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Hyperinsulinaemia triggered by dietary conjugated linoleic acid is associated with a decrease in leptin and adiponectin plasma levels and pancreatic beta cell hyperplasia in the mouse

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Abstract *Aims/hypothesis:* Dietary supplementation with conjugated linoleic acids (CLA) has a fat-reducing effect in various species, but induces severe hyperinsulinaemia and hepatic steatosis in the mouse. This study aimed to determine the causes of the deleterious effects of CLA on insulin homeostasis. *Methods:* The chronology of adipose and liver weight, hepatic triglyceride accumulation and selected blood parameters, including lipids, insulin, leptin and adiponectin, was determined in C57BL/6J female mice fed a 1% isomeric mixture of CLA for various periods of time ranging from 2 to 28 days. Insulin secretion was measured in 1-h static incubations of pancreatic islets, and pancreas morphometric parameters were determined in mice fed CLA for 28 days. *Results:* Plasma levels of leptin and adiponectin sharply decreased after 2 days of CLA feeding, although adipose tissue mass only decreased after day 6. Hyperinsulinaemia developed at day 6 and consistently worsened up to day 28, in parallel with increases in hepatic lipid content. Islets from CLA-fed mice displayed three- to four-fold increased rates of glucose-stimulated insulin secretion, both in the absence and presence of isobutyl methylxanthine or carbachol. The increased insulin-releasing capacity of islets from CLA-fed mice was explained by an increase in beta cell mass and number. *Conclusions/*

interpretation: The data suggest that CLA supplementation induces a profound reduction of leptinaemia and adiponectinaemia, followed by hyperinsulinaemia due to the increased secretory capacity of pancreatic islets, leading, in turn, to liver steatosis. These observations cast doubt on the safety of dietary supplements containing CLA.

Keywords Adipokines · Conjugated linoleic acid · Hepatic steatosis · Insulin resistance · Insulin secretion · Pancreatic beta cells

Abbreviations *c:* *cis* · CLA: conjugated linoleic acid · PPAR γ : peroxisome proliferator-activated receptor- γ *trans* · *t:* *trans*

Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometric conjugated dienoic isomers that are produced by the biological or industrial hydrogenation of linoleic acid. Dietary sources (i.e. ruminant meat and dairy products) predominantly provide the *cis*-9,*trans*-11 CLA (*c9,t11*-CLA) isomer, which is also termed rumenic acid, while commercial preparations of CLA are predominantly composed of *c9,t11*-CLA and *t10,c12*-CLA. There has been growing interest in CLA following the finding that CLA-enriched diets produce a rapid decrease in fat mass in various animal models [1]. However, CLA feeding has been reported to be associated with deleterious side effects in the mouse, including the development of insulin resistance, hyperinsulinaemia and fatty liver [2, 3]. We have previously shown that this remarkable diet-induced phenotype is strictly dependent on the *t10,c12*-CLA isomer [3].

In humans, conflicting results have been reported with regard to the fat-reducing effects of CLA-enriched diets. Of 14 randomised placebo-controlled trials published, only three reported a reduction in fat stores in CLA-treated subjects [4] (for reviews see [5, 6]). In addition, some of the adverse effects reported in the mouse, primarily impairment of insulin sensitivity, have been observed in overweight

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subjects receiving the purified *t10,c12*-CLA isomer [7]. The hyperinsulinaemic effect of CLA brings into question the safety of dietary supplements containing this fatty acid, which are widely promoted as non-prescription weight-loss agents. This is of particular concern because experimental evidence suggests that chronic hyperinsulinaemia promotes hepatic fat accumulation. Indeed, among CLA-fed mice, fatty liver specifically developed in the hyperinsulinaemic mice receiving the *t10,c12*-CLA isomer [3], and normalisation of circulating insulin levels reduced hepatic fat deposition [2]. The mechanism involved in the insulin-mediated accumulation of lipid in the liver involves the stimulation of a sterol regulatory element binding protein-1 (SREBP-1)-dependent lipogenic pathway [3], as demonstrated in lipodystrophic and genetically leptin-deficient mice with hyperinsulinaemia [8].

The phenotype of CLA-fed mice is similar to that of fatless transgenic mice [9–11]. In these models the primary defect is the engineered absence of adipose tissue; all aspects of the phenotype are partially or completely reversed by transplantation of adipose tissue [12]. The beneficial effect of the replenishment of fat stores is mediated, at least in part, through the production of leptin and adiponectin, two adipose hormones that exert an insulin-sensitising effect and reduce ectopic fat deposition [13, 14]. However, in the context of CLA supplementation, it is not clear whether hyperinsulinaemia and hepatic steatosis are secondary to adipose tissue loss. To evaluate this, we determined the chronology of the CLA-induced alteration in adipose tissue weight, liver lipid content and selected blood parameters, including plasma lipids, insulin, leptin and adiponectin. In addition, the effect of CLA supplementation on insulin secretion and pancreatic morphometric parameters was assessed to determine the mechanism underlying CLA-induced hyperinsulinaemia.

Materials and methods

Experimental animals French guidelines for the use and care of laboratory animals were followed. Ten-week-old C57BL/6J female mice were purchased from DEPRE (Saint Douillard, France) and individually housed in a controlled environment (constant temperature and humidity, with darkness from 18.00 to 08.00 hours). To explore the effects of the CLA mixture found in commercial preparations on body composition, female mice were fed an unrestricted semi-synthetic diet (UAR, Villemoisson, France) containing either 3.9% sunflower oil (control diet), or 2.9% sunflower oil plus 1% (w/w) CLA mixture (47% *c9,t10*-CLA, 48% *t10,c12*-CLA; Tonalin CLA 90%; Natural Lipids, Hovdebygda, Norway) (Table 1). The diets were freshly prepared every day. Body mass was monitored at regular intervals. To determine the time course of the effects produced by CLA, a first series of experiments was performed in mice fed unrestricted amounts of the CLA-enriched diet for 2, 4, 6, 8, 10 or 28 days. In a second series of experiments, mice fed the 1% CLA supplement for 28 days were used to investigate the effect of the diet on the pancreas.

Table 1 Composition of the two diets (g/100 g of dry powder)

Ingredients	Control diet	CLA diet
Sunflower oil	3.9	2.9
<i>c9, t11</i> -CLA	0	0.47
<i>t10, c12</i> -CLA	0	0.48
<i>c9, c11</i> -CLA	0	0.02
<i>c10, c12</i> -CLA	0	0.01
Other CLA	0	0.02
Casein	18.1	18.1
Glucose and starch	67.7	67.7
Cellulose	4.6	4.6
Mineral and vitamin mix	5.7	5.7

Isofluran-anaesthetised animals were bled by section of axillary vessels. After the mice were killed, samples of pancreatic, hepatic and peri-uterine adipose tissue were collected, weighed, snap frozen in liquid nitrogen, and stored at -80°C until use. Freshly collected pancreatic samples were used for the functional and morphometric analyses.

Isolation of islets and static incubations Pancreases were digested by intraductal injection of collagenase, and isolated islets were purified by double-hand picking, as described previously [15]. After two washes of 15 min at 37°C in Krebs buffer containing 2.8 mmol/l glucose, islets were incubated for 60 min in the presence of 2.8 or 16.7 mmol/l glucose, either alone or with 0.1 mmol/l carbachol (a cholinergic agonist) or isobutyl methylxanthine (a phosphodiesterase inhibitor), which stimulate the kinase C or cyclic AMP pathways, respectively. Incubations were performed using five islets, and each condition was run in triplicate.

Islet triglyceride assay Triglyceride content was measured in three batches of 100 islets taken from three mice, after chloroform : methanol extraction, as described previously [16]. Briefly, 100 islets were resuspended in 3 ml of chloroform/methanol (2:1) and stored overnight at 4°C under nitrogen. After the addition of 2 ml of double-distilled H_2O , the tubes were vortexed and centrifuged ($3,000\times g$, 10 min, 4°C). The upper phase was discarded and then 750 μl of double-distilled H_2O was added to the lower phase. After centrifugation ($3,000\times g$, 10 min, 4°C), the lower phase was evaporated under nitrogen. The samples were resuspended in 50 μl of chloroform/methanol (2:1), and 10 μl was used in the triglyceride assay (GPO Trinder kit; Sigma Diagnostics, St Quentin Fallavier, France).

Immunocytochemistry Pancreases from four control mice and four CLA-fed mice were weighed and fixed in a 3.7% formalin solution for 6 h at 4°C . After overnight incubation in 20% sucrose, the pancreases were frozen in isopentane. Sections 10 μm thick were sliced on a cryostat at -20°C . Beta cells were detected using a monoclonal anti-insulin antibody (1:500, clone K36aC10; Sigma-Aldrich, St Quentin Fallavier, France) revealed by incubation with an fluorescein-isothiocyanate-conjugated anti-sheep antibody (1:400; Tebu-bio, Le Perray-en-Yvelines, France).

Islet morphometric analyses The areas of interest were measured with a Leica microscope (Rueil Malmaison, France) equipped with a video camera using Metamorph image analyser software. Detection of insulin immunostaining was performed for each fixed pancreas on five sections of approximately 1.4 mm² each, separated by 200 µm. Islets were defined as insulin-positive aggregates with a diameter >25 µm and were counted to determine islet density (i.e. the number of islets per square millimetre of pancreas). The percentage islet area was calculated as the ratio of the insulin-positive area to the total area of a covered section. Islet size (in square micrometres) was determined by dividing the size of the insulin-positive surface area by the number of islets present in the area. Beta cell mass was evaluated by multiplying pancreas weight by the percentage islet area. Beta cell density was determined by counting the number of nuclei in an insulin-positive area for at least ten insulin-stained areas. The size of the individual beta cells was then determined by dividing the size of the insulin-positive area by the number of nuclei.

Biochemical assays Total hepatic lipid content was assayed by the method of Delsal [17]. Briefly, liver (0.5 g) was homogenised and extracted twice with dimethoxymethane/methanol (4:1, v/v), and then settled and filtered. The filtrate was evaporated under vacuum to remove solvent. Dried extracts were weighed to estimate total hepatic lipid content. Blood glucose (Biotrol Diagnostics, Chennevieres, France), triglycerides (Sigma Diagnostics) and NEFA (Roche Diagnostics, Basel, Switzerland) were determined by enzymatic methods. Blood insulin (CIS Bio international Gif sur Yvette, France), leptin and adiponectin (Linco, St Charles, MO, USA) were assessed by RIAs.

Statistical analysis The results are expressed as means±SEM. The significance of differences between groups was determined by the Student's *t*-test. A *p* value of less than 0.05 was considered statistically significant.

Results

Time course of CLA-induced alterations in organ weight and blood parameters Mice were fed either a normal diet or a 1% CLA diet for a period of 2, 4, 6, 10 or 28 days. Peri-uterine adipose tissue mass was decreased at day 6 of CLA feeding. Frank lipoatrophy (a 70% reduction in adipose tissue mass) and a significant reduction in body mass were observed at day 28 (Table 2). Liver mass was altered in two stages. Liver mass was increased at days 2 and 4, then it was decreased at day 6. The second increase, which was more dramatic, was observed from day 10 until day 28 and could be partly explained by lipid accumulation in the liver (Table 2). In agreement with the literature, plasma NEFA and triglyceride concentrations were unaffected during the first week of CLA supplementation but decreased to 56 and 28% of their respective initial values after 28 days [18]. In contrast, glucose levels remained unchanged during the experimental period (Table 2). Although circulating leptin levels decreased during the CLA-enriched diet, the decreases were distinct from each other in terms of kinetics and magnitude (Fig. 1a). Adiponectin levels dropped sharply at day 2 (−70%) and continued to decrease, reaching 3% of the starting level by day 10. Leptin levels decreased less abruptly and remained roughly stable at around 50% of the initial level after day 6. Plasma insulin levels started to sharply increase 6 days after the initiation of the CLA-enriched diet (Fig. 1b, Table 2), and this was followed by a substantial increase in hepatic lipid content, initially detected at day 10 (Table 2). Moreover, plasma insulin levels were positively correlated with hepatic lipid content in CLA-fed mice (Fig. 1c).

Chronic CLA supplementation enhances insulin secretion To explore whether the hyperinsulinaemia induced by CLA dietary supplementation could be accounted for by an increase in insulin secretion, pancreatic islets were isolated from mice subjected to a 1% CLA diet for 28 days. Glucose-stimulated insulin secretion was measured in 1-h static incubations. In islets from control mice, the presence of 16.7 mmol/l glucose increased insulin release by a factor of four compared with that observed in the presence of 2.8

Table 2 Time course of the effects of CLA feeding on selected body composition and blood markers in mice

Day	0	2	4	6	10	28
Body weight (g)	19.3±0.3	19.9±0.6	19.8±0.6	19.6±0.5	19.1±0.5	18.0±0.6 ^a
Relative peri-uterine adipose tissue mass (% body weight)	1.00±0.08	0.81±0.19	0.93±0.13	0.72±0.08 ^a	0.71±0.06 ^b	0.15±0.13 ^c
Relative liver mass (% body weight)	4.73±0.15	6.58±0.47 ^a	6.53±0.62 ^a	5.27±0.26	6.12±0.42 ^a	14.73±0.78 ^c
Hepatic lipid content (mg/g)	45.9±6.1	59.7±5.5	53.2±3.9	36.3±9.5	74.0±8.6 ^a	239.9±12.10 ^c
Triglycerides (mmol/l)	0.46±0.09	0.65±0.05	0.60±0.10	0.60±0.08	0.54±0.10 ^b	0.13±0.02 ^c
NEFA (mmol/l)	0.55±0.07	0.56±0.06	0.70±0.13	0.39±0.03 ^a	0.41±0.05 ^b	0.31±0.06 ^b
Glucose (g/l)	2.0±0.1	1.7±0.2	1.7±0.1	1.8±0.1	1.8±0.1	2.4±0.4
Insulin (µU/ml)	18±3	20±3	30±9	32±3 ^a	76±28 ^a	4,886±1,556 ^b

C57BL/6J mice were fed for 2, 4, 6, 8, 10 or 28 days on a normolipidic diet containing 1% CLA (g/100 g dry powder). The data are means±SEM, *n*=5

^a*p*<0.05; ^b*p*<0.01; ^c*p*<0.001

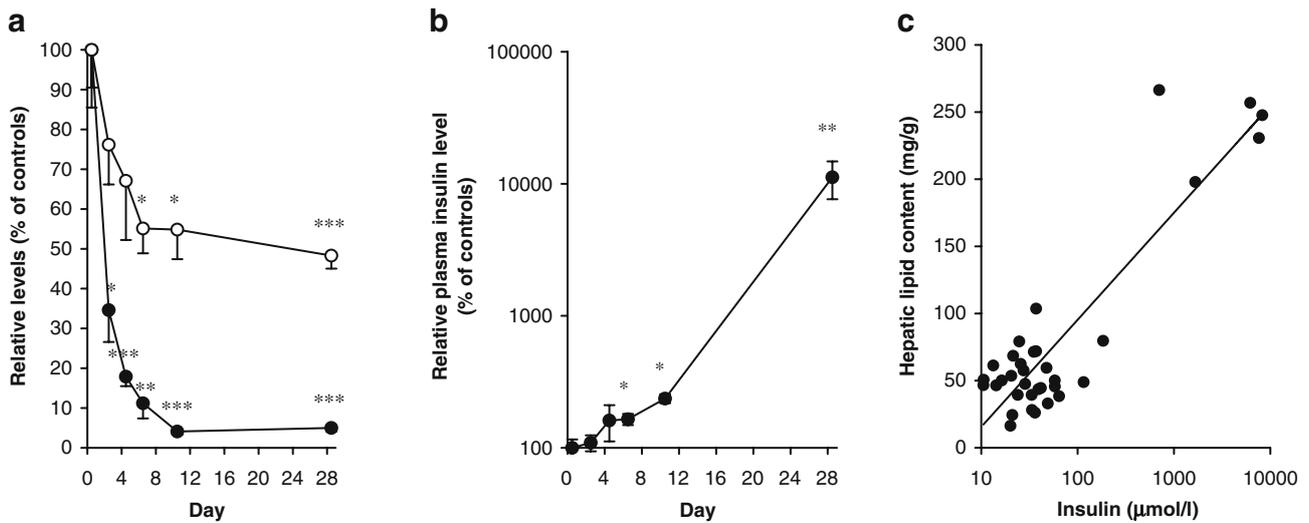


Fig. 1 Time course of the effects of CLA supplementation on body composition and selected endocrine parameters. C57Bl/6J mice were fed for 2, 4, 6, 8, 10 or 28 days on a normolipidic diet containing 1% CLA (g/100 g dry powder). **a** Plasma leptin (open circles) and

adiponectin (closed circles) levels. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. **b** Plasma insulin levels. The values shown are means \pm SEM, $n = 5$. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. **c** Correlation between relative hepatic lipid content and plasma insulin, $r = 0.82$, $p < 0.001$

mol/l and this effect was potentiated when the cyclic AMP or kinase C pathways were stimulated by isobutyl methylxanthine (14-fold increase) and carbachol (29-fold increase) respectively (Fig. 2). The rates of glucose-stimulated insulin secretion in pancreatic islets from CLA-fed mice were three

to four times higher than those in islets from mice fed a normal diet, regardless of the experimental conditions. These findings demonstrate that CLA supplementation increases the overall capacity of the islets to produce insulin and does not alter their responsiveness to glucose or other secretagogues.

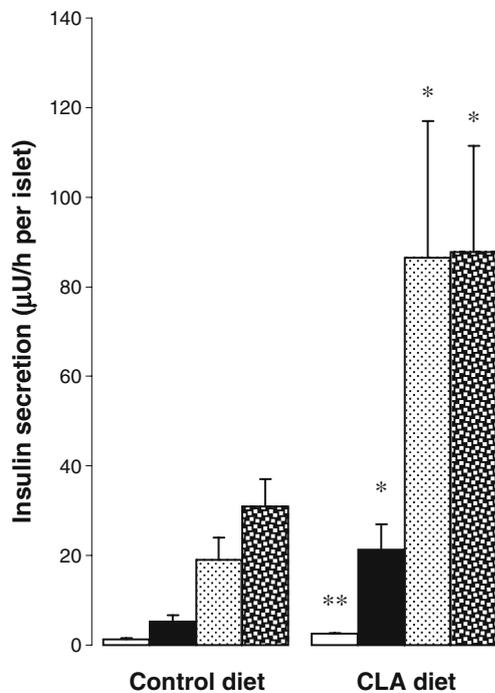


Fig. 2 Insulin secretion by isolated pancreatic islets. Insulin secretion was measured in static incubations of isolated islets from mice fed either the standard diet (control) or the CLA-enriched diet for 4 weeks. Incubations were performed in the presence of 2.8 mmol/l (open bars) or 16.7 mmol/l (black bars) of glucose alone, or 16.7 mmol/l glucose plus 0.1 mmol/l isobutyl methylxanthine (dotted bars) or carbachol (hatched bars). The data are means \pm SEM, $n = 8$. * $p < 0.05$; ** $p < 0.01$

CLA supplementation does not modify islet triglyceride content Islet triglyceride levels were determined to assess whether the changes observed in insulin secretion were caused by the ectopic accumulation of lipids in the islets of CLA-fed mice. The amounts of triglyceride were not significantly different in islets from CLA-fed and chow-fed mice (61 ± 6 vs 79 ± 32 ng per islet, $n = 3$ in each group, NS). These data were confirmed by oil-red-O labelling of islets in pancreatic sections (data not shown). Thus, the CLA diet did not significantly alter lipid homeostasis in mouse pancreatic islets.

The CLA-enriched diet increases beta cell mass and number A morphometric analysis was performed to explore whether CLA-mediated hyperinsulinaemia is caused by morphological alterations in pancreas tissue. As shown in Table 3, the pancreatic mass and the islet density (i.e. the number of islets per square millimetre of pancreatic tissue) were unchanged by CLA supplementation. In contrast, the size of individual islets was increased by a factor of 2.4. Representative micrographs showing this morphological change are provided in Fig. 3. Immunostaining for insulin demonstrated that these large islets were mainly composed of beta cells, and that beta cell mass was increased by nearly three-fold in the pancreases of CLA-fed mice (Table 3). Since increased beta cell mass is likely to be accounted for by an increased number of beta cells, we counted the number of nuclei present in an insulin-positive area to determine the beta cell number per pancreatic area. A three-

Table 3 Comparative analysis of islets and beta cell morphometric parameters in control and CLA-fed mice

Parameter	Control diet	CLA diet
Relative pancreatic mass (% body mass)	1.23±0.03	1.35±0.05
Islet area/pancreas area (%)	0.46±0.09	1.57±0.14 ^b
Islet density (number per mm ²)	0.96±0.14	1.29±0.16
Islet size (µm ²)	5,031±1,220	12,424±978 ^a
Beta cell mass (mg)	1.20±0.33	3.50±0.28 ^a
Beta cell density (nuclei per mm ²)	16,033±1,106	47,880±2,329 ^c
Beta cell size (µm ²)	30.43±2.65	33.94±2.40

Data are the means±SEM of the values for four different animals in each group

^a $p<0.05$; ^b $p<0.01$; ^c $p<0.001$

fold increase in the number of beta cells was found (Table 3), demonstrating that the CLA-dependent increase of islet size is a hyperplastic phenomenon.

Discussion

Dietary supplementation with CLA induces a complex response in the mouse, including marked changes in liver and adipose tissue weight and profound alterations in several endocrine blood parameters. In the present study we have demonstrated that the earliest response to CLA feeding is a sharp decrease in plasma adiponectin, followed by a less marked fall in plasma leptin. These alterations in circulating adipokine levels occur prior to any detectable reduction in the peri-uterine adipose tissue depots. Thus, in this model, the reduced production of leptin and adiponectin by adipose tissue is not linked to decreased adipose tissue mass, suggesting a mechanism of regulation that is independent of adipose cell size. A few factors are currently known to downregulate adiponectin and/or leptin gene expression in mature adipose cells. Cell culture experiments using mouse or human adipose cells have shown a marked and rapid (within 1 day) decrease in levels of leptin [19] and adiponectin mRNAs [20] in the presence of TNF- α . IL-6 has also

recently been shown to have a significant inhibitory effect on adiponectin mRNA levels [21]. Whether CLA interferes locally with these, or other cytokines produced by adipose tissue, to alter the expression of the genes encoding adiponectin and leptin remains to be determined. Moreover, since adiponectin is a target gene of peroxisome proliferator-activated receptor- γ (PPAR γ) [22], the CLA-mediated downregulation of this nuclear receptor [2, 23] may contribute to a decrease adiponectin level.

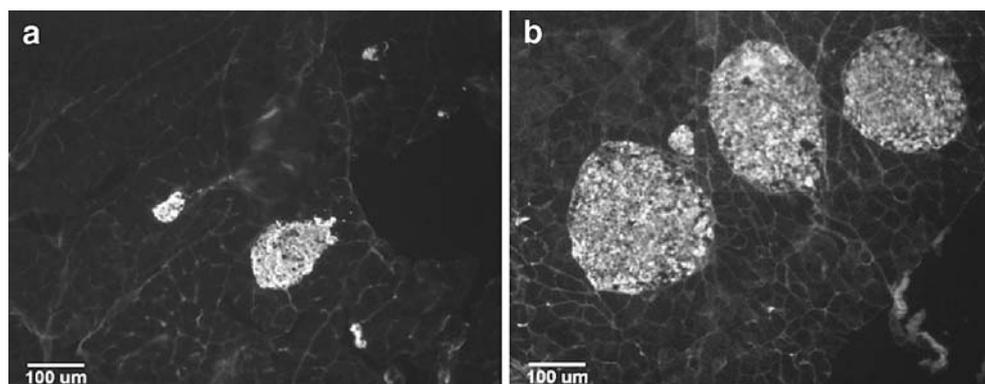
A previous study observed a similar profound reduction in plasma adiponectin in hyperinsulinaemic mice fed the purified *t*10,*c*12-CLA isomer for 28 days (3.3 ± 0.4 vs 76.7 ± 13 µg/ml in control mice, $n=8$, $p<0.001$), but not in normoinsulinaemic mice fed the *c*9,*t*11-CLA isomer (73.3 ± 17.2 µg/ml, $n=8$, NS; H. Poirier unpublished data). Circulating levels of leptin have also been observed to be specifically reduced by supplementation with *t*10,*c*12-CLA [3]. The results of the present study reveal that the marked reduction in the circulating levels of these two adipokines preceded the rise in insulinaemia in CLA-fed mice. Thus, the time course and isomer specificity of the CLA-induced decrease in adiponectin and leptin plasma levels imply a causal relationship with the development of hyperinsulinaemia, as in rodent models of lipodystrophy [13, 14]. In support of this proposal, leptin infusion reversed hyperinsulinaemia in CLA-supplemented mice [2].

Our time course study indicates that liver steatosis occurs several days after hyperinsulinaemia. Moreover, insulin levels are closely correlated with hepatic lipid content. These observations support a role for chronic hyperinsulinaemia in the promotion of hepatic fat deposition [8]. The early and marked reduction in circulating adiponectin levels could also contribute to the development of fatty liver in CLA-fed mice by reducing NEFA β -oxidation [24]. Feeding CLA to adiponectin-deficient mice should help clarify the role of this adipokine in CLA-induced hepatic triglyceride accumulation.

The present data demonstrate that, in addition to its effects on the liver and several blood parameters, dietary CLA supplementation drastically alters the capacity of pancreatic islets to secrete insulin. Islets isolated from CLA-fed mice showed a marked increase in glucose-stimulated insulin secretion and maximal insulin secretion capacity in the presence of two distinct agents known to potentiate glucose-stimulated insulin secretion. This augmentation of insulin

Fig. 3 Immunostaining of insulin in pancreatic islets from control and CLA-fed mice.

C57Bl/6J mice were fed a normolipidic diet containing 0% CLA (a) or 1% of CLA (b) for 28 days. Beta cells were stained using a monoclonal anti-insulin antibody and a fluorescein-isothiocyanate-conjugated anti-sheep antibody. Representative sections are shown from control and CLA-fed mice ($n=4$)



secretion does not rely on ectopic triglyceride accumulation in the islet, but rather on an expansion of islet mass. Indeed, the results of morphometric analysis of pancreatic tissue in this study are the first to demonstrate that CLA feeding triggers a remarkable increase in beta cell mass and number (Table 3). Moreover, the magnitude of the increase in basal, glucose-stimulated and potentiated insulin secretion in response to CLA (three- to four-fold) is similar to that of the increase in islet size (2.4-fold). This result suggests that enhanced glucose-stimulated insulin secretion in isolated islets is mainly accounted for by beta cell hyperplasia. Although the mechanism that leads to beta cell proliferation in response to CLA remains to be determined, two possibilities, which are not mutually exclusive, have been suggested by recent evidence. First, it has been shown that CLA decreases PPAR γ expression and activation in adipocytes [25, 26]. Since the targeted elimination of the PPAR γ gene in beta cells is associated with significant islet hyperplasia [27], CLA may enhance beta cell proliferation by interfering with the PPAR γ signalling pathway. Second, the hyperplastic response of beta cells may be an indirect consequence of the insulin resistance induced by CLA feeding [2, 28]. Indeed, a compensatory expansion of beta cell mass is found in insulin-resistant rodents [11, 29].

The magnitude of the increase in insulinaemia (100-fold) in mice fed CLA for 28 days greatly exceeded the three- to four-fold higher capacity of islets *ex vivo* to release insulin and the 2.4-fold increase in beta cell mass in the pancreas. This implicates reduced insulin clearance in the development of hyperinsulinaemia in response to a CLA-supplemented diet, possibly through impaired liver function secondary to fat accumulation.

Taken together, our data suggest the following sequence of events in response to dietary supplementation with a commercial mixture of CLA. The first event appears to be a marked reduction in the production of adiponectin and leptin by adipose tissue. In contrast to rodent models of lipotrophy, these changes occur with no detectable alteration of adipose tissue mass, even though they produce similar qualitative and quantitative alterations in circulating levels of the two adipose hormones. The profound reduction of leptinaemia and adiponectinaemia may favour peripheral insulin resistance and compensatory hyperinsulinaemia because of the increased islet secretory capacity. Finally, chronic hyperinsulinaemia and hypo adiponectinaemia may promote increased fat deposition in the liver, with deleterious consequences for hepatic function.

The female C57Bl/6J mice used in this study represent a highly CLA-responsive model that is particularly suitable for studying the actions and consequences of CLA on body composition and circulating parameters. However, it also constitutes a limitation in that it prevents the direct extrapolation of these results to other species. Given that CLA-mediated increases in plasma insulin levels and insulin resistance have been reported in humans [7, 30], the effect of CLA on insulin homeostasis may not be restricted to the mouse. Furthermore, if the early and profound decreases in leptin and adiponectin levels documented here in CLA-fed mice also occur in humans, this could have deleterious

consequences for whole body insulin sensitivity and favour the development of fatty liver. Indeed, a study on obese men reported that *l10,c12*-CLA supplementation induces hyperinsulinaemia, which is related to impaired insulin sensitivity and is correlated with adiponectin concentrations [31]. This raises the question of the safety of the CLA supplements used for weight management, which are easily available without medical prescription.

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