The Director General

Maisons-Alfort, 7 October 2011

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on a "safety assessment of the use of an oil enriched with Conjugated Linoleic Acid (CLA)"

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ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its Opinions are made public.

1. REVIEW OF THE REQUEST

On Tuesday 12 July 2011, the French Agency for Food, Environmental and Occupational Health & Safety received a request from the Directorate General for Competition, Consumer Affairs and Fraud Control to undertake the following expert appraisal: safety assessment of the use of an oil enriched with Conjugated Linoleic Acid (CLA).

2. BACKGROUND AND PURPOSE OF THE REQUEST

Discussions are currently underway in the European Union regarding a draft authorisation for a CLA mixture to be used for enrichment purposes in various food matrices. This draft follows from diverging Opinions issued by various Member States and the positive Opinion issued by EFSA on 26 May 2010 in the framework of hearings on novel foods. However, the recent publication of an original article[1] and a review[2] highlighting increased lipid risk factors for cardiovascular disease led the Member States to request a new assessment of the product in July 2011.
At the same time, in May 2011, the Australian and New Zealand authorities rejected an application for authorisation of another product made with CLA whose composition was very similar to that of the product concerned by these assessments.
That said, the European Commission does not consider that the Australian ban was founded on new scientific information, but rather on a different interpretation of the information already assessed by EFSA in 2010.
The Commission has therefore requested that the Member States send it any new scientific information that has not been assessed by EFSA that would warrant a new request to the European Agency, in order to propose a new draft authorisation in October.

[1] Wanders, A.J., Brouwer I.A., E. Siebelink *et al.*, Effect of a high intake of conjugated linoleic acid on lipoprotein levels in healthy human subjects. PLoS One, 2010; 5: e9000.
[2] Brouwer, I.A., Wanders A.J., and Katan M.B., Effect of animal and industrial *trans* fatty acids on HDL and LDL cholesterol levels in humans--a quantitative review. *PLoS One*, 2010; 5: e9434.

At the Agency, a report on the benefits and risks related to *trans* fatty acids (AFSSA, 2005) and particularly CLAs was published in 2005. Various Opinions on the subject were also issued in 2007, 2008 and 2011 (AFSSA, 2007, 2008; ANSES 2011).

The bibliographic research focused on the 2005-2011 period in order to identify scientific articles that had been published since the Agency's report on *trans* fatty acids and that were not cited by EFSA in its 2009 Opinion, or articles that had been published after the EFSA report. It concentrated on the effects of CLAs on cardiovascular health and insulin resistance/diabetes, as these had been the risks subject to reservations expressed by the Member States. Research focused primarily on human studies, but also on animal and *in vitro* studies to back up human data or when the latter were lacking. More specifically, 3 types of risks were assessed, which were the main risks described in the literature and examined in the EFSA Opinion:

- risks related to a change in circulating lipoproteins (elevated LDL-C/HDL-C ratio);
- risks related to increased insulin resistance, particularly in diabetics;
- risks related to enhanced markers of oxidative stress and inflammation (C-reactive protein, isoprostanes, prostaglandin F2α, etc.).

3. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with the French standard NF X 50-110 "Quality in Expert Appraisal Activities – General Requirements of Competence for Expert Appraisals (May 2003)".

The collective expert appraisal was undertaken by the Expert Committee (CES) on Human Nutrition, which met on 15 September 2011, on the basis of initial reports written by 5 expert *rapporteurs*.

4. ANALYSIS AND CONCLUSION OF THE CES

▪ Definition of CLAs

Conjugated Linoleic Acids (CLAs) are conjugated isomers of linoleic acid. Isomer 18:2 c9,t11c9,t11 (rumenic acid) is naturally the quantitatively predominant CLA, particularly in dairy products; this isomer is often combined in equal proportions with isomer 18:2 t10,c12 in synthetic products.
The primary origin of natural CLAs is ruminal biohydrogenation (Chilliard *et al.* 2001). Dairy products are the main source of dietary CLAs, and particularly rumenic acid, which accounts for 80-93% of the total CLAs in milk fat (Chin *et al.* 1992; Lavillonnière *et al.* 1998). Small quantities of CLAs can also come from heating (Chin *et al.* 1992; Juanéda *et al.* 2003; Juanéda *et al.* 2001) and the catalytic hydrogenation of fats (Mossoba *et al.* 1991). The levels that have been measured in vegetable oils are relatively low: from 0.01 to 0.04 g/100 g in various refined oils (Chin *et al.* 1992) and from 0.2 to 1.1% of total fatty acids in margarines, depending on the nature of the raw material and the degree of hydrogenation (Mossoba *et al.* 1991).
CLA consumption in the French population is estimated at 0.18 g/day for women and 0.21 g/day for men, and at 0.37 g/day and 0.44 g/day for high consumers (women and men) (97.5th percentile) (AFSSA 2005).

▪ Effects of CLAs in terms of risks related to a change in circulating lipids

Of the intervention studies examining the effects of CLAs on circulating lipids that have been published since 2005, 4 were not cited by EFSA in its 2009 Opinion and 6 have been published since. They have been taken into account in this assessment and are presented in **Table 1**.

- <u>Effects of rumenic acid</u>

Out of the 5 intervention studies examining rumenic acid, 3 showed no significant effects on circulating lipids (total cholesterol, LDL-C, HDL-C, triglycerides) with this fatty acid, with intakes ranging from 0.10 to 1.0 g/day (Venkatramanan *et al.* 2010; Brown *et al.* 2011; Sofi *et al.* 2010). Two studies reported a significant increase in the LDL-C/HDL-C ratio after consumption of higher doses (1.42 g/day and 2.6 g/day) of rumenic acid (Tricon *et al.* 2006; Desroches *et al.* 2005). However, it is important to underline that in these studies, rumenic acid intake came from dairy products that had been enriched with this fatty acid through a cow feeding protocol that also increased the level of mono-*trans* fatty acids in the milk (13.4 g/100 g versus 0.61 g/100g in standard milk for the study by Tricon *et al.* and 5.05 g/day versus 0.81 g/day in the control diet used in the study by Desroches *et al.*, 2005).

It is considered that 18:1-*trans* fatty acids tend to increase the LDL-C/HDL-C ratio (AFSSA 2005). However, small quantities of total mono-*trans* fatty acids and the proportions of the various isomers naturally found in milk (i.e. during the conventional feeding of dairy cows) do not have harmful effects on circulating lipids (Malpuech-Brugere *et al.* 2010; Motard-Belanger *et al.* 2008). It is therefore advisable not to increase these levels of mono-*trans* fatty acids above the levels recommended by AFSSA for *trans* fatty acids (2005).

Moreover, a case-control study was undertaken in Costa Rica where pasture-grazing cows have milk that is particularly rich in rumenic acid (Smit *et al.* 2010). This study reported an inverse relationship between the level of rumenic acid in the adipose tissue and risk of myocardial infarction, while dairy intake was not associated with risk of infarction (Smit *et al.* 2010).

- Effects of CLA isomer mixtures

Two recent studies investigated CLA mixtures in which rumenic acid was predominant (80%). The study by Sluijs *et al.* (2010) did not report any significant effects on blood lipids after consumption of 3.1 g/day total CLA (2.5 g rumenic acid + 0.6 g 18:2 t10,c12) (Sluijs *et al.* 2010), whereas the study by Wanders *et al.* (2010) reported an increase in total cholesterol, LDL, triglycerides and the LDL-C/HDL-C ratio after consumption of an extremely high dose of CLA (24.2 g/day) (Wanders *et al.* 2010).
As for equimolar mixtures of isomers c9,t11 and t10,c12, four new studies have been published since 2005. One study showed a significant decrease in HDL-C in overweight children (Racine *et al.* 2010), whereas two studies showed no effects in overweight (Venkatramanan *et al.* 2010) or obese (Steck *et al.* 2007) adults, with doses that were comparable (2.4; 3.2; 2.3 g/day) or higher (6.4 g/day). Another study did not show any effects with these mixtures in healthy adults (Lambert *et al.* 2007).

- Brouwer's meta-analysis

The meta-analysis undertaken by Brouwer *et al.*, 2010, which led the Member States to request a new assessment, analysed 13 intervention studies fulfilling several selection criteria. This meta-analysis reported that the LDL-C/HDL-C ratio increases by 0.043 (95% CI 0.012-0.074) for each percentage point of dietary energy ingested as CLA regardless of the isomer composition when replacing a monounsaturated fatty acid. This ratio increases by a factor of 0.056 when considering only studies using equimolar mixtures of isomers c9,t11 and t10,c12. However, this study contains numerous biases:

1. In order to homogenise and be able to compare the studies, the authors recalculated what the effect on lipoprotein would be if *trans* fatty acids replaced *cis* fatty acids (mainly oleic acid), according to the equation of Mensink *et al.* (Mensink *et al.* 2003). Beyond mathematical approximation, the use of this method warrants further justification, since oleic acid has an impact on plasma lipids. In fact, compared to mono-*trans* fatty acids and saturated fatty acids, oleic acid lowers LDL-C and total cholesterol (Gardner and Kraemer 1995).
2. While there are a considerable number of studies examining *trans* fatty acids of technological origin, with a sufficiently wide intake range to assess whether there is a proportional dose-response effect (intakes accounting for 0.5 to 11% of energy intake, 24 studies), this is less the case for CLAs for which there are fewer studies and the doses are more similar (12 studies with intakes accounting for 0.5 to 2.5% of energy intake, and only one at 7%) and even less so for ruminant *trans* fatty acids (6 studies, also with very close intakes, ranging from 0.5 to 2.5% of energy intake, and only one at 6.5% of energy intake).
3. The principal bias lies in the method used to calculate the link between *trans* fatty acid intakes and blood lipoproteins (LDL-C/HDL-C ratio, LDL-C, HDL-C). The authors make the assumption that this relationship is linear, i.e. that the risk is directly proportional to fatty acid intakes, with no threshold. And yet while there is a clear linear relationship, in agreement with the calculation method with forced origin, for *trans* fatty acids of technological origin, this is absolutely not the case for ruminant *trans* fatty acids and even less so for CLAs where it is obvious that the calculated linear relationship is purely artificial. It should also be noted that since no statistical significance value for this relationship has been reported, it is not possible to evaluate the truthfulness of this assumption, which nevertheless determines the interpretation relating to ruminant *trans* fatty acids and CLAs provided by the authors.
4. As far as CLAs are concerned, the data provided do not give any indications about the effects of 50:50 or 80:20 mixtures of isomers c9,t11 and t10,c12, or the isomers separately. Indeed, in the predictive regression system, CLAs are considered all together, as can be seen in the figures. Moreover, the studies investigating CLAs in particular do not mention the population status, such as the degree of obesity, type-2 diabetes, etc. which could make comparison difficult on account of a very different baseline lipoprotein status.

Therefore, it is not possible to conclude that there is a link between CLA intakes and cardiovascular risk factors on the basis of this meta-analysis.

- Conclusion on the effects of CLAs on circulating lipids

The recent studies do not highlight any harmful effects of rumenic acid except when it comes from dairy products enriched with rumenic acid as part of a special diet for cows. And yet this type of diet also increases the level of 18:1-*trans* fatty acids considered as increasing the LDL-C/HDL-C ratio when they are consumed in quantities greater than those consumed through standard milk (AFSSA 2005), which is the case in these studies.
The new studies undertaken with CLA mixtures containing isomers c9,t11 and t10,c12 show an absence of effect in the best case and adverse effects on HDL-C in the worst case, even after exclusion of the study by Wanders *et al.* (2010) in which intakes were extremely high.
Thus, in light of these new data, ambiguities still remain as to the harmful effects of these mixtures on circulating lipids.

Table 1: effects of CLAs on plasma lipids in humans

	reference	subjects	CLA (g/day)	duration	effects
Rumenic acid (RA)	Tricon *et al.*, 2006	32 healthy men	0.15 *vs.* 1.42 (dairy products enriched with RA through a special animal diet)	cross-over study 2*6 weeks	No significant effect on: TC, LDL-C, HDL-C, TG **↗ LDL-C/HDL-C (P=0.02)**
	Desroches *et al.*, 2005	16 overweight men	0.2 *vs.* 2.6 (Butter enriched with RA through a special animal diet)	cross-over study 2*4 weeks with 8 weeks of washout	**decreased TC or TC/HDL-C ratio in the control group but not in the CLA group** **↗ LDL-C/HDL-C (p=0.03)**
	Sofi *et al.*, 2010	10 healthy men and women	0.04 *vs* 0.10[1] (dairy products naturally rich in RA)	cross-over study 2*10 weeks	No significant effect on: TC, LDL, HDL, TG LDL-C/HDL-C: no information
	Brown *et al.*, 2011	18 normal-weight or overweight women	0.28 *vs* 0.85[2] (milk from pasture-fed cattle rich in RA vs. milk from grain-fed cattle low in RA)	Parallel study 8 weeks	No significant effect on: TC, LDL, HDL, TG LDL-C/HDL-C: no information
	Smit *et al.*, 2010	3626 subjects	CLA in the adipose tissue	Case-control study (cardiovascular incident)	Inverse relationship between rumenic acid level and prior heart disease
Mixture of c9,t11/ t10,c12 (80/20)	Sluijs *et al.*, 2010	346 overweight men and women	3.1	Parallel study 6 months	No significant effect on: TC, LDL, HDL, TG, LDL-C/HDL-C
	Wanders *et al.*, 2010	61 healthy men and women	24.2	cross-over study 2*3 weeks	significant effects: **↗ TC, LDL-C, LDL-C/HDL-C (P<0.001), TG (p=0.05).** **↘ HDL-C (p<0.001)**
Mixture of c9,t11/ t10,c12 (50/50)	Racine *et al.*, 2010	53 overweight children	2.4	Parallel study 6 months	No significant effect on: TC, LDL, HDL, TG **↘ HDL-C (p=0.05)**
	Lambert *et al.*, 2007	64 healthy men and women	2.57	Parallel study 12 weeks	No significant effect on: TC, LDL, HDL TG did not decrease as in the control group (p=0.013), non-significant effect after correction for body composition
	Steck *et al.*, 2007	48 obese men and women	3.2 or 6.4	Parallel, randomised, double-blind, placebo-controlled study 12 weeks	No significant effect on: TC, LDL-C, HDL-C, TG with 3.2 g/day and 6.4 g/day
c9,t11 vs 50/50 CLA mixture	Venkatramanan *et al.*, 2010	15 overweight men and women	Control milk: 0.2 naturally enriched milk: 1.0 g/day RA Milk + 50/50 mixture: 2.3 g/day	Cross-over study 3*8 weeks	No significant effect on: TC, LDL, HDL, TG LDL-C/HDL-C: no information

[1] Intakes calculated by the expert *rapporteurs*. The entire fatty acid profile varied since changes in experimental diets were the result of changes in the diets of dairy cows.
[2] Intakes calculated by the expert *rapporteurs* on the basis of intakes provided by the authors expressed as methyl esters.

- **Effects of CLAs in terms of risks related to increased insulin resistance (Table 2)**

  - Effects of rumenic acid (c9,t11)

The study by Brown *et al.* (2011) mentioned above also tested the effect of rumenic acid on insulin resistance. A diet enriched with c9,t11 did not change body composition or insulin sensitivity measured by an Oral Glucose Tolerance Test (OGTT). However, there was a change in insulin kinetics after treatment with rumenic acid, suggesting improved insulin sensitivity, but the area under the insulin curve was not significantly affected.

  - Effects of CLA isomer mixtures

The study by Sluijs, also mentioned above, reported that 3.1 g/day of an 80/20 CLA mixture (2.5 g/day c9,t11 and 0.6 g/day t10,c12) did not have an effect on body composition or the HOMA-IR insulin resistance index (Sluijs *et al.* 2010).

Four new studies assessing the effects of 50/50 mixtures of isomers c9,t11 and t10,c12 on insulin resistance have been identified. Two studies showed no effect for doses of 2.5 and 3.4 g/day CLA (Joseph *et al.* 2011; Racine *et al.* 2010). However, one study suggested an improvement in insulin sensitivity with 2.6 g/day of a CLA mixture, in women only, who had normal body weights and exercised regularly (Lambert *et al.* 2007). Therefore, this effect cannot be attributed solely to CLAs. On the other hand, one study reported decreased insulin sensitivity after ingestion of 4 g/day CLA in non-diabetic overweight subjects (Thrush *et al.* 2007). Lastly, another study assessed the effect of a CLA mixture combined with EPA and DHA with the idea that the latter would cancel out the decrease in insulin sensitivity that is sometimes observed with CLAs. This combination resulted in a decrease in insulin sensitivity only in obese elderly subjects and there was no effect on this parameter in young subjects and slim elderly subjects. Under the assumption that EPA and DHA have no effects on insulin sensitivity in humans (for review (Poudyal *et al.* 2011)), this harmful effect would be attributable to the consumption of the CLA mixtures.

  - Effects of isomer t10,c12

The harmful effect of t10,c12 on insulin sensitivity has clearly been demonstrated *in vitro* in human adipocytes. Two recent studies showed that 50 µM of t10,c12 for 48 hrs. reduced insulin-stimulated glucose uptake in adipocytes by 50% (Kennedy *et al.* 2010a; Kennedy *et al.* 2009). This effect of isomer t10,c12 on the reduction in glucose use has also been reported in muscle cells (C2C12), and was accompanied by an inhibition of the myogenic differentiation process (Hommelberg *et al.* 2010).

In mice, while a beneficial effect of isomer c9,t11 on insulin sensitivity has been described, the effect of t10,c12 on insulin resistance was clearly highlighted in a recent study (Halade *et al.* 2010). It was a study undertaken in 6-month-old female C57Bl6/J mice with an intake of rumenic acid only, isomer t10,c12 only or an equimolar mixture of the two compounds, as 0.5% of the total diet, for 6 months, compared to a control. The results showed no effects on glucose, insulin, HOMA or R-QUICKI after supplementation with rumenic acid. On the other hand, when isomer t10,c12 alone or in a mixture was added to the diet, the same parameters increased significantly, underlining the development of insulin resistance in this model.

  - Conclusion on the effects of CLAs on insulin sensitivity

These new studies do not indicate that CLA mixtures have beneficial effects on glucose homeostasis and may indeed suggest a risk related to the consumption of such mixtures in obese subjects, who are the most likely to consume food supplements containing CLAs or foods enriched with CLAs.
In light of the harmful effects of isomer t10,c12 that have been observed in animals, and the lack of scientific data on this isomer in humans, the safety of its consumption, alone or in a mixture, cannot be guaranteed.

Table 2: Effects of CLAs on the plasma parameters of glucose homeostasis and insulin resistance in humans

	reference	subjects	CLA (g/day)	duration	effects
Rumenic acid (RA)	Brown *et al.*, 2011	18 normal-weight or overweight women	0.28 *vs* 0.85[2] (milk from pasture-fed cattle rich in RA vs. milk from grain-fed cattle low in RA)	Parallel study 8 weeks	No effects on plasma glucose or glucagon No difference in the area under the insulin curve but slight change in kinetics that may suggest an improvement in insulin sensitivity
80/20 CLA mixture (c9,t11/t10,c12)	Sluijs *et al.*, 2010	346 overweight men and women	3.1 (2.5 g RA + 0.6 g t10,c12)	Parallel study 6 months	No significant effects on glucose, insulin or HOMA-IR
50/50 CLA mixture (c9,t11/t10,c12)	Racine *et al.*, 2010	53 overweight children	2.4 g/day	Parallel study 6 months	No effects on glucose, insulin or HOMA-IR
	Joseph *et al.*, 2011	36 overweight men	3.5 g/day	Cross-over study 3*8 weeks	No effects on insulin sensitivity (HOMA-IR) or adiponectin
	Thrush *et al.*, 2007	9 non-diabetic overweight subjects	4 g/day	Pre- and post-treatment study 12 weeks	**Reduced insulin sensitivity (p≤0.01) estimated by OGTT**
	Lambert *et al.*, 2007	62 healthy men and women who exercise more than 3 times/week	2.6 g/day	Parallel study 12 weeks	No effects on HOMA-IR or QUICKI but change in insulin curve kinetics suggesting better insulin sensitivity and a smaller increase in free fatty acids.
	Ahren *et al.*, 2009	49 normal-weight or obese men	2.1 g/day CLA + 3 g/day EPA + DHA	Cross-over study 2*12 weeks 12 weeks of washout	**↘ insulin sensitivity in the oldest obese subjects estimated with OGTT**

HOMA-IR, homeostasis model assessment, index used to estimate insulin resistance, OGTT, oral glucose tolerance test, QUICKI, quantitative insulin sensitivity check model, insulin-sensitivity index

Effects of CLAs in terms of risks related to increased markers of inflammation

Not only have the data related to inflammatory markers mentioned in the EFSA reports been updated, but the effects of CLA supplementation on plasma leukocytes and inflammation of the white adipose tissue have also been studied.

In fact, while in humans, the effects of CLA mixtures are controversial (two recent meta-analyses reported reduced adipose mass (Whigham *et al.* 2007) (FSANZ, 2009, Review Report, Intakes of *trans* fatty acids in New Zealand and Australia) whereas EFSA refused a claim on the effect of CLAs on weight loss), in animals, and particularly in mice, CLA supplementation reduces body fat mass. In mice, CLAs have a drastic effect on the adipose tissue and cause inflammation in a period of a few days (LaRosa *et al.* 2006; Poirier *et al.* 2005). These results have also been found *in vitro* and confirm that only isomer t10,c12 rapidly reduces the adipose tissue and causes inflammation (Kennedy *et al.* 2010b). As a result, in the context of the request, it was relevant to produce a bibliographic summary of the data that have been published regarding the effect of CLAs on inflammation of the adipose tissue in humans.

- Inflammatory markers **(Table 1 in the Annex)**
  - CRP

Six studies published after the EFSA Opinion and two studies published in 2007-2008 but not cited by EFSA analysed the effects of CLAs on plasma concentrations of CRP in healthy or overweight humans. Of these 8 studies, 7 undertaken in healthy or overweight people (Mullen *et al.* 2007; Pfeuffer *et al.* 2011; Venkatramanan *et al.* 2010; Joseph *et al.* 2011; Sluijs *et al.* 2010; Smit *et al.* 2010) or people living with rheumatoid arthritis (Aryaeian *et al.* 2008) did not observe effects with a CLA mixture containing variable proportions of the two main isomers on this inflammatory marker. However, 12-week supplementation in obese subjects (BMI from 30 to 35 kg/m^2) with 6.4 g/day of an equimolar mixture of c9,t11 and t10,c12-CLA significantly increased plasma concentrations of CRP (0 *vs.* 12 weeks, Control group: 11.2 mg/L ± 10.7 *vs* 9.6 mg/L ± 7.7; CLA group: 7.2 mg/L ± 3.5 *vs* 10.3 mg/L ± 6.7) (Steck *et al.* 2007). In this study, the effect was not found with 3.2 g/day CLA.

This result suggests that supplementation with a high dose of CLA (6.4 g/day) may increase the inflammatory marker CRP.

  - Inflammatory cytokines

Circulating concentrations:

Most of the studies that have recently been published or that were not mentioned in the EFSA report have not highlighted effects of supplementation with a CLA mixture in the form of capsules or in food on concentrations of inflammatory cytokines such as IL-6, TNF-α, fibrinogen and MCP-1 (Joseph *et al.* 2011; Mullen *et al.* 2007; Nugent *et al.* 2005; Pfeuffer *et al.* 2011; Smit *et al.* 2011; Venkatramanan *et al.* 2010). Only the study by Steck *et al.* that was published in 2007 reported that supplementation for 12 weeks with 6.4 g/day CLA induced a significant increase in plasma concentrations of IL-6 (0 weeks *vs.* 12 weeks: Control group: 2078.0 pg/L ± 1289.3 *vs* 1713.0 pg/L ± 905.4; CLA group: 1632.1 pg/L ± 776.1 *vs* 2071.1 pg/L ± 1131.6) (Steck *et al.* 2007). As in the case of CRP, the authors underline the significance of the dose when observing possible effects on inflammatory markers.

A publication investigating the effect of daily consumption, for 10 weeks, of 200 g of cheese naturally rich in rumenic acid reported a decrease in IL-6 inflammatory cytokines (Control group vs. CLA: 4.58 pg/mL ± 0.94 *vs* 8.08 pg/mL ± 1.57) and TNF-α (53.58 pg/mL ± 25.67 *vs* 32.09 pg/mL ±17.42) (Sofi *et al.* 2010). Since human data on the specific effect of isomers on these inflammatory markers are scarce, it is not possible to draw a conclusion as to a potential beneficial effect of rumenic acid.

These results concur with those obtained in animals as numerous studies have not observed increases in plasma concentrations of inflammatory cytokines (Poirier *et al.* 2006).

Expression in adipose tissue

In mice, it has been demonstrated that supplementation with a mixture containing isomer t10,c12 induces a significant increase in the expression of TNF-α, IL-6 and/or MCP-1 in the white adipose tissue (LaRosa *et al.* 2006; Poirier *et al.* 2006; Tsuboyama-Kasaoka *et al.* 2000). This induction is associated with rapid macrophage infiltration (Poirier *et al.* 2006). The data from numerous *in vitro* studies suggest the following mechanism of regulation: isomer t10,c12 activates the NFkB pathway which increases the production of

inflammatory cytokines. The cytokines activate the ERK1/2 pathway, which reduces the activity of Peroxisome Proliferator Activated-Receptor gamma (PPARγ) and the expression of its target genes (Brown *et al.* 2004; Brown and McIntosh 2003; Kennedy *et al.* 2008; Poirier *et al.* 2006). This cascade of events decreases glucose uptake and triglyceride synthesis, thereby resulting in insulin resistance and intense lipolysis (Kennedy *et al.* 2010b). However, increased expression of inflammatory cytokines in the adipose tissue is not associated with an increase in their plasma concentration.

In humans, to our knowledge, no studies that have been published to date mention an effect of CLAs on the level of inflammatory cytokines and macrophages in the adipose tissue of healthy or obese subjects.

As for PPARγ, a study published in 2007 mentioned that its expression increased in the white adipose tissue of healthy men who had been supplemented for 14 weeks with 3.76 g of a mixture containing an equal proportion of the two main isomers (35%/35%) (Nazare *et al.* 2007). Conversely, another study reported a decrease in PPARγ gene expression in the adipose tissue of subjects supplemented with 4.25 g fatty acids containing 3.2 g of either an equimolar mixture of the main isomers, or one of the two purified isomers, or linoleic acid (control group). The effect of each supplementation was analysed for 28 days in all of the participants who were homozygous for PPARγ2 Ala12Ala (15 individuals) or PPARγ2 Pro12Pro (23 individuals). This study's results confirm that rumenic acid induces effects that are far less significant than those observed with isomer t10,c12. However, decreased expression of PPARγ does not appear to affect the expression of its target genes (Herrmann *et al.* 2009).

As a result, due to the scarcity of published data on humans, it is not currently possible to definitively conclude that supplementation with a high dose of t10,c12 does not induce inflammation of the adipose tissue as has been observed in mice.

Furthermore, *in vivo* and *in vitro* studies have shown that isomer t10,c12 activates reticulum stress and inflammation (LaRosa *et al.* 2007). The endoplasmic reticulum (ER) is a key cellular compartment for the biosynthesis of all proteins produced in the cells. The flow of proteins through the ER must be closely controlled, in order to prevent perturbation of homeostasis and ER stress. ER stress triggers a cascade of signals known as UPR - Unfolded Protein Response, which modulates cell functioning and can lead to apoptosis. This increase in ER stress under the action of t10,c12 also leads to apoptosis in the adipocytes (Ou *et al.* 2008). The molecular mechanisms involved in the pro-inflammatory effect of isomer t10,c12 have recently been studied in human adipocytes and these studies suggest that the c-Jun kinase signalling pathway is a target of CLA (Martinez *et al.* 2011; Kennedy *et al.* 2009).

- Leukocytes (Table 2 in the Annex)

Leukocytes (granulocytes, lymphocytes and monocytes) are immune system cells that increase in number in case of infection or inflammatory reaction.
Several teams have studied the effect of CLAs on the number of circulating leukocytes (Gaullier *et al.* 2004; Gaullier *et al.* 2005; Larsen *et al.* 2006; Whigham *et al.* 2004). Four out of five studies reported a significant increase in the number of leukocytes. Only the study by Whigham *et al.* that was published in 2004, using a very high dose (6 g/day) of an equimolar mixture of the two main CLA isomers, did not observe effects on lymphocytes, neutrophils or monocytes in obese subjects after one year of supplementation (Whigham *et al.* 2004).
The effect of CLAs on circulating concentrations of leukocytes should be analysed with caution, since in general, studies have not distinguished between the various classes of leukocytes, and the variations obtained have been small.

- Lipid peroxidation markers (Table 3 in the Annex)
  - 8-iso-prostaglandin F2α produced by non-enzymatic peroxidation (oxidative stress marker) and 15-keto-prostaglandin F2α produced by enzymatic peroxidation (inflammatory marker)

Recent studies have confirmed that CLAs increase lipid peroxidation markers. In fact, urinary concentrations of 8-iso-prostaglandin F2α(8-iso-PGF2α) increased by 170% during supplementation for 3 weeks with a CLA mixture containing a c9,t11/t10,c12 ratio of 80/20 in the form of enriched margarine and yoghurts (7% of total energy consumed) (Smit *et al.* 2011). The CLA mixture therefore had a much more significant effect than that found in the case of *trans* fatty acids (mainly C18:1, *trans*) contained in a hydrogenated oil as the latter increased urinary concentrations of 8-iso-PGF2α by only 19% (Smit *et al.* 2011).
Furthermore, in 2008, Turpeinen *et al.* demonstrated that supplementation for 12 weeks with capsules containing a 2 g CLA mixture mainly in the form of rumenic acid (65.3% rumenic acid and 8.5% t10,c12)

increased urinary excretion of 8-iso-PGF2α and 15-keto-prostaglandin-F2α respectively by around 60% and 85% (Turpeinen *et al.* 2008).
Lastly, supplementation with 4.5 g/day of an equimolar CLA mixture for 4 weeks increased urinary markers of lipid peroxidation in overweight men (Pfeuffer *et al.* 2011). However, CLAs did not modify endothelial functions, or other markers of metabolic syndrome or oxidative stress. As a result, the authors suggest that CLAs do not increase cardiovascular risk and that F2-isoprostanes in this context may not indicate an increase in oxidative stress.
The induction of prostaglandins 2α has also been found *in vitro* in human adipocyte cultures (Kennedy *et al.* 2009), mouse adipocytes (Hargrave-Barnes *et al.* 2008) and 3T3-L1 adipocytes treated with isomer t10,c12 (Jiang *et al.* 2011). The level of triglycerides found in these cells appears to be influenced by the increase in concentrations of COX2-mRNA. These F2 prostaglandins appear to inhibit adipogenesis by reducing PPARγ activity.

- Other markers of lipid peroxidation

A recent study that analysed the effects of CLA supplementation (2.4 g/day of an equimolar mixture) for 8 weeks did not report any changes in lipid peroxidation or antioxidant metabolism when estimating several parameters such as the Total Radical-trapping Antioxidant Potential (TRAP), the activity of enzymes such as superoxide dismutase, catalase and glutathione peroxidase, and the concentration of fat-soluble antioxidant vitamins (Kim *et al.* 2011).
Oxidised LDL levels did not appear to be altered by CLA supplementation with an equimolar proportion of the two main isomers or enriched with rumenic acid. The authors suggest that CLAs do not affect oxidative stress levels in overweight humans (Joseph *et al.* 2011).

- Conclusion on markers of inflammation and oxidative stress

An analysis of the recent literature (published after the EFSA Opinions or not mentioned in these reports) confirms that daily supplementation with a 50/50 mixture of the two main isomers (c9,t11 and t10,c12) increases the oxidative stress markers produced by non-enzymatic lipid peroxidation (8-iso-prostaglandin F2α). The increase in lipid peroxidation in humans is maximum under the effect of isomer t10,c12. This increase can be significant (+170%) and greater than that observed with an equivalent quantity of *trans* fatty acids such as C18:1,*trans*. However, the stability of other oxidative stress markers (circulating concentration of oxidised LDL, dismutase activity), the lack of effect of vitamin E on the level of 8-iso-prostaglandins F2α observed in subjects supplemented with CLAs (Smedman, 2004), and the alteration of isoprostane catabolism by CLAs due to competition with β-oxydation in peroxisomes (Iannone, 2009) suggest that the increase in lipid peroxidation markers is not only a reflection of increased oxidative stress.
Moreover, this analysis corroborates the previous one as to the increase in certain inflammatory markers such as 15-keto-prostaglandin F2α, and in some studies the circulating concentration of CRP. However, CLAs do not appear to have an impact on plasma concentrations of inflammatory cytokines in *in vivo* studies undertaken in humans and animals. Furthermore, studies have reported that circulating concentrations of leukocytes increase slightly during supplementation with CLAs.
Lastly, recent studies showing inflammation of the white adipose tissue in mice and activation of reticulum stress in human adipocyte cultures should be taken into consideration for a new assessment of the effects of CLA mixtures in humans.

- **Conclusion of the CES**

On the basis of studies not included in the EFSA 2009 Opinion or published afterwards, the CES on Human Nutrition has assessed the risks related to CLA consumption:

- risks related to a change in circulating lipoproteins (elevated LDL-C/HDL-C ratio);
- risks related to increased insulin resistance, particularly in diabetics;
- risks related to increased inflammatory markers.

This assessment revealed that none of these studies reported beneficial effects of mixtures of isomers c9,t11 and t10,c12 on lipid risk factors for cardiovascular disease (LDL-C, HDL-C, triglycerides, LDL-C/HDL-C). However, adverse or harmful effects were sometimes reported, particularly an elevated LDL-C/HDL-C ratio.

As far as insulin resistance is concerned, numerous *in vitro* and animal studies have shown a harmful effect for isomer t10,c12. However, no studies undertaken in humans are available to assess the relevance of these results for humans. Because the new studies undertaken with equimolar mixtures of isomers c9,t11 (rumenic

acid) and t10,c12 are contradictory and report, in half of cases, a harmful effect on insulin sensitivity, they confirm the reservations previously expressed by AFSSA in its report on *trans* fatty acids (AFSSA 2005) and its Opinions of 23 March 2007 and 11 July 2008.

Lastly, regarding inflammation and oxidative stress, the new data in the literature confirm that in humans, consumption of a CLA mixture containing 50% rumenic acid and 50% t10,c12 increases:

- markers of oxidative stress (8-iso-prostaglandin F2α). The observed effect appears greater than that found through smoking (Tomey *et al.* 2007) or from the equivalent consumption of *trans* fatty acids (C18:1, *trans*).
- certain markers of inflammation (increase in plasma levels of CRP in some studies, levels of 15-keto-prostaglandin F2α and the number of circulating leukocytes).

However, these results should be qualified since:

- Some authors suggest that in this context, the increase in 8-iso-prostaglandin F2α levels is not solely a reflection of increased oxidative stress.
- The increase in circulating concentrations of CRP and leukocytes that has been observed in individuals supplemented with CLA has been small.

Isomer t10,c12 has also been found to cause inflammation of the white adipose tissue *in vivo* in mice and *in vitro* in human adipocyte cultures.

As with insulin resistance, it appears that isomer t10,c12 is responsible for the main identified effects such as the increase in markers of lipid peroxidation and inflammation. As expressed by AFSSA in 2005, studies not reporting risks related to "equal mixtures of 18:2 c9,t11 and t10,c12 cannot obscure the results obtained for 18:2 t10,c12. It appears difficult to accept the argument according to which the effects of one of the products cancel out those of the other".

Thus, the new data do not report any beneficial effects on the analysed parameters, but sometimes report harmful effects with CLA mixtures. If these harmful effects are combined, the risk of cardiovascular disease and metabolic syndrome could increase. The CES on Human Nutrition therefore considers that, on the basis of the new available data, the risks related to the consumption of CLA mixtures remain ambiguous.

Thus, the CES on Human Nutrition considers that these new data justify a new request to the European Agency, in order to propose a new draft authorisation for a novel ingredient made with CLA.

5. THE AGENCY'S CONCLUSION AND RECOMMENDATIONS

ANSES endorses all of the conclusions of the CES on Human Nutrition.

The Director General

Marc MORTUREUX

KEYWORDS

Keywords:
Rumenic acid, insulin resistance, insulin sensitivity, cardiovascular, inflammation, risk

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Annex(es)

Table 1: Human studies analysing the impact of supplementation with a CLA mixture and/or one of the two main isomers on markers of systemic inflammation. The references cited in the EFSA report (EFSA Journal 2010; (5):1600) are indicated in the grey cells.

Reference	Study design	CLA isomers, doses and duration	Results
(Smedman *et al.* 2005)	53 women and men aged 23-63 years were randomised either in a control group (placebo olive oil) or in a CLA group. Average of BMi: 25	CLA: 4.2g /day (purity: 76%) C9,t11/t10,c12-CLA: 1/1 3 months	CLA supplementation increased levels of C-reactive protein but not TNF-a, VCAM-1 plasma level.
(Watras *et al.* 2007)	40 healthy, overweight subjects (age: 18-44 years; BMi: 25-30).	placebo-controlled study of 3.2 g/day CLA 6 months.	CLA did not affect insulin resistance, blood lipids or markers of liver function or markers of inflammation, with the exception of a significant decrease in a biomarker for endothelial dysfunction.
(Gaullier *et al.* 2007)	118 healthy, overweight and obese adults. (BMI: 28–32 kg/m2) were randomized in 2 groups: control and CLA groups.	CLA: 3.4g/day (4.5g Clarinol) c9,t11 (37.5 %) and t10,c12 (38 %) 6 months	CLA supplementation increased levels of C-reactive protein but not TNF-a or IL-6. However, CRP levels in the CLA group appear to be in the normal range.
(Moloney *et al.* 2004)	32 subjects with stable, diet-controlled type 2 diabetes were randomized in 2 groups: control and CLA groups.	CLA: 3.0 g/d c9,t11 CLA and t10,c12: 1/1 8 weeks.	C-reactive protein and interleukin 6 plasma levels were stable
(Naumann *et al.* 2006)	92 middle aged (35–65 years) healthy men (n = 51) and women (n = 41) classified as having the LDL phenotype B were included in the study. All subjects were moderately overweight (BMI 25–32.5 kg/m2)	Group 1: 3 g of high oleic acid sunflower oil. Group 2: 3 g of c9, t11 CLA (80%); Group 3: 3 g of t10, c12 CLA (80%). 13 weeks	C-reactive protein plasma level was stable

(Ramakers *et al.* 2005)	42 women and men with a high risk of coronary heart disease characterized by moderate overweight (body-mass index, 25-32.5 kg/m2) in combination with LDL-phenotype B were randomized in control and CLA group.	3 g of c9,t11 or t10,c12-CLA 13 weeks	C-reactive protein plasma level was stable
(Song *et al.* 2005)	28 healthy male and female participants aged 25–50 y received either high oleic sunflower oil (reference) or CLA-triglyceride form. A 12-week washout period followed the intervention period.	CLA: 3g/day c9,t11/t10,c12: 1/1 12 weeks.	CRP plasma level was not affected by CLA supplementation. CLA supplementation decreased the levels of the proinflammatory cytokines, TNF-a and IL-1b, but increased the levels of the anti-inflammatory cytokine, IL-10.
(Mullen *et al.* 2007)	A double-blind placebo controlled intervention trial in a cohort of healthy middle-aged male volunteers (50 years old, BMI 26)	CLA: 2.2 g 50:50 isomeric of (c9, t11)-CLA and (t10, c12)-CLA or placebo 8 weeks.	Inflammatory markers associated with CVD, including IL-6, CRP and fibrinogen, were not affected by CLA supplementation.
(Aryaeian *et al.* 2008)	A randomized, double blind placebo-controlled trial was conducted in 87 patients with active Rheumatoid arthritis.	CLA: 2.5 g equivalent to 2 g mixture of c9,t11 and t10,c12 (50/50); Group E: vitamin E: 400 mg; Group CE: CLAs and vitamin E 12 weeks	CRP level was not modified by CLA.

(Joseph *et al.* 2011)	A double-blinded, 3-phase crossover trial was conducted in 36 overweight (BMI: 25 kg/m^2), borderline hypercholesterolemic [LDL-cholesterol (C): 2.5 mmol/L] men aged 18–60 y. During three 8-wk phases, each separated by a 4-wk washout period, 27 participants consumed under supervision in random order 3.5 g/d of safflower oil (control), a 50:50 mixture of t10, c12 and c9, t11 CLA:Clarinol G-80, and c9, t11 isomer:c9, t11 CLA	Control: 3.5 g/d of safflower oil, 3.5 g/d of a 50:50 mixture of t10, c12 and c9, t11 CLA oil (containing 2.8 g of total CLA), 3.5 g/d of c9,t11 CLA (c9, t11 CLA oil, containing 2.7 g of total CLA) 8 weeks	The current study did not show such an increase in circulating hs-CRP after 8 wk of both CLA treatments compared with control. None of the 2 other serum inflammatory markers (TNF-a and IL-6) analyzed varied upon CLA intake. CLA supplementation for 8 wk did not modify plasma Ox-LDL, suggesting that CLA supplementation does not affect oxidative status in men.
(Venkatramanan *et al.* 2010)	A randomized, 3-phase, crossover, single-blind clinical trial was carried out in moderately overweight, borderline hyperlipidemic individuals who consumed (1) milk naturally enriched in CLA; (2) milk enriched with a synthetic mixture of t10, c12 and c9, t11 CLA isomers providing 1.3 g/d of CLA; or (3) untreated milk as a control providing 0.2 g/d CLA. Dietary phases were each in duration and were separated by 4-week washout periods	Group 1: in CLA (4.2%) containing c-9, t-11 only providing 1.3 g/d of CLA; Group 2: milk enriched with a 4.2% synthetic mixture of t-10, c-12 and c9,t11 CLA isomers providing 1.3 g/d of CLA; Group 3: untreated milk as a control providing 0.2 g/d CLA. 8 weeks	CLA consumption did not significantly affect plasma alanine transaminase, total bilirubin, C-reactive protein, or tumor necrosis factor-alpha concentrations.
(Sluijs *et al.* 2010)	In a double-blind, randomized, placebo-controlled, parallel group trial, 401 subjects, aged 40–70 years and with a BMI=25, received either CLA or placebo supplements.	4 g CLA/da 2.5 g c9,t11 CLA/day and 0.6 g t10,c12 CLA/day or placebo supplements 6 months	There was no effect of c9,t11 CLA supplementation on blood pressure, body composition, insulin resistance, or concentrations of lipid, glucose, and C-reactive protein.

(Pfeuffer *et al.* 2011)	85 overweight men (aged 45-68 years, body mass index 25-35 kg/m2) were randomized to receive the CLA isomeric mixture, safflower oil, heated safflower oil, or olive oil in double-blind study.	CLA: 4.5 g/day CLA isomeric mixture (c9,t11/t10,c12:1/1) 4 weeks	CRP plasma level was stable
(Nugent *et al.* 2005)	A total of 55 healthy volunteers (n=20 males, n=35 females) were randomised into one of three study groups who received 3 g/day of a fatty acid blend containing a 50:50 c9, t11: t10, c12 CLA isomer blend, and 80:20 c9, t11: t10, c12 CLA isomer blend or linoleic acid (control, 2 g linoleic acid).	3 g/day of a fatty acid blend containing: a 50:50 c9, t11/ t10, c12 CLA isomer blend (2 g CLA), or a 80:20 c9, t11: t10, c12 CLA isomer blend (1.76 g CLA) or linoleic acid (control, 2 g linoleic acid) 8 weeks	Supplementation with the 80:20 CLA isomer blend significantly (Pr0.05) enhanced PHA-induced lymphocyte proliferation. CLA decreased basal interleukin (IL)-2 secretion (Pr0.01) and increased PHA-induced IL-2 and tumor necrosis factor a (TNFa) production (P<0.01). However, these effects were not solely attributable to CLA as similar results were observed with linoleic acid.
(Smit *et al.* 2011)	25 men and 36 women study in double-blind, randomized, multiple cross over trial with 3 consecutive periods of 21 days. Control (oleic acid), industrial trans fatty acid then CLA.	Oleic sunflower oil, C18:1trans and CLA oil: (c9,t11/t10,c12: 6.9/1.5): 7% total energy of diet Lipids were incorporated in margarine and yogurt drinks 3 weeks	No effect of CLA on plasma levels of inflammatory markers (IL-6, TNF-a, CRP, MCP-1).
(Sofi *et al.* 2010)	Ten subjects (6 F; 4 M) with a median age of 51.5 followed for 10 weeks a diet containing 200 g/week of cheese naturally rich in CLA (intervention period) and for the same period a diet containing a commercially available cheese of the same quantity (placebo period).	A diet containing 200 g/week of cheese naturally rich in c9,t11-CLA 10 weeks	Dietary intervention with pecorino cheese with a grass naturally rich in cis-9, trans-11 CLA determined a consistent reduction of inflammatory cytokines: IL-6 et TNF-a.
(Steck *et al.* 2007)	38 participants (13 males and 35 females) (BMI between 30 and 35 kg/m^2 were randomized to receive placebo (8 g safflower oil/d), or CLA.	3.2 g/d CLA or 6.4 g/d CLA c9,11t/ t10,c12: 50:50 12 weeks	Significant decreases in serum HDL-cholesterol and sodium, hemoglobin, and hematocrit, and significant increases in serum alkaline phosphatase, C-reactive protein and IL-6 and white blood cells occurred in the 6.4 g/d CLA group, although all values remained within normal limits.

Table 2: Impact of CLA supplementation on the number of circulating leukocytes in humans.

Reference	Study design	CLA isomers, doses and duration	Results
(Gaullier *et al.* 2004)	180 healthy volunteer men and women aged 18–65 years were randomised in 3 groups.	Group control: 4.5 g olive oil Group FFA CLA: 4.5 g 80% CLA-Free Fatty Acids (3.6 g active CLA isomers), Group TG CLA: 4.5 g 76% CLA-triacylglycerol (3.4 g active isomers) 1 year	Leukocyte number was increased in both CLA groups (5.3 ± 1.62 vs 6.0 ± 1.69 leucocytes (10^9/L) after 12 months.
(Larsen *et al.* 2006)	122 obese healthy subjects (BMI 28) underwent an 8-wk dietary run-in with energy restriction (3300–4200 kJ/d). 101 subjects who lost .8% of their initial body weight were subsequently randomly assigned to a double-blind CLA (n . 51) or placebo (olive oil; n . 50) supplementation regime in combination with a modest hypocaloric diet of .1250 kJ/d.	The 2 treatment groups received either 4.5g CLA oil/day (3.4 g CLA/d) or 4.5 g olive oil) per day. c9,t11 CLA/t10,c12 CLA: 1/1 as triacylglycerols 1 year	Leukocyte number was increased in both CLA groups (6.21 ± 1.43 vs 6.36 ± 1.66 leucocytes (10^9/L) after 52 weeks.

(Gaullier *et al.* 2005)	The 134 (24 men and 110 women) subjects of (Gaullier *et al.* 2004) who had been randomised in 3 groups as in CLAFFA supplemented with CLA-FFA; group CLATG and group PLAC the placebo (olive oil) group, were supplemented with TG-CLA.	6 opaque soft gel capsules of CLA-TG 4.5 g (3.4 g CLA isomers; Natural Lipids) 12 (Gaullier 2004) + 12 months	Leukocytes were increased in groups supplemented in CLA and previously supplemented with CLAFFA and CLATG during 12 months (example CLATG: 5.30 ± 1.57 vs 6.19 ± 1.65 leucocytes (10^9/L) after 24 months) Thrombocytes levels at mo 24 had increased in groups CLAFFA and CLATG compared with mo 0 and in group PLAC compared with mo 12 (P . 0.001) In the present study, 2 of 134 subjects had increased levels of transaminases (ASAT and ALAT) at the end of the study. The transaminases levels returned to normal values 4 wk after ending the CLA treatment, indicating that a relation to CLA treatment cannot be excluded.
(Whigham *et al.* 2004)	Obese humans who were generally healthy were randomized, in double-blind study consisting of three phases in which subjects were given CLA or placebo. Phase 1 was a low calorie diet (13 kcal/kg desirable weight) for 12 weeks or until 10-20% of initial body weight was lost. In phase 2, from weeks 12 to 28, subjects were re-fed a diet providing 25-30 kcal/kg of desirable body weight. Phase 3 was open label, with subjects from both groups taking CLA from weeks 28 to 52.	given 6 g/day of CLA or placebo CLA: 6g/day c9,t10/T10,c12: 50/50 1 year	No effect of CLA supplementation on lymphocytes, neutrophiles and monocytes. ALAT and ASAT levels were decreased in CLA group.

Table 3: Human studies analysing the impact of supplementation with a CLA mixture and/or one of the two main isomers on markers of lipid peroxidation. The references cited in the EFSA report (EFSA Journal 2010; (5):1600) are indicated in the grey cells.

Reference	Study design	CLA isomers, doses and duration	Results
(Taylor *et al.* 2006)	Control group: 19 overweight white men (Placebo: olive oil) and CLA group: 21 healthy white men (35 to 60 years old; BMi > 27)	CLA: 4.5g/day c9,t11/t10,c12: 1/1 3 months	Plasma F2 isoprostanes was increased in the CLA groups compared to controls. No effect on TNF-a and CRP
(Basu *et al.* 2000b)	Control group: 25 healthy subjects (Placebo: olive oil) and CLA group: 28 healthy subjects (23 to 63 old; BMi: 25± 4; men/female: 25/26) n used for statistical analysis: 53	CLA: 4.2g/day c9,t11/t10,c12: 1/1 3 months	Plasma and urinary (x3) 8-iso-prostaglandin F2a levels were increased in the CLA groups compared to controls. Urinary 15-keto-dihydroprostaglandin F2a level was also increased in CLA group (2 fold).
(Basu *et al.* 2000a)	Control group: 10 men in metabolic syndrome (Placebo: olive oil, BMi: 31.4 ±1.9) and CLA group: 14 men with metabolic syndrome (mean age 53 years; BMi: 32.2±23.4)	CLA: 4.2g/day c9,t11-CLA/t10,c12-CLA: 1/1 1 month	Urinary 8-iso-prostaglandin F2a (marker of non-enzymatic lipid peroxidation) and 15-keto-dihydroprostaglandin F2a (marker of enzymatic lipid peroxidation and systemic inflammation) levels were increased in CLA group by 4 and 2, respectively.
(Riserus *et al.* 2004)	Control group: 12 abdominally obese white men (Placebo: olive oil, BMi: 30.4±2.5) and CLA group: 13 abdominally obese white men (around 56 years old; BMi: 30.6 ±2.0)	CLA: 3g/day c9,t11-CLA (83%), t10,c12-CLA (7.3%) 3 months	Urinary 8-iso-prostaglandin F2a (marker of non-enzymatic lipid peroxidation) and 15-keto-dihydroprostaglandin F2a (marker of enzymatic lipid peroxidation and systemic inflammation) levels were increased in CLA group.
(Riserus *et al.* 2002)	60 men with metabolic syndrome were randomized in 3 groups: Controls, t10,c12-CLA, mixture CLA) (around 56 years old; BMi: 30.6 ±2.0)	3.4g/day of olive oil (placebo), purified t10,c12-CLA (76.5%) or CLA mixture (c9,t11 and t10,c12-CLA: 1/1). 3 months	t10c12 CLA markedly increased urinary 8-iso-PGF 2. (578%) and 15-keto-dihydroprostaglandin F2a and plasma C-reactive protein (110%) compared with placebo. TNFa and IL-6 plasma levels were not affected.
(Raff *et al.* 2008)	38 healthy young men were randomized in 2 groups (placebo: butter, CLA group)	CLA oil: 5.5g/day C9,t11/t10,c12-CLA: 1/1 5 weeks	CLA supplementation markedly increased urinary 8-iso-PGF 2

(Tholstrup *et al.* 2008)	75 postmenopausal women were randomized in 3 groups: controls (olive oil), mixture CLA group, c9,t11-CLA group. BMi around 25.	Supplementation: 5.5g/day Mixture CLA group: 4.6g/day (c9,t11/t10,c12-CLA: 1/1) c9,t11-CLA group: 5.1g/day (85%) and t10,c12-CLA (7%) 16 weeks	MCP-1 and IL-6 plasma levels were stable. Plasma concentrations of CRP were increased only in CLA mix groups. Urinary 8-iso-PGF2a concentration was increased more in CLA mix group than in c9,t11-CLA group.
(Smedman *et al.* 2004)	60 men and women were randomized in 3 groups: control group without supplementation, cyclooxygenase 2 (COX-2) inhibitor group (rofecoxib) and a-tocopherol group. The 3 groups were subsequently randomized to take CLA mix or purified t10,c12-CLA	CLA mix (c9,t11/t10,c12: 1/1): 3.4g.day Purified t10,c12-CLA: 4g/day 4 weeks	Plasma and urinary concentration of 8-iso-PGF 2 and 15-keto-dihydroprostaglandin F2a were increased after CLA administration, with a significant larger increase in the t10,c12-CLA group. Only the supplementation with Cox-2 inhibitor counteracted the increase in plasma isoprostane level and 15-keto-dihydroprostaglandin F2a
(Smit *et al.* 2011)	25 men and 36 women study in double-blind, randomized, multiple cross over trial with 3 consecutive periods of 21 days. Control (oleic acid), industrial trans fatty acid then CLA.	Oleic sunflower oil, C18:1trans and CLA oil: (c9,t11/t10,c12: 6.9/1.5): 7% total energy of diet Lipids were incorporated in margarine and yogurt drinks 3 weeks	CLA and industrial trans fatty acid supplementation increased urinary 8-iso-PGF 2 level by + 170% and + 5%, respectively.
(Kim *et al.* 2011)	29 healthy overweight/obese participants (2 men, 27 women) were randomized in 2 groups	CLA: 2.4g/day C9/t11/t10,c12: 1/1 8 weeks	No effect of CLA on lipid peroxidation (plasma total radical-trapping antioxidant potential (TRAP) and antioxidant metabolism

(Turpeinen *et al.* 2008)	A randomised, placebo-controlled study, 40 subjects (20–46 years) with diagnosed birch pollen allergy received. The supplementation began 8 weeks before the birch pollen season and continued throughout the season.	2 g CLA/d in capsules, 65·3 % c9,t11-CLA and 8·5 % t10,c12 -CLA (n 20), or placebo (high-oleic acid sunflower-seed oil) (n 20) 12 weeks	Total plasma IgE and birch-specific IgE concentrations did not differ between groups, whereas plasma IgA, granulocyte macrophage colony-stimulating factor and eosinophil-derived neurotoxin concentrations were lower after CLA supplementation. Urinary excretion of 8-iso-PGF 2a and 15-keto-dihydro-PGF 2a , a primary PGF 2a metabolite increased in the CLA group.
(Pfeuffer *et al.* 2011)	85 overweight men (aged 45-68 years, body mass index 25-35 kg/m^2) were randomized to receive the CLA isomeric mixture, safflower oil, heated safflower oil, or olive oil in double-blind study.	CLA: 4.5 g/day CLA isomeric mixture (c9,t11/t10,c12:1/1) 4 weeks	The concentrations of the F(2)-isoprostane 8-iso-prostaglandin F (PGF)(2α) were increased in urine. Increased F(2)-isoprostane concentrations in this context may not indicate increased oxidative stress.
(Joseph *et al.* 2011)	A double-blinded, 3-phase crossover trial was conducted in 36 overweight (BMI: 25 kg/m2), borderline hypercholesterolemic [LDL-cholesterol (C): 2.5 mmol/L] men aged 18–60 y. During three 8-wk phases, each separated by a 4-wk washout period, 27 participants consumed under supervision in random order 3.5 g/d of safflower oil (control), a 50:50 mixture of trans 10, cis 12 and cis 9, trans 11 (c9, t11) CLA:Clarinol G-80, and c9, t11 isomer:c9, t11 CLA	Control: 3.5 g/d of safflower oil, 3.5 g/d of a 50:50 mixture of t10, c12 and c9, t11 CLA oil (containing 2.8 g of total CLA), 3.5 g/d of c9,t11 CLA (c9, t11 CLA oil, containing 2.7 g of total CLA) 8 weeks	CLA supplementation for 8 wk did not modify plasma Ox-LDL, suggesting that CLA supplementation does not affect oxidative status in men.