous: 27.52 ± 6.30%), and plasma membrane (normal: 15.30 ± 4.88%, cancerous:

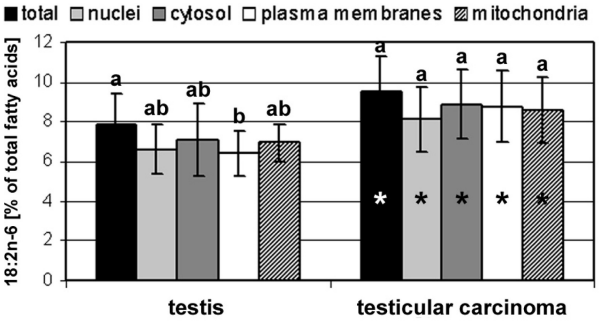

Figure 4. 18 : 2*n*-6 content in tissue samples of human testis and testicular carcinoma as well as in their subcellular fractions. Values are means ± SD ($n = 20$). Columns marked with different letters within one kind of tissue are significantly different ($P < 0.05$). *Corresponding normal and cancerous fractions were significantly different at $P < 0.05$.

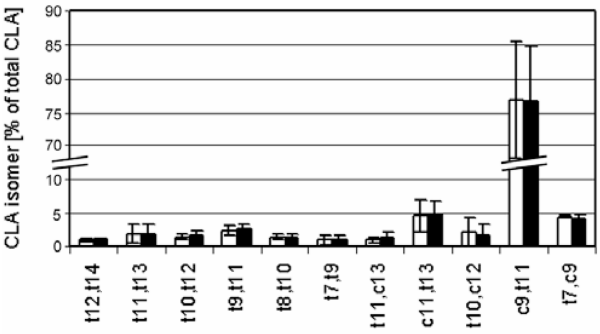

Figure 5. Contents of CLA isomers in tissue samples of human testis (white column) and testicular carcinoma (black column). Values are means ± SD ($n = 20$).

23.25 ± 6.47%) fractions. The 18 : 2*n*-6 content was significantly higher in total lipid extract (normal: 7.86 ± 1.57%, cancerous: 9.48 ± 1.77%) as well as in all subcellular fractions of TC. Regarding the single CLA isomers (Fig. 5), *cis*9,*trans*11-CLA was shown to be the prominent CLA isomer in normal as well as in cancerous testicular tissue with more than 75% of total CLA. Significant differences in the content between both types of tissues were not detected for any CLA isomer.

In addition to the CLA contents, the total fatty acid profiles were determined. 16 : 0, 18 : 0, 18 : 1*cis*9, 18 : 2*n*-6, and 20 : 4*n*-6 were found to be the major fatty acids in all subcellular fractions from both types of testicular tissue studied (data not shown). In detail, the contents of the long-chain *n*-6 (18 : 2*n*-6, 18 : 3*n*-6, 20 : 3*n*-6, and 20 : 4*n*-6) and *n*-3 (18 : 3*n*-3, 20 : 5*n*-3, and 22 : 6*n*-3) PUFAs were examined (Figs. 6A–C). The 18 : 2*n*-6 (normal: 7.86 ± 1.57%, cancerous: 9.48 ± 1.77%), 18 : 3*n*-3 (normal: 0.16 ± 0.04%, cancerous: 0.28 ± 0.12%), and 20 : 5*n*-3 (normal: 0.18 ± 0.05%,

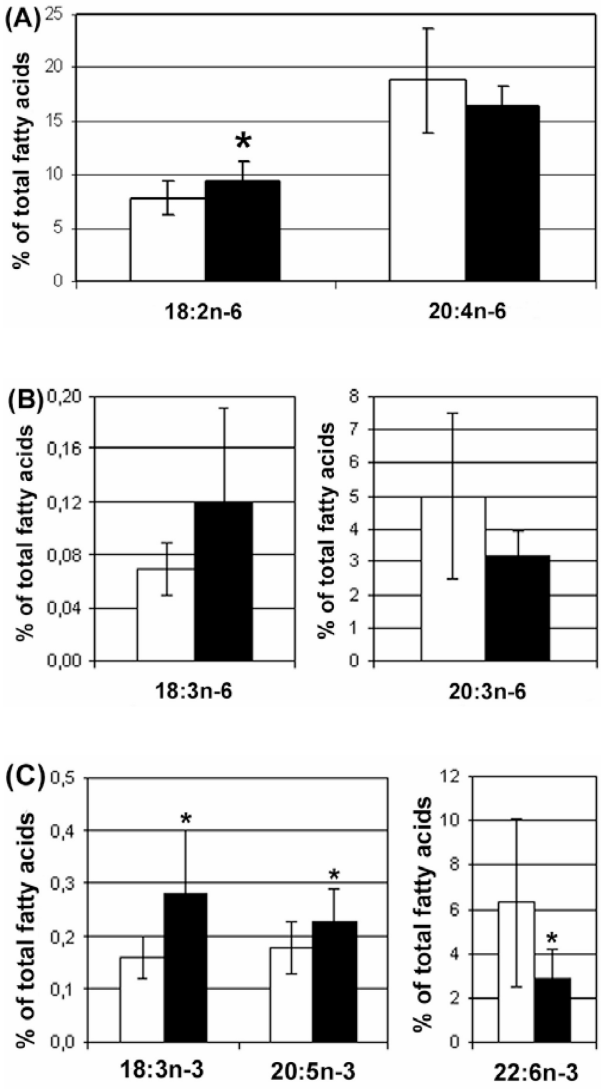

Figure 6. Contents of (A) 18 : 2*n*-6 and 20 : 4*n*-6, (B) 18 : 3*n*-6 and 20 : 3*n*-6, and (C) 18 : 3*n*-3, 20 : 5*n*-3, and 22 : 6*n*-3 in tissue samples of human testis (white column) and testicular carcinoma (black column). Values are means ± SD ($n = 20$). *Corresponding normal and cancerous fractions were significantly different at $P < 0.05$.

cancerous: 0.23 ± 0.06%) contents were found to be significantly higher in TC than in normal testicular tissue, whereas the 22 : 6*n*-3 content (normal: 6.29 ± 3.81%, cancerous: 2.81 ± 1.35%) was found to be significantly lower. The contents of 20 : 4*n*-6 (normal: 18.80 ± 4.82%, cancerous: 16.40 ± 1.90%) and 20 : 3*n*-6 (normal: 4.99 ± 2.49%, cancerous: 3.18 ± 0.74%) tended to be lower, whereas the content of 18 : 3*n*-6 (normal: 0.07 ± 0.02%, cancerous: 0.12 ± 0.07%) tended to be higher in TC than in normal testicular tissue. Due to high standard deviations, these differences were not significant.

4 Discussion

CLA contents in the subcellular fractions of the normal and cancerous testicular tissues studied were close to the limit of detection, due to the small amounts of tissue specimens available. Nevertheless, CLA contents in % of total fatty acids could reliably be determined in the total tissues as well as in all subcellular fractions. However, due to the fact that the patients studied are inhomogeneous in their anthropomorphical and clinical data, the standard deviations are partially quite high. Nevertheless, significant differences between normal and cancerous testicular tissue were detectable, which emphasizes the relevance of these differences.

The total CLA content was shown to be significantly higher in TC than in normal testicular tissue, but no differences in the contents of single CLA isomers were detected. In the literature, contradictory results are described. Chajès *et al.* [6] found no significant difference in mean CLA levels between patients with invasive breast carcinoma and patients with benign breast pathologies, whereas Lavillonnière and Bougnoux [20] showed that the CLA content of breast tissue was higher in women with benign disease than in women with carcinoma of the breast. However, these two studies had a different study design than the study described here. They both examined the CLA content of breast adipose tissue, but none of them determined the CLA content of carcinomas. In an earlier study [21], we compared the CLA content of kidney and renal cell carcinoma (RCC). No significant difference in total CLA content between renal tissue and RCC could be observed. However, the content of *cis*11,*trans*13-CLA was shown to be significantly increased in RCC.

To examine the CLA content of TC more precisely, the total CLA content was measured not only in human TC and corresponding unaffected testicular tissue of human tumor-bearing testes, but also in subcellular fractions of these tissues. The CLA content was found to be significantly lower in the mitochondrial fraction of TC.

Demizieux *et al.* [17] examined the mitochondrial oxidizability of *cis*9,*trans*11- and *trans*10,*cis*12-CLA. They showed that both CLA isomers are oxidized to a marked lesser extent than 18 : 2*n*-6 and are capable of accumulating inside the mitochondrial matrix and of interfering with the oxidation of usual fatty acids at a step close to the beginning of the β-oxidative cycle in rat liver mitochondria. However, Banni *et al.* [22] recently described the detection of conjugated C16 PUFA in rat tissues as possible partial peroxisomal β-oxidation products of naturally occurring CLA. As the fraction designated here as mitochondrial fraction also contains a great part of the peroxisomes [18], the lower amount of CLA in the mitochondrial fraction of TC leads to the speculation that peroxisomes in TC show a stronger β-oxidation activity of CLA in comparison to normal testicular tissue.

Palacios *et al.* [23] showed that CLA is an effective protector of rat liver mitochondria from peroxidative damage. Because CLA is esterified in phospholipids, it may represent a heretofore unrecognized *in situ* defense mechanism against membrane attack by oxygen radicals [24]. One might speculate that increasing the mitochondrial CLA level by dietary CLA intake could inhibit peroxidative mitochondrial cell damage during carcinogenesis in TC. In summary, the decrease in CLA content in the mitochondrial fraction of TC might be an indication of an increased partial peroxisomal β-oxidation of CLA and a resulting decrease in defense against membrane attack by oxygen radicals. The physiological properties of conjugated C16 PUFA need to be evaluated.

In contrast to TC, the total CLA content in RCC was found to be significantly increased in the plasma membrane fraction in comparison to the plasma membrane fraction of normal renal tissue [21], whereas no significant differences in the mitochondrial fractions of these two types of tissues were detectable.

Comparison of the incorporation of CLA into the subcellular fractions studied revealed that CLA is incorporated into the plasma membrane fractions of both tissues to a lesser extent than into the nuclear and cytosolic fractions, although no significant differences could be detected. This result is in agreement with our study of CLA in subcellular fractions of normal renal tissue and RCC where it was shown that CLA is best incorporated into nuclei and cytosol and significantly less into plasma membranes and mitochondria [21].

The subcellular distribution of total CLA was compared with the distribution of MUFAs and 18 : 2*n*-6. Corresponding to the examination of the normal and cancerous renal tissues [21] it turned out that CLA and MUFA are similarly distributed, whereas 18 : 2*n*-6 shows a different distribution pattern. The most prominent CLA isomers in testicular tissue and TC are the *cis*,*trans*- and *trans*,*cis*-isomers, respectively. As *cis*,*trans* and *trans*,*cis* unsaturated fatty acids show a stereochemical structure similar to that of *cis*-MUFA, their similar distribution pattern is easily explained. The differing subcellular distribution pattern of 18 : 2*n*-6 and CLA, supporting the results of Banni *et al.* [15] who found that 18 : 2*n*-6 is distributed mainly in phospholipids, whereas CLA is incorporated primarily in neutral lipids of rat liver, is underlining the different physiological properties of these two fatty acids.

Apart from CLA, the contents of long-chain *n*-6 and *n*-3 PUFAs were determined in testis and TC, because one mechanism that has been suggested for the effect of PUFA

on cancer risk is the increased metabolism of 18 : 2*n*-6 into 20 : 4*n*-6 and eicosanoids by the tumor tissue [25]. To our knowledge, there is no literature describing the fatty acid composition of human TC. We found significant and tendential differences in *n*-6 and *n*-3 fatty acid incorporation between normal testicular tissue and TC. These results indicate a changed availability of substrates for the cyclooxygenase (COX) or lipoxygenase (LOX) pathways generating eicosanoids. Eicosanoids have been implicated in the pathogenesis of a variety of human diseases, including cancer, and are considered to be important in tumor promotion, progression, and metastasis [26].

COX-1, -2 and LOX are reported to be only slightly expressed in testicular tissue, but markedly expressed in TC [27, 28]. Therefore, the reduced content of 20:4*n*-6 shown in this study might be due to an increased metabolism of this fatty acid into eicosanoids. COX-1, -2 and LOX inhibitors were shown to inhibit the growth of TC cells [27, 28]. These results suggest that the metabolism of *n*-6 and *n*-3 PUFAs into eicosanoids *via* the COX and LOX pathways are essential for cell growth of TC cells. Further understanding of the cause and/or consequence of the differences in PUFA metabolism between normal testicular tissue and TC may lead to a better understanding of TC. Banni *et al.* [12] found that CLA inhibits the elongation and desaturation of 18 : 2*n*-6 into 20 : 4*n*-6. In that case, a diet enriched in CLA might be a useful tool in preventing TC. However, the involvement of CLA in preventing TC cannot be definitively demonstrated from the design of this experiment. In future work it should be examined if eicosanoids are effectively increased in the tumor.

Studies about dietary factors and the risk of TC are rare. Ganmaa *et al.* [11] found a diet rich in cheese, animal fats, and milk to be positively correlated with the incidence of TC at ages 20 – 39. Sigurdson *et al.* [29] reported that high total fat consumption increases TC risk in young men. As cheese, milk, and fat of ruminants are rich in CLA, these studies seem to be controversial to the expected beneficial health effects of CLA. One possible explanation for the lack of an inhibitory effect of ruminant fat on the incidence of TC is that a high consumption of meat is accompanied by an increased intake of 20 : 4*n*-6, which is discussed to have carcinogenic properties [28]. Furthermore, it is very likely that the amount of CLA stored in human tissues is too low to induce any protective effect on tumor development. CLA remains at a level in which inhibition of tumor growth has never been documented in animals.

This work was supported by grants from the "Deutsche Forschungsgemeinschaft" (DFG).

5 References

[1] Fritsche, J., Steinhart, H., Amounts of conjugated linoleic acid (CLA) in german foods and evaluation of daily intake. *Z. Lebensm. Unters. Forsch. A* 1998, *206*, 77 – 82.

[2] Ip, C., Banni, S., Angioni, E., Carta, G., *et al.*, Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *J. Nutr.* 1999, *129*, 2135 – 2142.

[3] Palombo, J. D., Ganguly, A., Bistrian, B. R., Menard, M. P., The antiproliferative effects of biologically active isomers of conjugated linoleic acid on human colorectal and prostatic cancer cells. *Cancer Lett.* 2002, *177*, 163 – 172.

[4] O'Shea, M., Lawless, F., Stanton, C., Devery, R., Conjugated linoleic acid in bovine milk fat: a food-based approach to cancer prevention. *Trends Food Sci. Technol.* 1998, *9*, 192 – 196.

[5] Aro, A., Mannisto, S., Salminen, I., Ovaskainen, M. L., *et al.*, Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. *Nutr. Cancer* 2000, *38*, 151 – 157.

[6] Chajès, V., Lavillonnière, F., Ferrari, P., Jourdan, M.-L., *et al.*, Conjugated linoleic acid content in breast adipose tissue is not associated with the relative risk of breast cancer in a population of French patients. *Cancer Epidemiol. Biomarkers Prevention* 2002, *11*, 672 – 673.

[7] Chajès, V., Lavillonnière, F., Maillard, V., Giraudeau, B., *et al.*, Conjugated linoleic acid content in breast adipose tissue of breast cancer patients and the risk of metastasis. *Nutr. Cancer* 2003, *45*, 17 – 23.

[8] Voorrips, L. E., Brants, H. A. M., Kardinaal, A. F. M., Hiddink, G. J., *et al.*, Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands cohort study on diet and cancer. *Am. J. Clin. Nutr.* 2002, *76*, 873 – 882.

[9] Schmoll, H. J., Hossfeld, D. K., Cancer of the testis, in: Love, R. R. (Ed.), *Manual of Clinical Oncology*, Springer-Verlag, Berlin 1994, pp. 395 – 408.

[10] Bonner, M. R., McCann, S. E., Moysich, K. B., Dietary factors and the risk of testicular cancer. *Nutr. Cancer* 2002, *44*, 35 – 43.

[11] Ganmaa, D., Li, X.-M., Wang, J., Qin, L.-Q., *et al.*, Incidence and mortality of testicular and prostatic cancers in relation to world dietary practices. *Int. J. Cancer* 2002, *98*, 262 – 267.

[12] Banni, S., Angioni, E., Casu, V., Melis, M. P., *et al.*, Decrease in linoleic acid metabolites as a potential mechanism in cancer risk reduction by conjugated linoleic acid. *Carcinogenesis* 1999, *20*, 1019 – 1024.

[13] Kavanaugh, C. J., Liu, K. L., Belury, M. A., Effect of dietary conjugated linoleic acid on phorbol ester-induced PGE(2) production and hyperplasia in mouse epidermis. *Nutr. Cancer* 1999, *33*, 132 – 138.

[14] Sugano, M., Tsujita, A., Yamasaki, M., Yamada, K., *et al.*, Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats. *J. Nutr. Biochem.* 1997, *8*, 38 – 43.

[15] Banni, S., Carta, G., Angioni, E., Murru, E., *et al.*, Distribution of conjugated linoleic acid and metabolites in different lipid fractions in the rat liver. *J. Lipid Res.* 2001, *42*, 1056 – 1061.

[16] Demarée, S. R., Gilbert, C. D., Mersmann, H. J., Smith, S. B., Conjugated linoleic acid differentially modifies fatty acid composition in subcellular fractions of muscle and adipose tissue but not adiposity of postweanling pigs. *J. Nutr.* 2002, *132*, 3272 – 3279.

[17] Demizieux, L., Degrace, P., Gresti, J., Loreau, O., *et al.*, Conjugated linoleic acid isomers in mitochondria: Evidence for an alteration of fatty acid oxidation. *J. Lipid Res.* 2002, *43*, 2112 – 2122.

[18] Hoffmann, K., Blaudszun, J., Brunken, C., Höpker, W.-W., *et al.*, New application of a subcellular fractionation method to kidney and testis for the determination of conjugated linoleic acid in selected cell organelles of healthy and cancerous human tissues. *Anal. Bioanal. Chem.* 2005, *381*, 1138 – 1144.

[19] Pfalzgraf, A., Timm, M., Steinhart, H., Gehalte von *trans*-Fettsäuren in Lebensmitteln. *Z. Ernährungswiss.* 1994, *33*, 24 – 43.

[20] Lavillonnière, F., Bougnoux, P., Conjugated linoleic acid (CLA) and the risk of breast cancer, in: Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W., Nelson, G. J. (Eds.), *Advances in Conjugated Linoleic Acid Research*, Vol. 1, AOCS Press, Champaign, USA 1999, pp. 276 – 282.

[21] Hoffmann, K., Blaudszun, J., Brunken, C., Höpker, W.-W., *et al.*, Distribution of polyunsaturated fatty acids including conjugated linoleic acids in total and subcellular fractions from healthy and cancerous parts of human kidneys. *Lipids* 2005, *40*, 309 – 315.

[22] Banni, S., Petroni, A., Blasevich, M., Carta, G., *et al.*, Detection of conjugated C16 PUFAs in rat tissues as possible partial beta-oxidation products of naturally occurring conjugated linoleic acid and its metabolites. *Biochim. Biophys. Acta* 2004, *1682*, 120 – 127.

[23] Palacios, A., Piergiacomi, V., Catalá, A., Antioxidant effect of conjugated linoleic acid and vitamin A during non enzymatic lipid peroxidation of rat liver microsomes and mitochondria. *Mol. Cell. Biochem.* 2003, *250*, 107 – 113.

[24] Pariza, M. W., Ha, Y. L., Newly recognized anticarcinogenic fatty acids. *Basic Life Sci.* 1990, *52*, 167 – 170.

[25] Bartsch, H., Nair, J., Owen, R. W., Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: Emerging evidence for their role as risk modifiers. *Carcinogenesis* 1999, *20*, 2209 – 2218.

[26] Bawa, S., An update on the beneficial roles of conjugated linoleic acid (CLA) in modulating human health: Mechanisms of action – A review, *Pol. J. Food Nutr. Sci.* 2003, *12*, 3 – 13.

[27] Hase, T., Yoshimura, R., Matsuyama, M., Kawahito, Y., *et al.*, Cyclooxygenase-1 and -2 in human testicular tumours. *Eur. J. Cancer* 2003, *39*, 2043 – 2049.

[28] Yoshimura, R., Matsuyama, M., Mitsuhashi, M., Takemoto, Y., *et al.*, Relationship between lipoxygenase and human testicular cancer. *Int. J. Mol. Med.* 2004, *13*, 289 – 393.

[29] Sigurdson, A. J., Chang, S., Annegers, J. F., Duphorne, C. M., *et al.*, A case-control study of diet and testicular carcinoma. *Nutr. Cancer* 1999, *34*, 20 – 26.

