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Review

Conjugated linoleic acids (CLAs) and white adipose tissue: how both *in vitro* and *in vivo* studies tell the story of a relationship

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Summary. The distribution of adipose tissue in mammals is dependent on genetic and environmental factors, and in health the fundamental role of adipocytes is to store triacylglycerol during energetic excess and to mobilize this reserve during energy expenditure or reduced food intake. This requires an accurate balance, which is maintained through the interactions of several regulatory factors, as well as dietary manipulations. Dietary supplementation with CLAs (conjugated linoleic acids) is regarded as promising in many mammalian species for obtaining good body mass repartition and diminution of fat depots.

CLAs are a group of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid, naturally present in foods originating from ruminant species, and normally present in human adipose tissue. CLAs can, however, also be obtained as commercial supplements, usually containing synthetically prepared isomeric mixtures, and as dietary supplements CLAs are widely used by obese people, above all in the USA and Europe. CLAs are claimed to have protective effects against human degenerative pathologies, such as cancer, atherosclerosis, and diabetes, as well as showing beneficial effects on immune functions and food and energy intakes. The mechanisms of action of CLAs are not fully clarified at present, because *in vitro* and *in vivo* studies are not always in agreement, and possibly because CLAs act in different ways and with different consequences when administered in the diet to different species. The present review summarizes the ascertained mechanisms of action of CLAs, the mammalian species of major interest in which important studies have been conducted, and the future prospects for the use of CLAs in both humans and food animal species.

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The following topics will be discussed, taking evidence from both *in vitro* and *in vivo* studies, to provide a possible rationale for the therapeutic or dietary utilization of CLAs: decreased energy/food intake, increased energy expenditure, decreased pre-adipocyte differentiation and proliferation, and increased apoptosis of adipocytes. All of these parameters, in turn, affect decreased lipogenesis and increased lipolysis.

For the future, interactions with individual hormonal substrates, changes in gene expression of proteins involved in lipid metabolism, and anti-tumorigenic effects will possibly constitute areas for scientific development and deepening of knowledge of dietary CLAs.

Key words: Conjugated linoleic acids, Adipose tissue, Adipocytes, Pre-adipocytes, Histometry

Introduction

The distribution of adipose tissue in mammals depends on both genetic and environmental factors. The bodily total and regional distribution of white adipose tissue are usually gender-related, and depend on the number of adipocytes as well as their metabolic status and degree of filling with depot fat. In healthy individuals, the primary role of adipocytes is to store triacylglycerol during periods of caloric excess, and to mobilize this reserve when expenditure exceeds intake. Mature adipocytes are uniquely equipped to perform these functions. They possess the full complement of enzymes and regulatory proteins needed to carry out both lipolysis and *de novo* lipogenesis.

Growth and development are greater during the foetal and neonatal periods than at any other time in an animal's life, and correct nutrient partitioning during the rapid growth phases may be the key to achieving a favourable mammalian body composition. This is true for both humans and food animal species.

During the lifetime of an animal, the intricate regulation of adipocyte development and acquired functions is a result of the actions of many regulatory factors, including hormones, growth factors, modulators of inflammation, and transcription of various genes. Dietary manipulation may alter the activity of some of these factors, thus altering adipose tissue development and/or localization (Sugano et al., 2001; Cameron-Smith et al., 2003).

The object of this review is to indicate which dietary manipulations might alter the development of white adipose tissue, so that favourable consequences could be detected both in food animal species and in humans who eat them. As the rationale of dietary manipulations is often derived from *in vitro* studies, research referring to cultured adipocytes has also been considered. Conjugated linoleic acids (CLAs) have received unique attention in these respects, on the basis of their potential to produce effects on mammalian body mass repartition (Sugano et al., 2001; Belury, 2002; Brown and McIntosh, 2003; Noci et al., 2005). As CLAs are fatty acids, and their natural target is the adipose tissue, special attention has been paid in this review to those morpho-functional aspects of white adipose tissue cells, and possible changes in them, relating to dietary manipulations, which may lead to important functional interpretations of the metabolic level of fat depots. Possible effects on the structure of white adipose tissue have also been examined, because these could be indicators, together with other evidence, of the safety of CLA dietary manipulation.

What are CLAs?

Conjugated linoleic acids (CLAs) are a group of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid. CLAs are produced naturally in the rumen of ruminant mammals by symbiotic fermentative bacteria, which transform linoleic acid, obtained from plants, into CLAs. Once synthesized, CLAs are either absorbed or further metabolised. Mahfouz et al. (1980) and Pollard et al. (1980) described desaturation of *trans* monoenes to *cis, trans* 18:2 derivatives by Δ-9 desaturase. *Trans*11 18:1 (*trans*-vaccenic acid, TVA) is the predominant *trans* monoene in ruminant fat, which is formed by incomplete biohydrogenation of dietary fatty acids in the rumen (Noble et al., 1974). The most abundant isomer of CLAs in natural foods (ruminant meat, particularly lamb, and dairy products) is *cis*-9, *trans*-11 (*c*9, *t*11, also called rumenic acid) (Ha et al., 1987; Kramer et al., 1998; Ma et al., 1999), and humans who eat ruminant products store CLAs in adipose tissue, possibly in a gender-related manner (Jiang et al., 1999).

Research on the biological functions and putative health benefits of CLA dates back to the 1980s, when Ha et al. (1987) made the fundamental observation that CLA mixtures, isolated from either grilled beef or produced by base-catalysed isomerisation of linoleic acid, inhibited chemically induced skin neoplasia in mice. This discovery led to many studies that emphasised the protective effects of CLAs against different cancers, atherosclerosis and hypertension, and diabetes, as well as their beneficial effects on immune functions, weight gain, feed efficiency and body composition (Ha et al., 1987; Chin et al., 1992; Smedman and Vessby, 2001; Belury, 2002; Inoue et al., 2004; Lock et al., 2004; McLeod et al., 2004; Corino et al., 2005; Miglietta et al., 2005).

Reported effects of dietary CLAs on feed intake have not been consistent, and show important species-related differences. A diminished feed intake, only demonstrable in some species and in some trials, and possibly resulting from a slight organoleptic effect of CLAs, is not now regarded as sufficient *per se* for producing a significant reduction in fat mass (House et al., 2005a). *In vitro* studies (Park et al., 1997; Evans et al., 2002) have analysed the effects of CLA on energy metabolism, showing that the *t*10, *c*12 CLA isomer increased fatty acid oxidation in 3T3-L1 adipocytes, thereby suggesting a putative mechanism by which it lowers triglyceride content. Similar effects on rat fat depots have also been reported *in vivo* (Sergiel et al., 2001). The anti-obesity, anti-atherogenic, and anti-diabetic effects of CLA, as well as a reportedly better balance between bodily fat and muscle, obtained as a result of their dietary administration, are supported by studies in animals (Park et al., 1997; Yamasaki et al., 2003; Bhattacharya et al., 2005). This has led in turn to the widespread use of dietary CLA in the United States and Europe, especially among obese human individuals, often without a stated rationale for dietary intervention (House et al., 2005a).

Commercial CLA supplements are synthetically prepared isomeric mixtures obtained from linoleic acid-enriched vegetable oils, usually containing equal amounts of the two predominant isomers, *cis*-9, *trans*-11 (c9, t11) and *trans*-10, *cis*-12 (*t*10, *c*12). There are several indications that various isoforms might have different biological actions (Gaullier et al., 2002; Martin and Valeille, 2002; Smedman et al., 2004; Thijssen et al., 2005). The *c*9, *t*11-isomer was implicated as the active form responsible for the protective effects against tumorigenesis (Ha et al., 1987; Ip et al., 1999; Pariza et al., 2001; Lock et al., 2004). The *t*10, *c*12-isomer seems to be the active form that affects energy metabolism and body fat deposition and composition (Park et al., 1999; Pariza et al., 2001; Ryder et al., 2001; Riserus et al., 2002). Thijssen et al. (2005) observed that both CLA isomers are responsible for enhanced plasma fatty profiles, but have different effects on plasma desaturation indices.

CLAs show a possible anti-obesity effect in animals, especially in mice (Blankson et al., 2000; Sisk et al., 2001; Smedman and Vessby, 2001). However, the effects of CLAs on body weight and composition in humans (Berven et al., 2000; Blankson et al., 2000; Haugen and Alexander, 2004), and their long-term maintenance, are

heterogeneous and less significant than in animal models, in part because an optimal dose has not yet been clearly defined for humans (House et al., 2005a). Recently it has been proposed that a distinction between overweight and obesity is essential when considering the problem of weight reduction in humans, with major long-term effects being easier to achieve in the former (Malpuech-Brugere et al., 2004; Gaullier et al., 2005). Recent reports confirm that dietary CLA may cause adverse metabolic effects on human carbohydrate and lipid metabolism (Brown and McIntosh, 2003; Haugen and Alexander, 2004; Terpstra, 2004; Bhattacharya et al., 2005; Poirier et al., 2005). For this reason, research is necessary on the mechanisms of action of CLAs on various target tissues, and possibly also comparative studies on different species.

CLAs reduce fat deposition in animals

Numerous data demonstrate that dietary CLAs modulate body composition, reducing the accumulation of adipose tissue in several mammalian species (House et al., 2005a), although there are species- and breed-specific differences. Park et al. (1997) demonstrated for the first time the adipose-mass lowering effect of CLAs in post-weanling growing mice (6 weeks old) that were fed a diet containing 0.5% CLA for 28-32 days. In this experiment, adipose tissue mass was reduced by over 50% compared with mice fed a control diet without CLA. Marked reductions in white fat deposition were also observed in the same species by West et al. (1998), and Takahashi et al. (2002). In a long-term study, the diet of female mice was supplemented with 1.0% CLA for 5 months, and the results showed a marked decrease of subcutaneous white adipose tissue (Tsuboyama-Kasaoka et al., 2000). Further work by Park and Pariza (2001) demonstrated that the dietary CLA reduction of adiposity could be sustained in mice even after CLA was removed from the diet. The mouse appears to be the mammalian species in which dietary CLA is most effective in fat mass reduction (Pariza et al., 2001); this is consistent among different strains of mice.

In Sprague-Dawley rats, the inclusion of 1.5% CLA in the diet for 3 weeks reduced the weight of the white adipose tissue depots (Yamasaki et al., 2003), but the reduction was not so striking as in mice. In addition, supplementation with 0.5% CLA for 5 weeks reduced fat pad weights in lean rats, but increased fat pad weights in obese Zucker rats (Sisk et al., 2001), indicating a possible interaction between rat genotype (which sustains hyperphagia in obese animals) and CLA. Recent data (Akahoshi et al., 2004) show that different dietary protein sources in rats fed CLA affected the delipidative effects of CLAs, showing the importance of interactions between dietary proteins and lipids.

Pigs have also been studied frequently, because of their importance as a food animal. Dugan et al. (1997) demonstrated for the first time in this species that dietary CLA diminished fat depots, thus favouring lean mass. Ostrowska et al. (1999) demonstrated that fat tissue deposition was reduced in a linear fashion as the amount of dietary CLA was increased in the diet of growing pigs. Dietary CLA decreased body fat accumulation in a dose-dependent manner in pigs experimentally fed for 8 weeks (Ostrowska et al., 2003). Corino et al. (2003) showed that dietary CLA supplementation in heavy pigs at either 0.25 or 0.5% (as-fed basis) caused a lower back-fat thickness than in control animals, whereas other tissue depots were not influenced by these treatments. It is conceivable that in the same species, the observed heterogeneous responses to dietary CLA supplementation in different trials may be attributed to different ages of the animals studied, and to the examination of different adipose tissue depots.

In another food animal species, the rabbit, feeding male and female New Zealand White individuals with either 0.25 or 0.5% of a CLA preparation reduced perirenal fat weights at slaughtering (Corino et al., 2002) without demonstrable gender-related differences, whereas Poulos et al. (2001) showed that male rat pups are more responsive to dietary CLA, resulting in reduced adipose and increased muscle masses compared with female pups.

In humans, dietary CLAs have been shown to reduce body fat mass, but not body mass index (BMI) (Malpuech-Brugere et al., 2004; Terpstra, 2004).

Mechanisms through which CLAs may influence adipose tissue depots

CLAs reduce fat-cell size

An increase in fat-cell size is likely to result in an increase of total body fat, while a reduction in the body fat mass may be expected as a consequence of a reduction in the size of adipose cells in the adipose tissue depots (Belury, 2002). In fact, several studies seem to attribute major importance to a decreased size of adipocytes, rather than a reduction in their number, as the basis for the reduction of total body fat mass as a consequence of CLA treatment. An *in vitro* study demonstrated that post-confluent cultures of 3T3-L1 pre-adipocytes supplemented with CLA had less triglyceride content and smaller cell size than cultures supplemented with similar amounts of linoleic acid (Evans et al., 2000). In agreement with this, Brown et al. (2001a) reported that post-confluent cultures of 3T3-L1 pre-adipocytes, which were treated with the *t*10, *c*12 CLA isomer during the first 6 days of differentiation, showed a decreased triglyceride content and reduced adipocyte size. The effects of the *t*10, *c*12 isomer were more evident than those of a crude mixture of CLA isomers (Evans et al., 2000; Brown et al., 2001b).

In an *in vivo* study after dietary supplementation with CLAs, Tsuboyama-Kasaoka et al. (2000) reported that adipose tissue depots from female mice fed 1% CLA had more small adipocytes and fewer large adipocytes than in control animals. In Sprague-Dawley

rats, dietary CLA reduced fat-pad weight in visceral adipose tissue sites, due to decreased size of the adipocytes rather than a decrease in their number (Azain et al., 2000; Poulos et al., 2001). Feeding 0.5% CLA reduced fat-pad weight due to decreased adipocyte size in lean male and female Zucker rats, but increased fat-pad weight in obese male and female Zucker rats, which paralleled the CLA-induced increase in the size of adipocytes (Sisk et al., 2001). In rabbits fed 0.5% CLAs (plus vitamin E), Di Giancamillo et al. (2002) found larger adipocytes sizes than in controls. The effects of dietary CLA in finishing pigs have been reviewed by Azain (2003), who emphasised that variations in body fat depots may account for differences in responsiveness. In heavy pigs whose diet was supplemented with 0.75% CLA for 3 months, Corino et al. (2005) recently found smaller adipocyte size than in controls, but did not observe any difference in size or number of adipocyte lipid droplets between control and CLA-treated adipose tissue cells. This observation indicates that CLA did not affect the normal mechanism of droplet size increase in pig adipose tissue cells. The same authors reported that structural and cytological details of adipocytes are not altered by dietary CLA; this is important for assessing the safety of the treatment for heavy pigs, both *per se* and as an animal model extensively used for studying many biological and medical problems.

CLAs alter pre-adipocyte proliferation

When energy intake is abundant and exceeds immediate requirements, fat depots usually increase, with an increase in the number of mature adipocytes contained in them. Differentiated adipocytes do not proliferate, but pre-adipocytes can proliferate and have the potential to differentiate into mature adipocytes, filling their cytoplasm with lipid. The result is visible as expanded fat depots.

Several reports (see Table 1) have documented, above all by in vitro studies, the anti-proliferative effects of CLAs on pre-adipocytes, which may possibly impede the expansion of fat depots (McNeel et al., 2003; Azain, 2004). The *t*10, *c*12 CLA isomer is considered responsible for this effect (Satory and Smith, 1999; Evans et al., 2000). Culturing 3T3-L1 pre-adipocytes is one of the few well-characterised model systems available to study *in vitro* proliferation and differentiation of adipose cells (MacDougald and Lane, 1995). Some authors (Brodie et al., 1999; Satory and Smith, 1999; Evans et al., 2000) observed that CLA decreased proliferation of 3T3-L1 pre-adipocytes in a dose-dependent manner (10 to 50 micromol/L CLA), and Brandebourg and Hu (2005) have recently obtained similar results in pig pre-adipocytes. On the other hand, Ding et al. (2000) and McNeel and Mersmann (2001) were not able to demonstrate a reduction in cell proliferation (and consequent differentiation) in cultured porcine pre-adipocytes treated with CLA. Poulos et al. (2001) did not observe any adipocyte proliferation after administration of dietary CLAs in rats, whereas recent studies conducted in heavy pigs report that dietary CLA inhibits the proliferation of pre-adipocytes compared to control animals (Corino et al., 2005).

There is no doubt that in trying to explain these discordant results, differences among species must be considered, as well as the sometimes profound differences between culture conditions and *in vivo* experiments, even when using cells of the same origin (porcine).

CLAs alter pre-adipocyte differentiation

Pre-adipocyte differentiation is mediated by a series of programmed changes in gene expression of adipocyte-specific proteins such as lipoprotein lipase (LPL) (Cornelius et al., 1994), and is regulated by transcription factors such as CCAATT/enhancer binding protein-α (C/EBP-α) and peroxisome proliferator activated receptor-γ (PPAR-γ) (MacDougald and Lane, 1995; Shao and Lazar, 1997; Choi et al., 2000; McNeel et al., 2003; Azain, 2004; Granlund et al., 2005). PPAR-γ is expressed in porcine adipose tissue (Houseknecht et

Table 1. Summary of the effects of dietary CLA treatment on pre-adipocyte proliferation.

REFERENCE	Substrates (*in vitro* / *in vivo*)	CLA isomers
Decrease in adipose tissue cell proliferation		
Brodie et al., 1999	3T3-L1 pre-adipocytes	mixture
Satory and Smith, 1999	Human pre-adipocytes	mixture
Evans et al., 2002	3T3-L1 pre-adipocytes	*t*10, *c*12
Brandebourg and Hu, 2005	Pig pre-adipocytes	*t*10, *c*12
Corino et al., 2005	Heavy pig adipose tissue	mixture
No influence on adipose tissue cell proliferation		
Ding et al., 2000	Porcine pre-adipocytes	*c*9, *t*11
McNeel and Mersmann, 2001	Porcine pre-adipocytes	*c*9, *t*11 *t*10, *c*12 mixture
Poulos et al., 2001	Rat adipose tissue	mixture

al., 1998a,b; Spurlock et al., 2000; Ding et al., 2001). The CLA isomers are ligands for PPAR-γ (Ding et al., 2000; Belury, 2002; McNeel et al., 2003), and have been shown to affect adipose tissue development and gene expression in vitro. Brandebourg and Hu (2005) have recently observed that the *t*10, *c*12 CLA isomer inhibits porcine pre-adipocyte differentiation by a mechanism that involves the downregulation of PPAR-γ mRNA. A dose-dependent decrease in 3T3-L1 pre-adipocyte differentiation was observed in 3T3-L1 cells treated with either *c*9, *t*11-CLA (Brodie et al., 1999), or *t*10, *c*12-CLA, or a mixture of CLA isomers (Evans et al., 2000). Treatment of human pre-adipocytes with *t*10, *c*12-CLA also decreased differentiation (Brown et al., 2001b; McNeel et al., 2003), whereas CLA treatment of porcine adipocytes shows the opposite effect on PPAR-γ or no effect at all (Ding et al., 2000; McNeel and Mersman, 2001). Satory and Smith (1999) observed a dose-dependent increase in lipid synthesis and a consequent increase in cell differentiation when 3T3-L1 pre-adipocytes were treated with a mixture of CLA isomers. At present, there is no clear explanation for these differences in results, and thus the effects of CLAs on pre-adipocyte differentiation obtained by *in vitro* studies are not clearly defined; they probably depend on differences in the exact experimental conditions, the cell lines studied, and perhaps the CLA isomer used.

As pre-adipocytes differentiate, the concentrations of mRNAs for key transcription factors are expected to increase. In fact, Evans et al. (2000) observed a reduced expression of C/EBPα in 3T3-L1 pre-adipocytes after CLA supplementation. According to the studies of Brown et al. (2003) and Kang et al. (2003), CLA can suppress the expression and activity of PPAR-γ. In 3T3-L1 cells treated with *c*9, *t*11-CLA, the decreased differentiation was accompanied by decreased concentrations of PPAR-γ and C/EBPa mRNAs (Brodie et al., 1999). In addition, LPL activity was decreased in CLA-treated 3T3-L1 adipocytes (West et al., 1998; Lin et al., 2001).

In association with a decrease in LPL activity, several *in vivo* and *in vitro* studies have reported that t10, c12-CLA decreases lipogenesis (Evans et al., 2002; Brown et al., 2003, 2004; Wang and Jones, 2004). Two studies (*in vivo* in mice, Xu et al., 2003; *in vitro* with human adipocytes, Brown et al., 2001b) attribute the predominant *t*10, *c*12-CLA delipidative effect to inhibition of lipogenesis, especially during the early response (4 days) to CLA supplementation, rather than to increased lipolysis. Lin et al. (2004) have recently reported that *t*10, *c*12-CLA was a more potent inhibitor of *de novo* lipogenesis than *c*9, *t*11-CLA in the mammary gland of lactating mice. At the gene expression level, both *in vivo* and *in vitro*, there is a reduction in acetyl-CoA carboxylase (ACC) (Tsuboyama-Kasaoka et al., 2000; Kang et al., 2003; Lin et al., 2004) following treatment with *t*10, *c*12-CLA.

In porcine cells cultured in CLA-containing medium, there was either a decrease in PPAR-γ mRNA concentration at 2 days, or a decrease in LPL mRNA concentration at 5 and 7 days (Ding et al., 2001). It is conceivable that, as with the putative effects upon differentiation, the effects of CLAs on transcription factor transcripts are also not homogeneous, depending greatly on variations in culture conditions.

The differentiation of pre-adipocytes into adipocytes is regarded as an essential step to get fully functioning cells. Pre-adipocytes might be a target for inhibition of differentiation, in which CLA may cause a decrease in the number of cells potentially able to become mature adipocytes, thus indirectly diminishing bodily fat mass. In particular, the *t*10, *c*12-CLA isomer might be able to impart its delipidative activity through both metabolism and cell-cycle control. Further research will be necessary to elucidate the basis for differences between species, and to confirm *in vivo* the observations made *in vitro*.

CLAs increase apoptosis in adipocytes

Initiating apoptosis is another important process that might be associated with the reductions of fat deposition and body fat mass induced by CLA supplementation

Table 2. Summary of the effects of dietary CLA treatment on apoptosis in adipose tissue.

REFERENCE	Substrates (*in vitro* / *in vivo*)	CLA isomers
Increase in adipose tissue cell apoptosis		
Tsuboyama-Kasaoka et al., 2000	Mouse adipose tissue	mixture
Miner et al., 2001	Mouse adipose tissue	mixture
Evans et al., 2000	3T3-L1 pre-adipocytes	mixture
Hargrave et al., 2002	Mouse adipose tissue	mixture
Hargrave et al., 2004	Mouse adipose tissue	mixture
Corino et al., 2005	Heavy pig adipose tissue	mixture
No influence on adipose tissue cell apoptosis		
Brown et al., 2003	Human pre-adipocytes	*c*9, *t*11 *t*10, *c*12
Brown et al., 2004	Human pre-adipocytes	*c*9, *t*11 *t*10, *c*12

(Table 2). CLA has been reported to increase programmed cell death, or apoptosis, in adipose tissue cells of mice (Tsuboyama-Kasaoka et al., 2000; Miner et al., 2001) and in cultured pre-adipocytes (Evans et al., 2000). This effect is possibly due to the *t*10, *c*12-CLA isomer, responsible for the loss of body fat in mice, as revealed by Hargrave et al. (2002). The same CLA isomer, however, appeared to be unable to influence apoptotic rates in cultured human adipocytes (Brown et al., 2003, 2004).

In a recent *in vivo* study, Hargrave et al. (2004) hypothesized that dietary CLA in mice can induce the DNA fragmentation characteristic of apoptosis. Corino et al. (2005) have recently observed that 0.75% dietary CLA increased the number of apoptotic adipocytes in the subcutaneous adipose tissue of heavy pigs. The same authors (Corino et al., 2005) have evaluated histochemically the putative involvement of oxidative stress in the established anti-adipogenic effect of CLA, and have interestingly concluded that nitric oxide (NO) produced in adipose tissue vessels may be involved in the CLA anti-adipogenic effect (Fig. 1). In other words, CLA might act in pigs by down-regulating a nitric oxide-mediated lipolytic pathway.

The mechanism or mechanisms by which CLA causes increased apoptosis in adipocytes might be species-specific, or possibly linked to other factors, such as gender or age. One potential mechanism that leads to apoptosis of adipocytes might involve tumour necrosis factor-α (TNF-α) (Tsuboyama-Kasaoka et al., 2000). Exposure to TNF-α was shown to induce apoptosis in human adipose cells (Prins et al., 1997). Dietary CLA in female mice resulted in increases in the level of TNF-α mRNA in white adipose tissue cells (Tsuboyama-Kasaoka et al., 2000). A marked increase in the TNF-α level in the adipocytes of mice after feeding CLA was consistent with increased apoptosis rates, as measured by the DNA fragmentation assay and DNA analysis (Tsuboyama-Kasaoka et al., 2000). However, studies in male Sprague-Dawley rats (Yamasaki et al., 2003) and mice (Akahoshi et al., 2002; Bhattacharya et al., 2005) have shown decreases of serum TNF-α levels and bodily fat deposition after dietary supplementation with 1.0-1.5% of CLA. More studies are undoubtedly needed to evaluate the relationships between fat deposition and TNF-a concentration in different tissue sites (and species) after CLA supplementation.

CLA modulates energy expenditure via uncoupling proteins

A further way in which CLAs may exert their effects on white adipose tissue and lipid metabolism is to enhance energy expenditure via the family of uncoupling proteins (UCPs), which have recently been thoroughly studied (Adams, 2000; Ealey et al., 2002; Rousset et al., 2004; Mostyn et al., 2005).

UCP1 is very abundant in the inner mitochondrial membrane of the multilocular adipose cells (brown adipose tissue), and is predominantly devoted to heat production, at least in small rodents (Argyropoulos and Harper, 2002; Rousset et al., 2004). UCP2 is almost ubiquitous, but is predominantly localized in white adipose cells, and UCP3 is abundantly expressed in skeletal muscle cells. Both UCP1 and UCP2 are believed to act on insulin secretion and fatty acid metabolism, acting as regulatory molecules that both indirectly affect adipose tissue depots, and limit free radical levels in cells (Adams, 2000; Rousset et al., 2004).

Dietary CLAs, specifically the *t*10, *c*12 CLA isomer, can enhance UCP2 expression in adipocytes of both white and brown adipose tissues (Tsuboyama-Kasaoka et al., 2000; Ryder et al., 2001; Ealey et al., 2002), possibly enhancing the respiratory pathways of triglycerides in this way. Even though West et al. (2000) were not able to demonstrate such an effect in murine cell lines, and such effects may be species-specific, the ability of CLAs to modulate mitochondrial uncoupling proteins appears promising for reducing fat and increasing lean mass. In fact, *in vivo* studies of rodents (Choi et al., 2004; House et al., 2005b) have recently shown a possible delipidating mechanism, linked to an increased expression of uncoupling proteins.

Conclusion and future perspectives

This review concentrates on CLA-linked effects on fat depots, body repartition between fat and lean masses, and putative mechanisms of action. Cell-culture studies demonstrate that CLAs can modify gene expression of proteins involved in adipose-cell metabolism, as well as affect functional activities of adipose tissue and, consequently, lipid metabolism. In many cases, studies have been conducted with CLA as the only lipid source

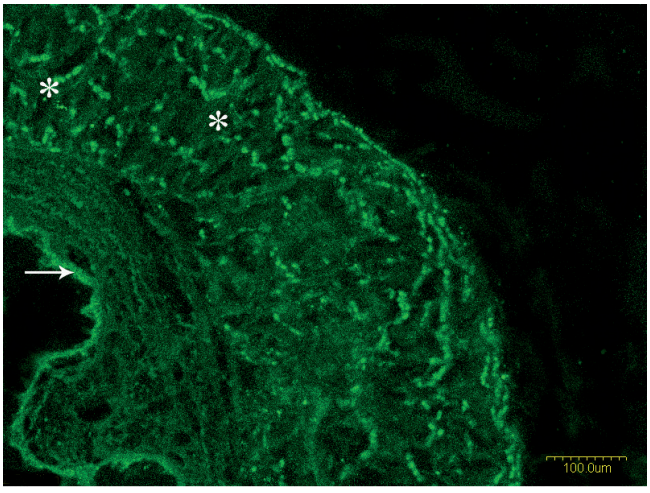

Fig. 1. Neuronal-NOS immunofluorescence in the endothelium (arrow) and in the vascular smooth muscle cells (asterisks) in a specimen of pig adipose tissue treated with dietary CLA. Scale bar: 100 μm.

in the culture media, and for this reason there is a need to perform additional work with mixed fatty acids to mimic the *in vivo* situation. *In vivo* studies produce less consistent results than cultures, both because of the different technical conditions used and the different biological substrates studied. On the whole, rodents appear strongly influenced by dietary CLA, which in these species clearly reduces adiposity. In the pig and the rabbit, fat depots are sometimes influenced by CLAs, but with differences which are possibly gender- and age-linked. In humans, CLAs appear to have an anti-overweight effect rather than an anti-obesity effect.

Mechanisms by which CLAs reduce body weight and fat accumulation are at present not fully understood, notwithstanding the large body of existing studies. However, both *in vitro* and *in vivo* studies indicate that putative mechanisms include: decreased energy/food intake, increased energy expenditure, decreased pre-adipocyte differentiation and proliferation, and increased apoptosis of adipocytes. All these factors, in turn, give rise to decreased lipogenesis and increased lipolysis. Additional *in vivo* studies on pure CLA isomers are needed to define clearly the short- and long-term effects of each individual CLA isomer (especially the *t*10, *c*12 CLA isomer), as well as possible side effects.

In addition to determining the real influence of dietary CLAs on adipose tissue, especially in humans, it is important to determine the extent of favourable modifications obtained, for potential application in the control of adiposity. In this case, it will be necessary to link CLA treatment to changes to hormonal substrates both in healthy individuals and in overweight and obese conditions. In particular, the reciprocal interactions between dietary CLA, and leptin reduction and insulin resistance, clearly need to be investigated further in different species, at both peripheral and central sites of integration. A general perturbation of lipid homeostasis also needs to be considered, such as that in mice, which provokes an excess of lipids within hepatocytes.

Apart from anti-over-weight effects, the anti-tumorigenic effects of CLAs, when applied to several cancer cell lines, appear promising for possible therapeutic use in humans.

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