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ORIGINAL CONTRIBUTION

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Conjugated linoleic acid effects on circulating hormones, metabolites and lipoproteins, and its proportion in fasting serum and erythrocyte membranes of swine

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Summary *Background:* Conjugated linoleic acids (CLA)[#] refer to a group of linoleic acid (18:2)-derived isomers with conjugated double bonds mostly at carbon atoms 9 and 11 or 10 and 12, and with all possible *cis* and *trans* combinations. CLA is a newly recognized nutrient that functions to regulate energy retention and metabolism and that causes a serum lipoprotein profile considered to be less atherogenic. However, rodent models that have been frequently used for these studies are only of limited use because of distinct differences in physiology, compared with man. Additionally, possible differences in food intake between the experimental groups remained often unconsidered in those studies. Thus, it can not be excluded that the beneficial effects of CLA reported in a series of studies may be due, at least partially, to differences in nutrient and energy ingested.

Aims of the study: This prompted us to undertake an investigation on the action of CLA by using a pig model and a feeding regimen with controlled amounts of food and antioxidants. The parameters used to assess CLA-specific action were selected hormones and metabolites involved in energy metabolism, individual lipoproteins and the appearance of CLA in fasting serum and erythrocyte membranes. Blood as an easily available biological sample was used for investigation.

Methods: For that purpose 16 adult female pigs were divided into two groups of 8 each, and were isoenergetically fed diets containing 0 (control diet) or 1% level of CLA (by weight) for 6 weeks. Plasma concentrations of total and free thyroxine (T4), total and free triiodothyronine (T3), and insulin were measured by radioimmunoassays. Plasma non-esterified fatty acids (NEFA), β-hydroxybutyrate, circulating blood ATP and other clinical chemical variables were determined using enzymatic assays. The concentration of α-tocopherol was determined by high performance liquid chromatography. The lipoproteins VLDL (density <1.019 kg/L), LDL (density 1.019 – 1.063 kg/L), and HDL (density >1.063 kg/L) were isolated by step-wise ultracentrifugation. Fatty acids of the dietary oils, serum and blood cell membranes were separated and quantified by gas chromatography.

Results: At week 6, body weights of the pigs fed the CLA-supplemented diet were not different from that of the controls. CLA-treated pigs exhibited a 37% higher concentration of fasting serum insulin than their controls receiving no CLA (P = 0.11). Circulating free and total T4 and T3 as well as serum levels of

β-hydroxybutyrate, α-tocopherol, protein, glucose, urea, creatinine and circulating blood ATP remained unaffected by CLA supplementation. Serum concentrations of non-esterified fatty acids were reduced by 38% in CLA-treated pigs relative to the controls, although this difference was not significant. CLA-treated pigs tended to have lower leukocyte counts in blood than their controls ($P < 0.1$). Erythrocyte and platelet counts, the hematocrit and hemoglobin concentration were similar between the groups. Serum of CLA-treated pigs showed a trend toward increased levels of triacylglycerols, cholesterol and phosphatidylcholine in the very low density and low density lipoproteins (LDL), without distinct changes in the high density lipoprotein fraction (HDL). The LDL cholesterol to HDL cholesterol ratio was significantly increased by CLA. When pigs were fed CLA at a dietary level of 1%, limited proportions of CLA appeared in fasting serum (1.6%) and erythrocyte membranes (1.1%).

Conclusions: Under the present experimental conditions there appeared to be parallels between the effects of CLA and the reported effects of *trans* fatty acids in the mode of action on lipoproteins and insulin. The failure to demonstrate significant beneficial effects of CLA on the lipoprotein profile which have been observed in other studies requires further research.

Key words Conjugated linoleic acid – hormones – metabolites – lipoproteins – fatty acids – swine

Abbreviations CLA, conjugated linoleic acids; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; T4, thyroxine; T3, triiodothyronine; NEFA, non-esterified fatty acids; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; FAMEs, fatty acid methyl esters; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Introduction

Conjugated linoleic acids (CLA) refer to a group of linoleic acid (18:2)-derived isomers with conjugated double bonds mostly at carbon atoms 9 and 11 or 10 and 12, and with all possible *cis* and *trans* combinations. CLA is a newly recognized nutrient that functions to regulate energy retention and metabolism (8, 31, 32). Recently reported experiments with CLA indicated enhanced lipolysis (32), an increase in lean body mass (13, 31, 32), and an inhibition of proliferation and differentiation of preadipocytes (5). Furthermore, CLA has been shown to reduce the development of atherosclerosis in rabbits (23) and hamsters (30) via hypolipidemic effects. The metabolic effects and lipid-lowering properties of CLA have been associated with relatively low levels of CLA in the diet ranging between 0.1 and 1.5% (by weight). However, most of these studies did not take into consideration the fact that CLA-fed animals often reduce voluntary food intake. Thus, the observed effects may be due at least partially to differences in feed intake between the control group and the CLA animals. However, an alignment of food intake between the CLA and the control group is necessary to limit the observed differences to the action of CLA alone and to make valid conclusions. Additionally, rodent models that have been frequently used for the CLA studies are only of limited use because of distinct differences in physiology and metabolism, compared with man. We suggest that the selection of pigs as an animal model reflects human physiological conditions much better than other animal models or *in vitro* assays. This prompted us to investigate the CLA mode of the action on selected hormones and metabolites involved in energy metabolism, individual lipoproteins and the appearance of CLA in fasting serum and erythrocyte membranes in pigs fed controlled amounts of diets with equalized levels of α-tocopherol. Blood as an easily available biological sample was used for investigation in order to find a marker specifically reacting on CLA consumption that can also be applied in human studies.

Materials and methods

Animals and diets

In this experiment, 16 adult female pigs with an average body weight of 186.4 ± 4.0 kg were divided into two groups of 8 each and were fed diets containing 0 (control diet) or 1% CLA (by weight) for 6 weeks. The control diet contained 1.65% sunflower oil and the CLA-fortified diet contained 1.65% of a CLA oil with 60.5% CLA isomers (Multi-Food GmbH, Buxtehude, Germany) at the expense of sunflower oil. All other components of the diet remained unchanged. Analysis of the isomeric composition (%, by weight) of the CLA-containing oil was done by the Institute of Food Chemistry and Biochemistry (University of Hamburg, Germany) using a gas chromatographic method (15). The CLA oil consisted of 34.6% *c*9,*t*11 plus *t*9,*c*11 CLA, 18.4% *t*10,*c*12 CLA, 5.4% *t*9,*t*11 CLA, and 2.1% *c*9,*c*11 isomers. The fatty acid composition of the control diet and the CLA-fortified diet is shown in Table 1. The amounts of individual diet fatty acids did not change during storage indicating lack of decomposition of unsaturated fatty acids by oxidative processes. The basal diet consisted of 55.35% barley, 20% maize starch, 5% soybean meal, 15% oat hull bran, 1% of a mixture of soybean oil, rape seed oil and sunflower oil (30:60:10, %, by weight), 1.65% of sunflower oil/CLA oil and 2% of a mineral-vitamin premix. The crude nutrient contents and gross energy of the diets were described recently (27). The difference in the dietary α-tocopherol levels between the sunflower oil (640 mg/kg) and the

Table 1 Composition of the major fatty acids (mol/100 mol fatty acids) in the control and CLA diet

Fatty acid	Control diet	CLA diet
16:0	15.3	13.1
16:1	0.28	0.31
18:0	5.89	3.42
18:1	28.5	27.5
18:2 n-6 (*c9,c12*)	44.7	23.3
18:3 n-6	0.71	0.40
18:3 n-3	3.94	1.80
22:1	0.69	0.34
CLA[1)]	n.d.[2)]	29.9

[1)] Conjugated linoleic acid consisting of the following CLA isomers:34.6% c9,*t11* plus t9,*c11*, 18.4% *t10,c12*, 5.4% *t9,t11* and 2.1% *c9,c11*
[2)] not detectable

Table 2 CLA effect on circulating insulin and thyroid hormones in fasting serum

Hormone	Control group	CLA group	P
Insulin (mU/L)	58.5 ± 21.3[1)]	79.9 ± 25.5	0.11
Total thyroxin (nmol/L)	52.3 ± 10.8	55.0 ± 6.9	0.57
Free thyroxin (pmol/L)	16.8 ± 5.7	16.3 ± 2.9	0.83
Total triiodothyronine (nmol/L)	1.04 ± 0.32	1.24 ± 0.39	0.31
Free triiodothyronine (pmol/L)	0.87 ± 0.12	0.86 ± 0.20	0.91

[1)] Values represent means ± SD, n = 8 for each group

Table 3 CLA effect on circulating blood ATP and serum concentrations of β-hydroxybutyrate, non-esterified fatty acids, α-tocopherol and other clinical-chemical parameters

Metabolites	Control group	CLA group	P
ATP (mmol/L)	822 ± 36[1)]	848 ± 43	0.22
β-Hydroxybutyrate (µmol/L)	5.48 ± 0.86	5.67 ± 1.54	0.77
Non-esterified fatty acids (µmol/L)	101 ± 61	63 ± 46	0.21
α-Tocopherol (µmol/L)	4.05 ± 0.51	3.85 ± 0.55	0.50
Total protein (g/L)	79.4 ± 4.9	80.9 ± 4.3	0.54
Albumin (g/L)	43.8 ± 3.2	46.2 ± 3.5	0.17
Creatinine (µmol/L)	206 ± 23	209 ± 28	0.83
Urea (mmol/L)	2.19 ± 0.47	2.43 ± 0.37	0.27
Glucose (mmol/L)	4.17 ± 0.54	4.12 ± 0.75	0.90

[1)] Values represent means ± SD, n = 8 for each group

CLA-containing oil (21 mg/kg) was made up by adding 619 mg α-tocopherol per kg CLA oil. The daily feed allowance matched the energetic maintenance requirement of 430 kJ metabolizable energy/kg $W^{0.75}$ (16). All pigs were treated in accordance with normal animal husbandry practices. The animals were kept under experimental conditions. The pigs were offered the diets in two equal portions per day. At each meal the sows were offered 5 L of water. At the end of the experimental period at week 6, 12 h after the last feeding, blood for lipid and hormone analysis was obtained by puncture of the ear vein.

Analyses

Blood for determination of serum hormones, lipoproteins and metabolites was collected into untreated tubes. Hematological parameters and ATP were determined from EDTA-treated blood and isolation of the erythrocyte membranes was done from heparin-treated blood. Measurement of ATP, β-hydroxybutyrate and the blood cells as well as isolation of serum lipoproteins and erythrocyte membranes were done immediately after sample collection. Serum samples for measurement of the other variables were stored at –80 °C until analyzed.

Plasma concentrations of total and free thyroxine (T4) and total and free triiodothyronine (T3) were measured by radioimmunoassay (RIA) kits (ICN Pharmaceuticals, Inc., Costa Mesa, CA). Plasma samples were assayed for insulin by a double-antibody RIA (ICN Biomedicals, Eschwege, Germany) using recombinant human insulin as a standard. The level of insulin has to be considered as a relative value because of the non-examined cross-reactivity. All hormone analyses were performed in duplicate. Total protein, albumin, creatinine, urea, and glucose in serum were determined by standardized procedures using an auto analyzer (Hitachi 704, Boehringer, Mannheim, Germany) and Boehringer kit reagents (Boehringer, Mannheim, Germany). Plasma non-esterified fatty acids (NEFA) were analyzed using an enzymatic kit assay based on acyl-CoA synthase (EC 6.2.1.3), acyl-CoA oxidase (EC 1.3.3.6), and peroxidase reactions (Boehringer, Cat. No. 1383175). Plasma β-hydroxybutyrate was determined using an enzymatic kit assay (Boehringer, Cat. No. 907979) based on an enzymatic colorimetric method of Bergmeyer and Bernt (4). Blood samples for ATP analysis were immediately deproteinized after sampling using a 12% (w/v) trichloroacetic acid. The 6000 rpm supernatant was used for enzymatic determination of ATP with a commercially available test kit (Sigma Chemical Co., St. Louis, MO, Cat. No. 366-A) and freshly prepared ATP standard solutions. Concentrations of α-tocopherol in the diet and serum were determined by high performance liquid chromatography (2). Tocopherols were extracted with n-hexane, separated on a LiChrosorb Si 60 column (5 µm particle size, 250 mm length, 4 mm internal diameter, Merck) with an n-hexane and 1,4-dioxane mixture

Table 4 CLA effect on blood cells and hematological measurements

Hematological variable	Control group	CLA group	P
White blood cells (10^9/L)	13.2 ± 2.0[1)]	11.1 ± 2.6	0.09
Red blood cells (10^{12}/L)	6.89 ± 0.59	6.79 ± 0.84	0.77
Platelets (10^9/L)	196 ± 53	156 ± 96	0.33
Hemoglobin concentration (g/L)	146 ± 14	148 ± 17	0.89
Hematocrit (%)	42.5 ± 3.5	42.8 ± 5.1	0.91
MCV (μm^3)	61.6 ± 1.9	63.2 ± 3.2	0.26
MCH (pg/cell)	21.2 ± 0.9	21.7 ± 1.1	0.28

[1)] Values represent means ± SD, n = 8 for each group

Table 5 CLA effect on serum lipids and lipoproteins

Lipid/lipoprotein	Control group	CLA group	P
Triacylglycerols			
Serum (µmol/L)	202 ± 101[1)]	279 ± 130	0.22
VLDL (µmol/L)	150 ± 84	206 ± 103	0.27
LDL (µmol/L)	40.4 ± 12.3	63.7 ± 24.4	0.03
HDL (µmol/L)	11.8 ± 11.3	13.1 ± 8.4	0.78
Total cholesterol			
Serum (mmol/L)	1.71 ± 0.24	1.78 ± 0.20	0.56
VLDL (µmol/L)	32.7 ± 21.4	54.4 ± 27.8	0.11
LDL (mmol/L)	0.75 ± 0.13	0.87 ± 0.15	0.10
HDL (mmol/L)	0.96 ± 0.19	0.87 ± 0.13	0.32
Phosphatidylcholine			
Serum (mmol/L)	1.17 ± 0.16	1.16 ± 0.12	0.88
VLDL (µmol/L)	28.5 ± 15.8	42.0 ± 25.0	0.23
LDL (µmol/L)	226 ± 40	254 ± 41	0.19
HDL (mmol/L)	0.94 ± 0.15	0.86 ± 0.10	0.20
Ratio $LDL_{Cholesterol}$/ $HDL_{Cholesterol}$	0.78 ± 0.15	0.98 ± 0.21	0.04

[1)] Values represent means ± SD, n = 8 for each group

(94:6, v/v) as an eluent (isocratically) and detected by fluorescence (excitation wavelength: 295 nm, emission wavelength: 320 nm). δ-Tocopherol was added as an internal standard.

Leukocyte, erythrocyte and platelet counts as well as hematocrit, hemoglobin concentration, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were determined with a Coulter Counter and a hemoglobinometer (Coulter Electronics GmbH, Krefeld, Germany).

The lipoproteins VLDL (density <1.019 kg/L), LDL (density 1.019 – 1.063 kg/L), and HDL (density >1.063 kg/L) were isolated by step-wise ultracentrifugation (230,000 x g for 20 h at 8 °C), according to Tiedink and Katan (38). The concentrations of triacylglycerols, total cholesterol and phosphatidylcholine, the major phospholipid class of serum lipoproteins were measured enzymatically using an auto analyzer (Model 704, Hitachi, Tokyo, Japan) and kit reagents (Boehringer, Mannheim, Germany).

Preparation of erythrocyte membranes for fatty acid analysis was done as described previously (36). Diet, serum and erythrocyte membrane lipids were extracted with a hexane/isopropanol mixture (3:2, v/v, containing butylated hydroxytoluene as an antioxidant) as described by Hara and Radin (18). Lipids were than methylated with a boron fluoride/methanol reagent (26). Fatty acid methyl esters (FAMEs) of the diet, serum and blood cell membranes were separated by gas chromatography using a Hewlett-Packard HP 5890 gas chromatographic system (Hewlett-Packard, Taufkirchen, Germany), fitted with an automatic on-column injector, a flame ionization detector, and a CP-Sil 88 capillary column (50 m x 0.25 mm internal diameter, film thickness 0.2 µm; Chrompack, Middleburg, The Netherlands). FAMEs were identified by comparing their retention times with those of individual purified standards, and quantified with heptadecanoic acid methyl ester as an internal standard (14). The total CLA concentration was determined by using a standard mixture consisting of *cis*- and *trans*-9,11 and –10,12-octadecadienoic acid methyl esters (Sigma Chemical Co., St. Louis, MO).

Statistics

The effect of dietary CLA was evaluated for statistical significance ($P < 0.05$) by the Student's *t*-test. All data in the present text are expressed as means ± standard deviations of the single values (SD).

Results

At the end of the experimental period at week 6, body weights of the pigs fed the CLA-supplemented diet were not different from that of the controls (190.5 ± 6 kg vs. 189.8 ± 5 kg). Pigs fed the CLA diet exhibited a 37% higher plasma concentration of insulin than their controls that received no CLA ($P = 0.11$, Table 2). In contrast, circulating concentrations of total and free T4 and T3 as well as serum levels of β-hydroxybutyrate, α-tocopherol, protein, glucose, urea, creatinine and ATP in blood re-

Table 6 CLA proportion and fatty acid composition (mol/100 mol fatty acids) of the fasting serum and erythrocyte membranes of control and CLA-treated pigs

Fatty acid	Fasting serum		Erythrocyte membrane	
	Control group	CLA group	Control group	CLA group
16:0	16.2 ± 1.0[1)]	17.3 ± 1.4	22.0 ± 0.3	22.2 ± 0.9
18:0	13.3 ± 1.1	12.7 ± 1.1	14.7 ± 0.3	14.8 ± 0.8
22:0	1.25 ± 0.27	0.87 ± 0.43	0.42 ± 0.13	0.36 ± 0.09
24:0	0.30 ± 0.02	0.27 ± 0.04	4.10 ± 0.20	4.20 ± 0.35
Total SFA	31.0 ± 1.7	31.1 ± 2.2	41.2 ± 0.2	41.5 ± 0.9
16:1	1.18 ± 0.20	1.23 ± 0.21	0.65 ± 0.05	0.60 ± 0.06*
18:1	19.7 ± 1.6	23.7 ± 1.5*	26.6 ± 3.2	28.5 ± 2.1
20:1	n.d.[2)]	n.d.	0.17 ± 0.04	0.16 ± 0.02
24:1	0.24 ± 0.03	0.26 ± 0.05	1.84 ± 0.07	1.90 ± 0.14
Total MUFA	21.1 ± 1.5	25.1 ± 1.6*	29.3 ± 3.1	31.2 ± 2.1
18:2 n-6	33.1 ± 2.3	29.9 ± 2.6*	16.2 ± 2.7	13.7 ± 1.7*
18:3 n-6	0.90 ± 0.10	0.65 ± 0.09*	0.61 ± 0.06	0.52 ± 0.07*
18:3 n-3	0.80 ± 0.12	0.69 ± 0.11	1.34 ± 0.12	1.15 ± 0.09*
20:2 n-6	0.36 ± 0.07	0.33 ± 0.07	0.33 ± 0.04	0.35 ± 0.04
20:3 n-6	0.70 ± 0.09	0.55 ± 0.12*	1.92 ± 0.16	1.94 ± 0.14
20:4 n-6	9.85 ± 1.22	7.73 ± 0.98*	5.82 ± 0.69	5.26 ± 0.38
22:4 n-6	0.48 ± 0.13	0.39 ± 0.12	1.35 ± 0.09	1.29 ± 0.15
22:5 n-3	0.86 ± 0.15	0.98 ± 0.14	1.09 ± 0.17	1.09 ± 0.05
22:6 n-3	0.75 ± 0.27	0.98 ± 0.41	0.88 ± 0.30	0.90 ± 0.35
Total PUFA	47.8 ± 1.5	42.1 ± 2.8*	29.5 ± 3.2	26.2 ± 1.8*
Total CLA	0.08 ± 0.04	1.59 ± 0.44*	0.05 ± 0.02	1.08 ± 0.24*
PUFA plus CLA	47.9 ± 1.6	43.7 ± 3.0*	29.6 ± 3.2	27.3 ± 1.9

[1)] Values represent means ± SD, n = 8 for each group; *indicates significant differences ($P < 0.05$);
[2)] not detectable

mained completely unaffected by CLA supplementation (Table 3). Serum concentration of non-esterified fatty acids showed a trend toward a reduced level of 38% in CLA-treated pigs relative to the controls. CLA-treated pigs tended to have fewer leukocytes in blood than their controls ($P < 0.1$), whereas erythrocyte and platelet counts, the hematocrit, the hemoglobin concentration and the corresponding MCV and MCH values remained unchanged by CLA consumption (Table 4). Serum of CLA-treated pigs showed a trend toward increased levels of triacylglycerols, cholesterol and phosphatidylcholine in the VLDL and LDL (Table 5). The lipid concentrations in the HDL fraction did not differ between the groups (Table 5). The LDL cholesterol to HDL cholesterol ratio was significantly increased in pigs fed the CLA-fortified diet compared to the control pigs receiving no CLA.

Only limited amounts of dietary CLA were incorporated into the fasting serum lipids and the erythrocyte membrane, although the proportion of CLA in serum and the erythrocyte membranes of pigs fed the CLA-fortified diet was increased by 20-fold and 22-fold, respectively, relative to the control animals (Table 6). Main effects of the CLA-fortified diet on fasting serum fatty acid composition were a slight reduction of the n-6 polyenoic fatty acids linoleic acid (18:2 n-6) and its elongation and desaturation products 18:3 (n-6), 20:3 (n-6) and 20:4 (n-6). When erythrocyte membranes were extracted, the fatty acid composition of swine erythrocyte membranes also remained at a very constant level (Table 6). Essentially, no statistical significant differences were observed with total saturated fatty acids (SFA) and total monounsaturated fatty acids (MUFA) concentrations. Only slight but statistically significant changes occurred with membrane polyunsaturated fatty acids (PUFA). The major effects of the CLA diet on fatty acids in red blood cell membranes were a slight reduction of the proportions of linoleic acid (18:2 n-6) and linolenic acid (18:3 n-6 and n-3) at the expense of CLA. The proportion of the membrane-associated arachidonic acid (20:4 n-6) was not significantly affected by CLA treatment.

Discussion

From the foregoing observations it is evident that a CLA mixture at a dietary level of 1% moderately affects lipoproteins, insulin and leukocytes in adult swine that were isoenergetically fed diets on maintenance level compared to animals fed sunflower oil. This study was the first to show that under the above described experimental conditions CLA may exhibit a rise in the LDL cholesterol to HDL cholesterol ratio, which has been found to be positively correlated with atherogenesis (24). This is in contrast to recent studies with rodents in which CLA-

fortified diets caused a serum lipoprotein profile that was considered to be less atherogenic (23, 28, 30), although data from Munday and coworkers (28) have shown that the addition of CLA to an atherogenic diet increased the development of aortic fatty streaks. In contrast, Sugano and coworkers (37) could not observe any differences in serum concentrations of total and HDL cholesterol in rats fed CLA at a dietary level of 1%. Thus, the effect of CLA on lipoproteins was a subject of some controversy. We assume that CLA responds differently to graded levels of energy intake and physiological state of the animal species used. This was confirmed by the fact that adult rats fed diets above the energetic maintenance requirement containing CLA levels of 1, 3 and 5% exhibited concomitant reductions of serum levels of cholesterol (35), while serum cholesterol of growing rats fed 3% CLA under conditions of enhanced fat mobilization remained unchanged, but serum levels of VLDL lipids decreased (34). Moreover, it is also difficult to compare the quality and extent of changes in the lipoprotein profile found in the present study with those from other experiments because the changeability of lipid variables also seems to depend on the composition of the diets used in the experiment. However, additional studies in this area are warranted to delineate the linkage between CLA, energy intake, composition of the basal nutrient ingredients and physiology of the animals used. Species differences may also contribute to the uncertainty. At this point an extrapolation of these findings to humans is complex. It is an interesting aspect that there appear to be parallels between the effects of CLA reported in this study and the effects of *trans* fatty acids. *Trans* fatty acids are reported to increase LDL cholesterol and/or to decrease HDL cholesterol in humans relative to monounsaturated or polyunsaturated fatty acids (21, 25, 29, 33). Additionally, the hydrogenation of linoleic acid to *trans* fatty acids has been shown to increase LDL and decrease HDL cholesterol relative to linoleic acid itself (40). Since the CLA intake in Germany has been calculated to be 0.35 g CLA/day for women and 0.43 g CLA/day for men (15) we suggest that CLA-induced influences on human lipoproteins might be negligible.

Additionally, despite the relatively high amounts of CLA ingested, of which 30% of the dietary fatty acids were CLA isomers, the proportions of CLA in the fasting serum and the erythrocyte membrane remained low relative to other fatty acids. Although, the magnitude of deposition of CLA in fasting serum and erythrocytes did not necessarily resemble that of dietary CLA, blood levels of CLA in CLA-treated pigs were considerably higher than that of sunflower-treated animals. Thus, we can conclude that blood levels of CLA may function as a marker for long-term ingestion of CLA. Other differences in fatty acid composition of serum and red blood cell counts may result from the differences in fatty acids provided with the diets, although a CLA-induced effect on fatty acid distribution can not be excluded. The presence of small amounts of CLA-isomers in swine fed the CLA-free control diet may be attributable to the fact that the intestinal tract of non-ruminants is capable of isomerizing free linoleic acid to CLA (9), but CLA may also be produced *in vivo* from free radical-mediated oxidation of linoleic acid (7, 12).

The present data demonstrate that the CLA diet tended to increase the basal circulating insulin level by 37% without changing the serum level of glucose. Houseknecht et al. (19) and Belury and Kempa-Steczko (3) provided initial evidence that CLA may act on glucose metabolism by normalizing impaired glucose tolerance and by improvement of hyperinsulinemia in rats. In general, previous data have shown that the fasting plasma insulin concentration was significantly lower following a polyenoic fatty acid-rich diet compared to diets enriched with saturated or monounsaturated fatty acids (22). Similar to the CLA-induced effect on lipoproteins, there also appear to be parallels between CLA and *trans* fatty acids in the mode of action on insulin secretion. Some authors have found that *trans* vaccenic and elaidic acids elicited a higher maximal insulin output than the respective *cis* isomers (1, 10) and it has been assumed that stimulated insulin secretion is caused by an increase in glucose oxidation. However, further research should be pursued to delineate whether modulation of insulin secretion can be elicited in animals treated with CLA.

Additionally, it was also obvious from the present data that the α-tocopherol level in serum and the μmol α-tocopherol per mmol total serum lipids (1.32 for the control group versus 1.20 for the CLA group) were not significantly affected by CLA, although recent data have shown that CLA is more susceptible to oxidation than linoleic acid (39) and may downregulate cellular antioxidant enzymes when cells were exposed to oxysterols (6). On the other hand inhibition of oxidative stress *in vitro* (17) and *in vivo* (20) suggests that the conjugated double bond system of CLA works as a potent antioxidant factor in tissues by efficiently trapping electrons (11). In our study, neither a significant decomposition nor an antioxidant-sparing effect of CLA in the serum could be observed.

In conclusion, the biological activity of CLA on metabolic events remains a subject of some controversy. In our study using the swine as a model with a nutritional status approaching equilibrium, CLA exhibited weak but detrimental effects on blood parameters, mainly on lipoproteins. The observed effects of CLA seems to resemble the effects of *trans* fatty acids, but an extrapolation to these findings from adult swine to humans might be complex.

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