Occurrence and Structures

Fatty acid isomers – and we will be mainly dealing with trans fatty acids in this review – may have two origins. These can be natural fatty acids but they may also be formed during technological treatments such as hydrogenation, refining or frying of oils [1, 2].

Trans monounsaturated fatty acids are mainly formed during the hydrogenation of oils to produce margarine and shortenings, but are also present in ruminant products such as milk and meat as a result of biohydrogenation in the rumen [2]. However, the isomer distribution presents some major differences (table 1). Vaccenic acid or 11t-18:1 is the major trans monoethylenic isomer in milk lipids while the trans ethylenic bond is more delocalized in the case of partially hydrogenated oils where \( \Delta 8, \Delta 9 \) and \( \Delta 10 \) are present in important quantities.

Trans polyunsaturated fatty acids (PUFAs) especially those arising from the two essential fatty acids (EFAs), linoleic (18:2 n-6) and linolenic (18:3 n-3) acids have been shown to be formed during the last step of refining which is the deodorization process [1] but also during frying of vegetable oils [3].

The two major isomers of 18:3n-3 are the 9c,12c,15t- and the 9t,12c,15c-18:3, while the ditrans isomers, especially the 9t,12c,15t-18:3 can be observed when the oil has been submitted to higher temperatures (above 220°C). Similarly, the two major isomers of linoleic acid are the monotrans isomers (9c,12t and 9t,12c-18:2), the ditrans isomer being only present when the oil has been submitted to high temperatures. Determination of the degree of isomerization (DI) of linoleic (DI 18:2) and of linolenic (DI 18:3) acids indicates that 18:3 is much more sensitive to isomerization than 18:2. Refined oils with DI 18:2 and DI 18:3 ranging from 0.3 to 3.3 and from 6.0 to 37.0, respectively, have been found in the market [4].

Fatty acid isomers without methylene interrupted double bonds but with conjugated double bonds have also been detected either as natural fatty acids...
in meat and milk from ruminants [5] and also as minor isomers as a result of catalytic hydrogenation or heat treatment procedures. In one case (ruminant products), the major isomer is rumenic acid or 9c,11t-18:2 while the fatty acids formed during heat treatment are a complex mixture of cc,ct,tc and tt isomers [6].

Trans Monoethylenic Fatty Acids

Metabolism and Effect on EFA Metabolism

The metabolism of trans isomeric fatty acids was intensively studied mainly in the early 1980s, both in vitro and in vivo, very often using EFA-deficient diets, which are not representative of the in vivo situation. In vitro it has been shown for example that most of the trans fatty acids could be desaturated by the Δ⁹-desaturase which is acting on stearic acid [7]. Consequently, number of trans-18:1 isomers present in partially hydrogenated oils are potential precursors of a number of 18:2 isomers and therefore of long-chain PUFAs.

In a study conducted by Turpeinen et al. [8], it was shown that feeding 3 g/day of vaccenic acid induced an increase in rumenic acid (9cis,11trans-18:2) in serum triacylglycerols. The rumenic acid content doubled within a few days and a steady state was achieved in 4–6 days. These data support the hypothesis that vaccenic acid may be desaturated into rumenic acid in humans as well as in rodents [9].

In vivo experiments showed that dietary trans fatty acids could be incorporated into nearly all lipid classes of various organs, but the concentration is highest in triacylglycerols. A decreased deposition of long-chain PUFAs after
a trans-18:1 intake, which could be explained by the inhibitory effect of trans isomers toward EFA conversions, has been shown in a number of studies [7]. However, some conflicting results on the effects of trans fatty acid on the conversion of linoleic acid and the deposition of long-chain PUFAs appeared in the literature. Perhaps the presence of an adequate amount of linoleic acid as well as the presence or not of linolenic acid would be a major factor which has been overlooked in many studies.

**Lipoprotein Metabolism in Humans**

Earlier studies (in the 1960s and 1970s) on the cholesterolemic effects of dietary trans fatty acids were difficult to interpret because the trans fatty acid intakes were in general accompanied by increased intakes of saturated fatty acids and lower intakes of PUFAs. Contradictory results were also published [for review see, 10].

Some years later, the discussion on the effects of trans fatty acids on serum lipoproteins started again after the publication by Mensink and Katan [11] in 1990. This study demonstrated that consumption of a trans fatty acid diet (11% of the energy intake) compared to oleic acid raised low-density lipoprotein (LDL) cholesterol concentration and decreased high-density lipoprotein (HDL) cholesterol concentration. However, the diet was very rich in trans. Furthermore, the trans fatty acids used were obtained by isomerization and not by hydrogenation. Recent studies indicate that trans-18:1 isomers increase the LDL cholesterol concentration as compared with oleic acid but the effects on HDL are less consistent.

To calculate the quantitative effects of trans-18:1 isomers, the data of different studies were combined by multiple regression analysis and carbohydrates were chosen as reference [12]. As outlined in figure 1, it was found that 1% of dietary energy as trans-18:1 isomers at the expense of carbohydrates results in an increase in LDL cholesterol of 0.034 mmol/l while the effects on HDL are very similar to those of carbohydrates.

On the contrary, very little has been published on the major ‘natural’ trans-18:1 isomers, vaccenic acid. It was recently concluded [13] from an animal study that the ratio of LDL/HDL cholesterol in plasma was significantly higher in hamsters fed vaccenic acid than in those fed elaidic acid. This was not in agreement with the conclusion drawn by an epidemiological study [14].

**Trans Polyunsaturated Fatty Acids**

**Methylene-Interrupted Fatty Acids**

For a detailed description of the metabolism and health impact of trans PUFAs, one is referred to the article by Sébédio and Chardigny [15] in which all the earlier experiments, mostly carried out in USA, are described. Here we will review studies carried out in the last decade by our group in Dijon, France.
Fatty Acid Isomers in Lipid Metabolism

Metabolism and Effect on EFA Metabolism

Both our group and others [16, 17] have demonstrated that some 18:2 and 18:3 isomers can be desaturated and elongated in isomers of arachidonic, eicosapentaenoic and docosahexaenoic acids. For example, the 2 monotrans isomers of linoleic acid can be converted into 5c,8c,11t,14c (11t-20:4) and 5c,8c,11c,14t-20:4 (14t-20:4). However, the 9t,12-18:2 is rather elongated into 11t,14c-20:2 than desaturated and elongated into 11t-20:4. Similarly, the 9c,12c,15t-18:3 can be desaturated and elongated into 17t-20:5 and 19t-22:6 [18].

Early studies [for review see, 15] on the effect of trans PUFAs on the metabolism of EFA were dealing with the 9t,12t-18:2 which is only found as a trace in human food. More recently, Berdeaux et al. [19] showed that the 9c,12t-18:2 could inhibit the Δ^6-desaturase of linoleic acid in liver microsomes of rats fed a fat-free diet. Blond et al. [20, 21] also showed a decrease in n-3 fatty acids (18:3, 20:5, 22:5 and 22:6) and an increase in 20:4n-6 in hepatic phospholipids of animals fed a diet containing trans isomers of linolenic acid. While the Δ^6-desaturase activity was not modified by trans isomers of 18:3n-3, the Δ^5-desaturation of dihomo-γ-linolenic acid in arachidonic acid was increased [21].

Effects of Trans PUFA on Arachidonic Acid Metabolism

As already described, monotrans isomers of 18:2n-6 and 18:3n-3 are converted into higher metabolites, including C20 and C22 PUFAs and the effects of these molecules were studied using either isolated metabolites from rat liver or fully synthesized isomers.

Fig. 1. Effects of exchanging dietary carbohydrates for trans-18:1 isomers on HDL- and LDL-cholesterol concentrations. From Mensink and Katan [11].
The effects of 14trans-20:4 on the metabolism of arachidonic acid in eicosanoids was studied recently by Berdeaux et al. [22] using rat platelets. It was shown that this structural analog of 20:3n-9 induced an inhibition of the conversion of 20:4n-6 into thromboxane B$_2$ or 12-hydroxyheptadecatrienoic acid (cyclooxygenase pathway) and increased the production of 12-hydroxyeicosatetraenoic acid (12-HETE) through the 12-lipoxygenase pathway (table 2). These data were well correlated with platelet aggregation. These data confirm a specific effect of the trans double bond located at the $\Delta^{14}$ position. Moreover, using radiolabeled 14trans-20:4, it was shown that it was metabolized by platelets into two metabolites. One of them is probably a product of the 12-lipoxygenase pathway, as its production is lowered when platelets are pre-incubated with baicalein, a selective 12-lipoxygenase inhibitor [22]. The production of this unknown metabolite is greatly enhanced when arachidonic acid or 12-HETE are present in the incubation medium. The data suggest that a sufficient 'peroxide tone' is needed to produce this unknown metabolite. They enhance the hypothesis about the origin of the metabolite, which might be a trans isomer of 12-HETE. However, its structure needs to be fully elucidated.

The effects of 17trans-20:5 and 19trans-22:6 were studied after incorporation into platelet lipids. Thrombin and collagen stimulation of platelets enriched in 20:5 or 22:6 PUFAs showed that the occurrence of a trans double bond at the (n-3) position decreased the anti-aggregant effects of both 20:5 and 22:6 fatty acids. Similarly, the stimulation of these platelets with U46619, a stable analog of thromboxane A$_2$, showed that platelets enriched in trans PUFAs were more sensitive than when enriched with cis PUFAs. These data suggest that the incorporation of trans PUFAs may modify the sensitivity of the thromboxane A$_2$ receptor of the platelet membranes. This hypothesis is enhanced by the lack of effect on the production of eicosanoids when platelets were triggered by collagen [23].

As several trans-18:3 isomers are converted in vivo into 20:5 isomers, we have carried out studies on the effects of 11trans-, 11trans- and 17trans-20:5
on platelet aggregation. However, this study was performed using washed rat platelets as human platelets are not devoid of long-chain trans PUFAs [24]. Washed rat platelets were stimulated by arachidonic acid in the presence of increasing quantities of EPA or one of its trans isomers [25]. It appeared that 20:5 isomers with a trans double bond at the Δ11 position were inhibitors of the cyclooxygenase activity and more anti-aggregant than EPA or 17trans-20:5 [25].

Besides the production of thromboxane by platelets, arachidonic acid is metabolized by endothelial cells into different eicosanoids. The most important one is prostacyclin (PGI2) which has strong anti-aggregant properties. Endothelial cells are also able to produce prostaglandin I3 (PGI3) from EPA, but the question of their ability to metabolize trans EPA has only recently been considered. Human endothelial cells may be harvested and cultured. These have been shown to be powerful tools to study vascular functions, without changing their ability to produce prostacyclin and prostaglandin E2 from arachidonic acid for instance. But such human endothelial cells contain trans fatty acids as do human platelets. We have therefore cultured animal cells from bovine aortae in order to study the effect of trans PUFAs on PGI2 production. Using this model, different trans EPA isomers have been studied. In the present review, we will only present data from 17trans-20:5, which is the major trans isomer of EPA resulting from the desaturation-elongation from the 15trans-18:3 isomer. Information about the effect of other trans EPA isomers may be found in the original paper [26]. After incorporation of 17trans-EPA in cell phospholipids, the production of PGI2 as measured by its metabolite 6keto-PGF1α was significantly reduced when compared with control cells. However, this decrease was of a similar magnitude as that after incorporation of EPA in these cells (table 3). We have also studied the metabolism of labelled 17trans-EPA in endothelial cells. This was of particular interest since platelets, which interact with endothelial cells, were able to produce several metabolites from this trans fatty acid. 17trans-EPA is converted into at least two metabolites [26]. One of these has the same retention volume as 6keto-PGF1α when analyzed by high-performance liquid chromatography.
Unfortunately, the identification of this metabolite was not possible due to the limited quantities obtained. However, its production was inhibited when cells were pre-incubated with indomethacin. This suggests that it results from the cyclooxygenase pathway and may be an isomer of 6keto-PGF$_{1a}$.

**Lipoproteins and 18:3 Isomers**

To our knowledge, only one human study (TransLinE) has been carried out to assess the health impact of trans-18:3 isomers. The volunteers consumed an experimental diet; the intakes of carbohydrate, protein and lipid are detailed elsewhere [27]. After a run-in period, the subjects were randomly allocated to 1 of 2 groups eating a diet low or high in trans $\alpha$-linolenic acid for 6 weeks (0.6% of energy, i.e. about 50% of $\alpha$-linolenic acid as trans isomers.)

As already discussed, trans isomers of oleic acid may raise serum cholesterol and reduce HDL cholesterol. It was therefore important to check whether trans isomers of $\alpha$-linolenic acid could interfere with cholesterol and lipoprotein metabolism in humans. The consumption of trans $\alpha$-linolenic acid did not change plasma total cholesterol (treatment effect high vs. low 1.7% (−2.2 to 5–7), $p = 0.41$). HDL cholesterol increased by 3.8% when the subjects continued to consume the low trans diet, but did not change on the high trans $\alpha$-linolenic acid diet (treatment effect $−2.7\% (−6.8$ to $1.6)$, $p = 0.22$). As a result the trans $\alpha$-linolenic acid isomer-rich diet raised the LDL to HDL cholesterol and the total to HDL cholesterol ratios by 5 and 8% respectively, while the inverse was observed on the low trans diet (fig. 2) [28]. Assuming that these parameters are good risk indicators for cardiovascular disease [29], trans $\alpha$-linolenic acid isomers would raise the risk by 8%.

**Conjugated Fatty Acids**

**Metabolism and Effect on Other Fatty Acid Metabolism**

In this review, we will exclusively cover the conjugated isomers of linoleic acid which are present either in meat and milk from ruminants but also in synthesized conjugated linoleic acid (CLA) fraction used for human studies.

Among the different positional and geometrical CLA isomers present in dairy products and meat from ruminants, only two isomers, 9c,11t- and 10t,12c-18:2 have been shown in vivo to be converted into conjugated isomers of 18:3, 20:3, and 20:4. It has been clearly shown [30] using rat feeding studies that these two CLAs can undergo desaturation and elongation while maintaining the conjugated diene structure.

The metabolites formed depend on the type of fatty acids present in the diet. When fed to rats along with a diet deficient in both linoleic and linolenic acids, both CLA isomers are transformed into conjugated isomers of arachidonic acid [9]. Again the conjugated 20:4 metabolite was also found in the liver of rats fed a high or low CLA-enriched butter fat, which are diets containing
low quantities of EFAs [31]. In tissues of rats fed an equilibrated diet and either pure or mixtures of CLA isomers, the 9c,11t isomer was converted mainly into 8c,11c,13t-20:3, whereas the 10t,12c-18:2 was converted into 6c,10t,12c-18:3 and into 6,10c-16:2. Banni et al. [32] also reported the presence of 8c,11c,13t-20:3 as the major CLA metabolite in liver lipids of rats fed either a low or high CLA-enriched butter fat diet, both of which contained a large amount of vaccenic acid, the precursor of 9c,11t-18:2 formed in the mammary gland by $\Delta^9$-desaturase. In vitro data [33, 34] fit very well with those obtained in vivo [for details see, 9].

CLAs and their metabolites have been shown to be incorporated mainly into neutral lipids from different tissues. This shows that for acylation, CLA is behaving more like oleic acid than like linoleic acid. When fed in the same quantity, more 9c,11t isomer than 10t,12c-18:2 was found in the different tissues studied. Similarly, very few CLA metabolites were incorporated into the phospholipids. On the contrary, using diets low in EFAs (butter fat), Banni et al. [32] demonstrated that the conjugated arachidonic acid was predominantly found in phosphatidylserine and phosphatidylinositol. On the contrary, arachidonic acid was incorporated mainly into phosphatidylcholine and to a lesser extent into phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol.

Not only are 9c,11t- and 10t,12c-18:2 isomers metabolized differently, but they also have distinct effects on the metabolism of other fatty acids. Although both isomers resulted in a decrease in the level of arachidonic acid in the liver.

**Fig. 2.** Effects of exchanging 0.6% (of energy) of linolenic acid for trans linolenic acid isomers in human subjects from three locations in Europe on total- to HDL-cholesterol levels. Adapted from Vermunt et al. [28].
phospholipids of rats, only the 10t,12c-18:2 induced an increase in the C\textsubscript{22} PUFAs in the liver lipids [30].

Interestingly, only the 10t,12c-18:2 increased the stearic acid content at the expense of 18:1n-9, which suggests an alteration in \(\Delta^9\)-desaturase activity [30]. This observation was confirmed by in vitro studies [35]. Lee et al. [36] reported that CLA depressed the expression of stearoyl-CoA desaturase (SCD) mRNA. These effects were correlated with a decrease in the 16:1/16:0 and 18:1/18:0 ratios in mice liver, which are considered to be indices of \(\Delta^9\) desaturation. Moreover, studies conducted with pure rumenic acid suggested that this effect on SCD was due mainly to other conjugated fatty acid isomers. Bretillon et al. [35] demonstrated that the effect of CLA on \(\Delta^9\)-desaturase activity was due to the 10t,12c isomer. In 3T3-L1 adipocytes, it was demonstrated that the trans10,cis12 isomer downregulated the expression of the SCD gene, which may be related to the effects of this isomer on body mass [37].

In addition to these effects on \(\Delta^9\) desaturation, Bretillon et al. [35] also reported some effects on \(\Delta^6\) desaturation. For this enzyme, the 9c,11t isomer appeared to be the most active, particularly on the conversion of linolenic acid. The effects on the \(\Delta^6\) desaturation of \(\alpha\)-linolenic acid were less important and observed only when rumenic acid was present at high concentrations.

**Effect on Arachidonic Acid Metabolism**

Various studies showed a decrease in \(\text{PGE}_2\) release after feeding CLA to animals. For example, CLA lowered the ex vivo production of \(\text{PGE}_2\) in bone organ culture of rats (tibia and femur) [38]. Also the reduction of serum \(\text{PGE}_2\) and splenic leukotriene B\textsubscript{4} (\(\text{LTB}_4\)) in CLA-supplemented rat was reported [39, 40]. CLA decreased \(\text{PGE}_2\) during a hypersensitivity type-I reaction. Dietary CLA treatment significantly reduced antigen-induced histamine and \(\text{PGE}_2\) release in pigs [41].

In a study on cultured keratinocytes, CLA decreased 12-0-tetradecanoylphorbol-13-acetate (TPA)-induced \(\text{PGE}_2\) synthesis compared to linoleic acid [42]. A recent study showed that dietary CLA significantly decreased \(\text{PGE}_2\) synthesis in the epidermis, which suggested that CLA modulated TPA-induced tumor promotion by a mechanism involving \(\text{PGE}_2\) [43]. CLA (mainly a mixture of 9c,11t- and 10,12c-18:2) also reduced brain \(\text{PGE}_2\) production without inhibition of cyclooxygenase gene expression in mice.

It is also clear from a study on human saphenous vein endothelial cells [44] that the effect of CLA is not restricted to \(\text{PGE}_2\) but can affect the production of all the prostaglandins in a similar manner. However, another report on human endothelial cells and platelets shows that in vitro, CLA may be either inhibitory or stimulatory, and this seems to depend not only on the specific CLA isomer but also whether cells are in the resting or stimulated state [45].

To investigate the mechanisms by which CLAs reduce prostaglandins, a recent study determined the ability of CLAs and specific isomers to alter the activity of a cyclooxygenase enzyme, prostaglandin H synthase (PGHS) [46].
It was shown that the different isomers of CLA inhibit PGHS differently: 9c,11t-C18:2 was the most effective one and 9c,11c-C18:2 was the least. As a consequence of the inhibition of PGHS the synthesis of prostaglandin D$_2$ (PGD$_2$), prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$), PGE$_2$, PGI$_2$ and thromboxane A$_2$ is diminished [46].

**Conclusion**

Fatty acid isomers of oleic, linoleic and linolenic acids may be of natural origin and/or formed during technological treatments of fats and oils. Some of these fatty acid isomers, especially the dienoic and trienoic fatty acids, may be good substrates for desaturation and elongation as their natural cis isomers, linoleic and linolenic acids. They can therefore interfere not only in the metabolism of EFAs but also in the oxidative metabolism of arachidonic acid.

Trans monoethylenic fatty acids such as elaidic acid and some conjugated linoleic acid may also play a role in the lipoprotein metabolism.

**References**

Fatty Acid Isomers in Lipid Metabolism

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Fatty Acid Isomers in Lipid Metabolism


Discussion

Dr. James: I congratulate you on the extraordinary ability that you have shown in isolating these particular isomers. I have run a big group in this field and it was really a major challenge to try to distinguish the impact of these subtle isometric changes on the desaturation and elongation, and so you have some very elegant data. What I am a little unclear about is that I understand the mechanism by which these particular isomers interact with desaturases and essentially operate sometimes in a major competitive and sometimes an irreversible mode. But I am a little confused as to how these isomers effect, for example, the low-density lipoprotein cholesterol. What is the mechanism by which it is inducing these changes? In pregnant women the essential fatty acids are being transported as beautifully selective transport proteins through the placenta. What evidence do we have as to whether these particular isomers in any way have differential binding properties and therefore transport through the different fatty acid-binding proteins, because this could have enormous implications. There has been one paper, I think from Germany, implying that the cis-isomers and trans-isomers of fatty acids are associated with low birth weight in children and babies. Therefore how we are dealing with a fundamental interaction with not just enzymes but transport as well?

Dr. Sébédio: You have raised two important questions. The mechanism by which these fatty acids can act on lipoproteins: what we know and has been published is that the trans 18:1 isomers can act on the cholesteryl ester transfer protein. The cholesteryl ester transfer protein would be one of the mechanisms but this has not been studied for the trans polyenes because it is a lot of work to synthesize most of these molecules. They cannot be bought and must be synthesized or isolated. We did not do the work on the cholesterol transfer protein or the plasma phospholipid transfer protein. About the different facility for the binding of fatty acid proteins I don't have the answer either.

Dr. James: Has there been any follow-up, or have I got it completely wrong that there was a proposition that women who ate a lot of these isomers were producing small babies?

Dr. Sébédio: I think some data have been published. Very interestingly we did some work with the hospital in Dijon with fatty acids. We first isolated them in oils; first in
frying oils with trans-polyunsaturated fatty acids, and after that we just looked in different supermarkets and different stores and what was on the market as far as the oils were concerned. There were some oils of very good quality, for example rapeseed or soybean, with little isomerization of 18:3, and in some with very high isomerization of 18:3. We looked at the milk of women, and it was extraordinary that we found exactly the same fatty acid in the milk. After these fatty acids were found in women’s milk, in platelets they were elongated and desaturated, and we looked in the cord and these fatty acids were transferred to the babies. I don’t know what the effects on the babies are exactly, but I can tell you that in rats these trans 22:6n-3 isomers, these trans-isomers were found in the brain structure and in the retina where they have a major impact. After feeding them for 3 months to rats, the biwave amplitude of the electroretinographic response, which reflects the function of the photoreceptors, is decreased by about 30–35%.

Dr. James: That is very interesting because when we first looked at this originally in the early 1980s with the British Committee looking at the problem of cardiovascular disease, we were extremely worried by the data showing the interactions of these potential isomers on the desaturases and the whole issue of the very high rates of heart disease. For example in the UK it was a big issue because we suddenly discovered that the German system for processing very unsaturated fish oils, for example, was completely different from the British system. The German system went essentially with complete hydrogenation then producing a mixture of the classic normal oil and saturate, whereas the British went for partial hydrogenation. So there was the whole stream of different isomers, and the more we looked at the in vitro data on this, the more terrified we became of what the system might be in the UK. So it is very interesting to see that.

Dr. Sébédio: Especially that the in vitro data fit well with what we observed in vivo in animals and also in humans. Blood tests were used in Germany to identify these isomers in babies.

Dr. Angkatavanich: My question is about the adverse effects of conjugated linoleic acid (CLA). I came across information from Riserus et al. [1] from Norway that 10trans-CLA will increase the level of C-reactive protein which is very bad from a cardiovascular point of view. Did you find any kind of related data on that? My second question is about CLA and the efficacy in reducing body weight.

Dr. Sébédio: CLA and body weight: what I can say is that there are numerous studies which have been carried out on different animal species like rats, mice or pigs. Right now it looks as though CLA can reduce body fat mass, especially the 10trans, 12 cis isomer, the one I have put on the slide, not the natural one, even if you can still find a little in the milk but there are small isomers in the milk. So it seems to work on growing animals and it is species-dependent. Now as to CLA in human experiments, right now there are about 12–14 different human interventional studies which have been published. You spoke of Riserus et al. [1], and there are also groups in the USA and in northern Europe doing basic work on mixtures of isomers. Some people found that it was reducing fat mass to a level of about 6 g/day of the CLA mixture. Riserus et al. found that in most cases it didn’t do anything to the fat mass. So that was for the synthetic isomer mixture. In the experiments that we recently completed, we wanted to know if the isomer was working in animals was effective in humans. So we synthesized both isomers using fractional crystallization to isolate them and made the corresponding triglycerides. It was completely different as the other studies were done on the free fatty acids. We fed them for about 4 months at levels of 1.5 and 3 g/day and did not find any difference. The question is still open as to whether these isomers can do something or not, because the problem is that people use different types of isomer mixtures. In a study we did [2], we did not see anything. As far as the C-reactive protein was concerned, we looked at different things and we did not see anything wrong.
as far as a secondary action of these isomers. But I know that some people saw that and it depends on what was fed.

**Dr. Komindr:** The effect on the weight, is it a dose response?

**Dr. Sébédio:** The first study I think was done in Norway. It was a dose-response study starting from 1.5 g to something like 9 g. In one part there was a dose response and in the middle there was a point at which it was not acting.

**Dr. Tantibhaedhyangkul:** What is the level of triglycerides? You did not mention the very low-density lipoprotein triglycerides. Do you know the mechanism?

**Dr. Sébédio:** No.

**Dr. Tantibhaedhyangkul:** Do you think it involves the exchange between triglycerides and cholesterol among lipoproteins?

**Dr. Sébédio:** I cannot answer you on this point.

**Dr. Go:** I also would like to congratulate you, it is fantastic work on fatty acid isomers. I want to pick up the comment you made with regard to CLA and the bad effect on the heart or the CLA effect on the carcinogenesis pathway. In the United States the Food and Drug Administration requires nutritional labeling: the amount of CLA in the food. The question that always arises is whether the analysis methodology is good enough for a standardization of what could appear on the box for the CLA upper/lower level measurement. Is there actually a threshold response that we should be concerned with? Is all CLA bad or just a certain amount is bad?

**Dr. Sébédio:** I am sorry I did not exactly understand the second question.

**Dr. Go:** The question is how much CLA? For example if I were a consumer and were looking at the amount of CLA on the box, is there a threshold response, an upper/lower limit that I should be concerned with? How much CLA should I consume per day? How should I use that information?

**Dr. Sébédio:** Concerning the first question: in Europe we had a concerted action on CLA. Dr. Christie from Scotland was to develop a whole range of analytical methods; for example, there is the gas chromatographic method and high-performance liquid chromatography on silver nitrate method. Now everything is calibrated, and we have tested the analytical methods with different industries and different laboratories. We have prepared some samples of CLA and had them analyzed. There was a long study going on, and if you are interested there is a site about CLA on the web and there is the CLA Newsletter called ‘What Is Going on CLA’. All these methods are published and are available to the public and everybody can use them. But there are very complex methods in order to be able to quantify down to the isomer, not only the total quantity but the isomer distribution. As to your question about the upper and lower limit, it is very hard now to say how much CLA we should consume, or should we consume CLA, or should we increase CLA in the food. I don't think we know enough. We know enough about animal experiments but I don't think we know enough on human experiments to be able to give guidelines for CLA consumption a the moment. This is my own feeling. Americans are consuming less CLA than Europeans, and even in Europe there are differences between northern and southern Europe because the main source is milk.

**Dr. Komindr:** Do you have any data on the effect of the cooking process, the cooking oils, vegetable oils, on linoleic acid? How much would turn to CLA? Deep fry not stir fry.

**Dr. Sébédio:** We don't have any data on stir fry but on deep fry we have some data and you can produce up to about 0.2–0.3% CLA in an oil which contains 18:2, and it follows the isomerization of linoleic acid in fact. This is below 180°C. When you go up to about 210–220°C you can produce up to about 1%, but it is not the same mixture as I showed you. It is basically out of this 1%; it is basically 70–80% of the trans-trans 9,11 and 10,12 isomers.

**Dr. Schiffrin:** I would like to ask you a very general question. Have you studied or is it known if specific trans-isomers are incorporated in macrophage, monocytes and
phospholipids and generate eicosanoids or proinflammatory mediators in different ways? We could then imagine that these trans-isomers could be a nutritional way to modulate the inflammation or to interfere with inflammation.

Dr. Sébédo: No, we have not done these studies, but to get the answer we would have to. It is possible, we still have some isomers which have been isolated and produced, so it is possible but we didn’t do the studies.

Dr. Tappy: You quickly showed some data indicating that trans-fatty acids might more readily be oxidized than other fatty acids. My first question is: was that during pure fat meals or as part of a mixed meal? Did it replace other fats or non-lipid substrates during these experiments?

Dr. Sébédo: The study that I showed you was done during the studies carried out with Drs. Mensink and Beaufrère feeding CLA at 1.5 and 3 g/day. At the end of the 4-month feeding studies, we did a 48-hour oxidation study. So people did not have a meal for a few hours, they had a load of a $^{13}$C-labeled compound, and we did exactly the same thing with CLA. They received a $^{13}$C load which was mixed in an oil which is rich in 18:1. Breath samples were collected in bags before and after ingestion and after that we looked at the isotopic enrichment through gas chromatography-mass spectrometry, and they had a meal after a few hours but they didn’t have a meal before.

Dr. James: Can I come back on that. I was intrigued that if in fact you have some of these isomers that are more readily oxidized than others, the implication is that you either have a block in the uptake as through processing into tissues so that it then becomes available for catabolism, or else you are implying that certain isomers are selectively processed very readily through catabolic pathways, and then I guess it is going to alter appreciably the level to which the body stores differentiate the different isomers. Is that right?

Dr. Sébédo: Yes, I do agree with you and the bioavailability of the isomers is probably different, the 9:11 and for example the 10:12, some are stored more in different tissues than the others. We saw that the 9:11 was more oxidized than the 10:12 and than oleic acid. So if you want to supplement some food or whatever, you have to take that into account.

Dr. Tantibhaedhyangkul: Reading a paper published many years ago, I was intrigued that pomegranate, which is a fruit, contains 70% of total fatty acids as punicic acid [3]. I was intrigued why a fruit contains such a high level, 70% of the total fatty acids as CLA.

Dr. Sébédo: I don’t know why, I don’t have any answer.

References
