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**Insulin secretion after dietary supplementation with conjugated linoleic acids and *n-3* polyunsaturated fatty acids in normal and insulin-resistant mice**

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Running title: Dietary CLAs and *n-3* PUFAs and islet function in mice

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## Abstract

Conjugated linoleic acids (CLAs) and *n-3* polyunsaturated fatty acids (PUFAs) improve insulin sensitivity in insulin-resistant rodents. However, the effects of these fatty acids on insulin secretion are not known, but are of importance to completely understand their influence on glucose homeostasis. We therefore examined islet function after dietary supplementation consisting of 1% CLAs in combination with 1% *n-3* enriched PUFAs for 12 weeks to mice on a normal diet and to insulin-resistant mice fed a high-fat diet (58% fat). In the mice fed a normal diet, CLA/PUFA supplementation resulted in insulin resistance associated with low plasma adiponectin levels and low body fat content. Intravenous and oral glucose tolerance tests revealed a marked increase in insulin secretion, which nevertheless was insufficient to counteract the insulin resistance, resulting in glucose intolerance. In freshly isolated islets from mice fed the normal diet, both basal and glucose-stimulated insulin secretion were adaptively augmented by CLA/PUFA, and at a high glucose concentration this was accompanied by elevated glucose oxidation. In contrast, in high-fat fed mice, CLA/PUFA did not significantly affect insulin secretion, insulin resistance or glucose tolerance. It is concluded that dietary supplementation of CLA/PUFA in mice fed the normal diet augments insulin secretion, partly due to increased islet glucose oxidation, but that this augmentation is insufficient to counterbalance the induction of insulin resistance, resulting in glucose intolerance. Furthermore, the high-fat diet partly prevents the deleterious effects of CLA/PUFA but this dietary supplementation was not able to counteract high-fat diet-induced insulin resistance.

Keywords: Islets, insulin secretion, glucose oxidation, lipodystrophy, adiponectin

## Introduction

The global epidemic of obesity and type 2 diabetes has brought about the need for new dietary recommendations for the prevention of these diseases (16). Considerable interest has been focused on conjugated linoleic acids (CLAs) and *n*-3 polyunsaturated fatty acids (PUFAs), as they both show positive effects on energy metabolism in insulin resistance (11, 13, 24, 41). Several reports have shown that fish oil, which is rich in *n*-3 PUFAs, has beneficial effects on lipid and glucose metabolism (13, 15, 33, 34). For example, PUFA administration to rodents reduces lipid accumulation in peripheral tissues by suppressing lipid synthesis and up-regulating fatty acid oxidation (5, 28, 39). Likewise, CLA increases insulin sensitivity and reduces hyperglycemia, as well as body weight, in severely insulin-resistant Zucker diabetic rats (11, 35). Dietary supplementation with 1-1.5% CLA in mice has also been shown to reduce fat tissue mass suggesting that CLA may be useful as a weight-loss agent (8). However, long-term treatment with CLA in mice results in lipodystrophy and ablation of brown adipose tissue, and these findings are associated with hyperinsulinemia, liver steatosis and insulin resistance (7, 29, 36, 38). Recent studies performed in humans with type 2 diabetes, also demonstrated negative effects of CLA on insulin sensitivity and glycemic control (22, 32). Nevertheless, these adverse effects could, at least in mice, be reduced by increasing the amount of fat in the diet (7, 37). Furthermore, when CLA was given in combination with fish oil the adverse effects were reduced, or even reversed (14), supporting the notion that CLAs and *n*-3 PUFAs in combination may have positive effects on whole-body energy metabolism.

Glucose homeostasis cannot be fully understood without also understanding the islet function, since both insulin secretion and insulin sensitivity are involved (1). In particular, it is known that although insulin resistance is among the major risk factors for the development of type 2 diabetes in obesity, islet dysfunction is critical for the development of

hyperglycemia and glucose intolerance (17). It is thus important to study the influence of CLAs and PUFAs also on insulin secretion to fully appreciate the influence of dietary supplementation of these lipids on glucose metabolism. However, their effects on insulin secretion and islet function are currently not known, and were therefore examined in this study. As a model, we used female C57BL/6J mice fed a high-fat diet (58% by energy from lard), because they develop obesity, impaired glucose tolerance and insulin resistance, and thus represent a model for studying the early stages in the development of obesity and type 2 diabetes (42). We examined whether a combination of dietary CLAs and *n*-3 PUFAs could affect islet function in insulin resistance caused by the high-fat diet. As a control group we used mice fed a normal diet (11% fat by energy). It should be emphasized that we supplied fresh CLAs and *n*-3 PUFAs every day to avoid oxidation of the fatty acids.

## Material and Methods

### **Animals and study design**

Female C57BL/6J mice, weighing  $19.4 \pm 0.1$  g, were purchased from Taconic (Skensved, Denmark). The animals were maintained in a temperature-controlled room (22°C) on a 12-h light-dark cycle. The study was approved by the Animal Ethics Committee, Lund, Sweden. One week prior to the start of the CLA/PUFA supplementation, all mice were fed the normal diet (11% fat by energy, D12309, Research Diets Inc., New Brunswick, NJ). The mice were then divided into two groups, one continued on the normal diet and the other was given a high-fat diet (58% fat, D12310, Research Diets Inc.). These groups were then divided into two groups each, one group received the CLA/PUFA supplement, and the other a vegetable control fat mixture. The latter groups acted as control groups to study the effects of CLA/PUFA supplementation. The mice were fed the different diets ad libitum for 12 weeks.

Food intake was measured daily and body weight once a week. The intravenous glucose tolerance test (IVGTT) was performed after 8 weeks and the oral glucose tolerance test (OGTT) after 9 weeks in 19-22 mice from each dietary group. After 10 weeks on the diets, the body composition was determined with dual X-ray absorptiometry (DEXA) using a Lunar Piximus (Lunar, Madison, WI). Blood samples were taken from the intraorbital, retrobulbar plexus from nonfasted, anesthetized mice to measure basal plasma levels of glucose, insulin, leptin and adiponectin. Finally, after 12 weeks, the mice were sacrificed and islets were isolated for determination of islet function.

### **Preparation of diets**

The diets were supplemented with 1% CLAs and 1% PUFAs enriched in *n*-3 fatty acids. CLAs are a heterogeneous group of isomers of linoleic acid (c-9,c-12 octadecadienoic acid). Dietary CLAs are present in meat and dairy products and the major isomer in natural food is c-9,t-11, representing 73-93% of the total CLAs (19, 20). Commercial CLA preparations

usually contain a mixture of *c*-9,*t*-11 and *t*-10,*c*-12 isomers in equal amounts, and the *t*-10,*c*-12 isomer has been suggested to be the active form affecting energy metabolism and triglyceride synthesis (27, 35). The CLA preparation used in this study (Clarinol G-80, Loders Crocklaan Lipid Nutrition, Wormerveer, the Netherlands) contained 80% CLA, with equal amounts of the two isomers *c*-9,*t*-11 and *t*-10,*c*-12. The PUFA preparation was a fish oil mixture, containing 66% *n*-3 PUFAs, 4% other PUFAs, 19% monounsaturated fatty acids and 9% saturated fat (Pronova Biocare a.s., Lysaker, Norway). Fatty acids were provided both as free acids and bound in triglycerides. Control diets were supplemented with 2% vegetable oil (CIA Placebo, RP Scherer Ltd). The mice were provided with fresh food every day, in the afternoon (3-4 pm). Diets were prepared once a week, purged with nitrogen, and stored frozen in daily portions in sealed bottles to minimize oxidation of the fatty acids. This procedure, which is sometimes overlooked in experimental studies on dietary fatty acid supplementation, is important because oxidized fatty acids could induce undesirable effects. The CLA dose was chosen based on previous studies (7, 37). The PUFA dose was more difficult to determine, because we used *n*-3-enriched PUFAs, whereas previous studies have used fish oil. The PUFA preparation used in this study contains a similar amount of *n*-3 fatty acids to that found in 2-3% regular fish oil. A recent study demonstrated that 1.5-6% fish oil could decrease adverse effects, such as liver steatosis and hyperinsulinemia, caused by 1% CLA (14). Furthermore, the recommended intake of *n*-3 PUFAs for humans is between 0.2 and 1 g/day (6, 18). Thus, the *n*-3 PUFA dose (1%) used in this study is higher than the dietary recommendations for humans but within the estimated range of previous mouse studies. The mice were fed ad libitum and food not consumed within 24 h was removed and discarded.

### **IVGTT and OGTT**

In the IVGTT, 4-h-fasted mice were anesthetized with 20 mg/kg fluanison/0.8 mg/kg fentanyl (Hypnorm, Janssen, Beerse, Belgium) and 10 mg/kg midazolam (Dormicum,

Hoffman-LaRoche, Basel, Switzerland). A blood sample was drawn from the retrobulbar, intraorbital, capillary plexus, and D-glucose (1 g/kg) was injected intravenously into a tail vein (volume load 10 $\mu$ l/g). Additional blood samples were collected 1, 5, 10, 20, 50 and 75 min after the glucose injection. Following immediate centrifugation at 4°C, plasma was collected and stored at -20°C until analysis of glucose and insulin.

In the OGTT, 16-h-fasted anesthetized mice were given 150 mg D-glucose by intragastric gavage. Blood samples were collected 0, 15, 30, 60 and 120 min after glucose administration and handled as described above.

### **Islet insulin secretion and content**

Pancreatic islets were isolated by collagenase digestion and hand-picked under a microscope. Batches of freshly isolated islets were preincubated in HEPES balanced salt solution (HBSS) containing 125 mM NaCl, 5.9 mM KCl, 1.28 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 25 mM HEPES (pH 7.4), 3.3 mM glucose and 0.1% fatty-acid-free bovine albumin (Boehringer Mannheim, GmbH, Germany) for 60 min. Islets in groups of three were then incubated in 200  $\mu$ l HBSS with various glucose concentrations for 60 min at 37°C. After incubation, aliquots of 25  $\mu$ l were collected in duplicates and stored at -20°C until analysis of insulin.

For estimation of islet insulin content, batches of four islets were frozen and then sonicated in acidic ethanol (0.2 M HCl in 87.5% ethanol). The procedure was performed twice. The samples were then centrifuged and the total insulin content was measured in the supernatant.

### **Islet and liver triglyceride content**

Islets (200-300) and liver biopsies (100 mg) were homogenized in ice-cold 20 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA and 1% Triton X-100, pH 7.5. Triglycerides were extracted from the tissue homogenates with chloroform:methanol (2:1) and further processed according to Briaud et al. (4). The amount of extracted triglycerides was measured using a commercially



available kit (Infinity Triglycerides Liquid Stable Reagent, Thermo Electron, Melbourne, Australia), and the triglyceride content was correlated to the total protein, determined with the BCA Protein Assay kit (Pierce, Rockford, IL).

### **Assays of plasma samples**

Glucose was measured using the glucose oxidase method. Insulin was determined radioimmunochemically using a guinea pig anti-rat insulin antibody,  $^{125}\text{I}$ -labelled human insulin as a tracer and rat insulin as the standard (Linco Res., St Charles, MO). Plasma leptin was determined using a radioimmunoassay kit with mouse-specific anti-leptin antiserum and  $^{125}\text{I}$ -labelled mouse leptin as tracer (Linco Res.). Recombinant mouse leptin was used as the standard. Plasma adiponectin was measured using a radioimmunoassay kit with a multispecies rabbit anti-adiponectin antiserum and  $^{125}\text{I}$ -labelled murine adiponectin as tracer (Linco Res.). Recombinant adiponectin was used as the standard. Plasma triglycerides were measured using Infinity Triglycerides Liquid Stable Reagent from Thermo Electron and free fatty acids by the NEFA-C kit from Wako Chemicals GmbH (Neuss, Germany).

### **Fuel oxidation**

Islet palmitate and glucose oxidation were measured as previously described (2, 25). For palmitate oxidation, batches of 30 islets in quadruplicate were incubated in a reaction mixture consisting of 0.5 mM palmitic acid complexed to 1% fatty-acid-free bovine serum albumin, with 0.5  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]-palmitic acid (NEN, Boston, MA, specific activity 55 mCi/mmol), 0.8  $\mu\text{M}$  L-carnitine and 2.8 mM or 16.7 mM glucose. For glucose oxidation, islets were incubated with 0.1  $\mu\text{Ci}$  or 0.7  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]-glucose (NEN, specific activity 310 mCi/mmol) and 2.8 mM or 16.7 mM glucose, respectively. The reaction was terminated after 2 h and the amount of  $^{14}\text{CO}_2$  trapped with benzetonium hydroxide was determined by liquid scintillation counting.

### **Statistical analysis**

Data are presented as mean $\pm$ SE. Multiple comparisons between the different groups were performed by one-way ANOVA and Tukey's post hoc test to calculate statistical differences between the groups. Significant statistical difference was considered at  $P < 0.05$ .

Metabolic efficiency was calculated as the energy intake divided by the body weight gain. In the IVGTT, the acute insulin response (AIR) to intravenous glucose was calculated as the mean of suprabasal 1 and 5 min values, and the glucose elimination was quantified using the glucose elimination constant,  $K_G$ , calculated as the slope of the logarithmic transformation of circulating glucose between 1 and 20 min after the glucose bolus. In the OGTT, the early insulin response was defined as the increase in plasma insulin above basal at 15 min, and the glucose elimination rate was calculated between 30 and 60 min. Linear relationships were estimated using the Pearson moment correlation coefficient. The minimal modeling of glucose disappearance during IVGTT was employed to estimate the insulin sensitivity index,  $S_I$  (26). Statistical comparisons were performed with Student's unpaired and paired t-tests and, when multiple comparisons were performed, with ANOVA.

## Results

### **Food intake and body weight**

Body weight increased in mice fed the high-fat diet already after one week ( $21.6 \pm 0.3$  g vs.  $20.6 \pm 0.3$  g,  $P=0.024$ ). Addition of CLA/PUFA to the high-fat diet decreased the body weight gain during the first two weeks ( $22.6 \pm 0.2$  vs.  $21.9 \pm 0.2$  g,  $P=0.037$ ), and this difference was maintained throughout the 12-week experiment (Fig. 1A). In contrast, in the normal diet group, CLA/PUFA addition had no effect on body weight gain. The overall 12-week food intake was not significantly different between these two groups (Fig. 1B). Hence, the metabolic efficiency was reduced in mice fed the high-fat diet compared to mice on the normal diet, but CLA/PUFA had no effect (Fig. 1C).

### **Body fat content**

DEXA demonstrated a marked and significant decrease in the body fat content when CLA/PUFA was added to both the normal diet and the high-fat diet, the effect being more pronounced in the normal diet group (Table 1). In the mice fed the normal diet, the body fat content was reduced from  $19 \pm 4\%$  to  $12 \pm 2\%$  by CLA/PUFA supplementation ( $P < 0.001$ ). This was accompanied by an increase in lean body mass, resulting in a total body weight similar to that of the normal control mice (Table 1). The increase in body fat content caused by the high-fat diet was significantly reduced by CLA/PUFA, being  $23 \pm 5\%$  versus  $26 \pm 6\%$  of the total body weight ( $P < 0.01$ ; Table 1). Lean and total body weight were similar in the groups fed the high-fat diet.

### **Basal plasma parameters**

Inclusion of CLA/PUFA in the normal diet had no effect on basal plasma glucose, while the insulin levels were elevated (Table 1). In the high-fat-fed mice, both basal glucose and insulin levels were significantly elevated in the CLA/PUFA group. Taken together, these results indicate that CLA/PUFA supplementation induced insulin resistance in both diet groups.

Plasma triglycerides and free fatty acids were significantly reduced by CLA/PUFA in mice fed the normal diet, while there was no effect of CLA/PUFA on plasma lipids in high-fat-fed mice. In mice on the normal diet, both plasma leptin and adiponectin levels were significantly reduced, while in the high-fat-fed group, CLA/PUFA only reduced the adiponectin levels significantly, the leptin levels being similar to that of the high-fat-fed control mice (Table 1).

### **Intravenous glucose tolerance test**

The IVGTT was performed 8 weeks after the start of CLA/PUFA supplementation (Fig. 2). Table 1 shows the parameters obtained with model analysis. In mice fed the normal diet, the glucose elimination constant,  $K_G$ , was reduced after CLA/PUFA addition compared to the controls ( $P<0.001$ ). The AIR was, at the same time, markedly elevated ( $P<0.05$ ), while the insulin sensitivity index,  $S_I$ , was severely reduced in the CLA/PUFA-fed mice compared to control mice ( $P<0.001$ ) (Table 1). Hence, mice fed the normal diet with CLA/PUFA became glucose intolerant in spite of elevated insulin levels. In high-fat-fed mice  $K_G$ , AIR and  $S_I$  were similar in CLA/PUFA-fed mice compared to their control mice (Table 1). Hence, CLA/PUFA did not significantly affect glucose tolerance or the insulin response in high-fat-fed mice.

One-way ANOVA analysis of the insulin sensitivity data showed that mice fed the normal or high-fat diet with CLA/PUFA, as well as the high-fat control diet, had impaired insulin sensitivity compared to the normal-diet control group ( $F=12.4$ ;  $P<0.001$ ). Since adiponectin levels have previously been found to be reduced in insulin-resistant rodents, monkeys and humans (9, 10, 23), we performed a linear regression analysis between basal adiponectin levels and  $S_I$  in the different dietary groups. Adiponectin levels were found to correlate with  $S_I$  ( $r=0.436$ ,  $P=0.002$ ), supporting a relationship between the two parameters also in this model of insulin resistance.

### **Oral glucose tolerance test**

In normal mice, plasma glucose levels reached the maximum 30 min after the oral glucose challenge, after which a first-order kinetic of glucose elimination occurred until 60 min (Fig. 3A). The glucose elimination between 30 and 60 min was markedly reduced in the CLA/PUFA-fed mice, being  $4.0 \pm 0.4\%/min$  in control mice versus  $0.4 \pm 0.4\%/min$  in CLA/PUFA-fed mice ( $P < 0.001$ ), indicating severe glucose intolerance when CLAs and PUFAs were included in the diet. The 30-min insulin response to the oral glucose challenge was increased almost 3-fold, from  $4.0 \pm 0.6$  nM in controls to  $10.9 \pm 2$  nM in CLA/PUFA-fed mice (Fig. 3B), but was apparently insufficient to maintain normal glucose tolerance.

Glucose tolerance was not affected by CLA/PUFA in high-fat-fed mice (Fig. 3C and D), since glucose elimination between 30 and 60 min was  $1.4 \pm 0.3\%/min$  in control mice, and  $2.0 \pm 0.3\%/min$  in CLA/PUFA-fed mice ( $P > 0.05$ ). However, the 30-min insulin response was augmented by CLA/PUFA ingestion ( $3.5 \pm 0.4$  nM vs.  $2.1 \pm 0.2$  nM in control mice,  $P = 0.005$ ), indicating increased demand for insulin after CLA/PUFA supplementation to maintain normal glucose tolerance also in this diet group.

### **Islet insulin secretion and fuel oxidation**

In static incubation of freshly isolated islets from mice on the normal diet, both basal and glucose-stimulated insulin secretion were elevated in the CLA/PUFA group compared to the controls (Fig. 4A). In islets from high-fat-fed mice, insulin secretion was similar in the CLA/PUFA and the control group (Fig. 4B). The total islet insulin content was similar in all feeding groups (Table 1).

Fuel oxidation was measured in isolated islets from the different dietary groups. Glucose oxidation at 2.8 mM glucose was similar in all groups. At 16.7 mM glucose the oxidation of glucose was significantly increased in islets from normally fed mice given CLA/PUFA (Fig. 5A), while glucose oxidation in islets from high-fat, CLA/PUFA-fed mice

was similar to that in the control islets from high-fat-fed mice. Palmitate oxidation at the lower glucose level was elevated in islets from high-fat-fed mice compared to islets from normally fed mice, but no difference was seen after CLA/PUFA feeding in either of the dietary groups. At 16.7 mM glucose, an increased oxidation of fatty acids was observed in islets from mice fed the high-fat diet supplemented with CLA/PUFA ( $P>0.01$ ), while CLA/PUFA had no effect in islets from mice fed the normal diet (Fig. 5B).

### **Triglyceride levels in islets and liver**

In the normal-diet mice, liver triglycerides were similar in the two feeding groups, being  $124\pm 22$   $\mu\text{g}/\text{mg}$  protein in the controls and  $154\pm 62$   $\mu\text{g}/\text{mg}$  protein in the CLA/PUFA group. In high-fat-fed mice, there was a significant increase in liver triglyceride levels after CLA/PUFA supplementation ( $270\pm 70$  vs.  $114\pm 8$   $\mu\text{g}/\text{mg}$  protein in the controls,  $P=0.037$ ). Islet triglyceride levels were similar in all dietary groups (Table 1).

## Discussion

This study shows that dietary supplementations with CLAs and *n-3* PUFAs to mice with high-fat diet-induced insulin resistance had minor effects on glucose-stimulated insulin secretion and did not improve glucose tolerance. Thus, at the doses given, CLAs and PUFAs were not able to counteract the deleterious effects on islet function caused by high-fat feeding. In mice fed the normal diet, the CLA/PUFA supplementation caused elevated glucose-stimulated insulin secretion, which however was insufficient since these mice developed insulin resistance, possibly caused by the severe reduction in adipose tissue mass. These results were obtained after the evaluation of insulin secretion in the different dietary groups using both IVGTT and OGTT in combination with direct measurements of insulin secretion in freshly isolated islets. The main conclusion of the study is that islet compensation to insulin resistance is insufficient following CLA/PUFA supplementation and therefore, impaired glucose tolerance occurs.

It is well known that insulin secretion is inversely related to insulin sensitivity (1, 17), which supports that the increased insulin secretion, observed after CLA/PUFA addition to a normal diet, is an adaptive increase due to insulin resistance. Since the islet studies showed that glucose oxidation was enhanced, we suggest that this is one mechanism underlying islet adaptation to insulin resistance in this model. Recently, Poirier et al. observed hyperinsulinemia in combination with larger islets with increased  $\beta$ -cell mass in CLA-fed mice (30), indicating that CLA may alter both islet metabolism as well as  $\beta$ -cell proliferation, both being important for islet compensation in insulin resistance. However, in spite of the increased insulin secretion, glucose intolerance developed, indicating that the islet compensation was insufficient.

In islets isolated from high-fat, CLA/PUFA-fed mice, insulin secretion was similar to that in the high-fat-fed controls, and accordingly islet glucose oxidation was not altered. Since

increased accumulation of intracellular triglycerides is a phenomenon that has been shown to proceed islet dysfunction in the development of type 2 diabetes (3, 21, 31, 40), we measured islet triglyceride content. There was, however, no difference in islet triglyceride content between the dietary groups. These results indicate that CLAs and PUFAs interact with islet lipid metabolism since palmitate oxidation was elevated, both at low and high glucose concentrations, at least after high-fat feeding, although this did not result in any change in the total triglyceride content.

In our studies, 1% CLAs were given in combination with a preparation of 1% *n-3* enriched PUFAs derived from fish oil. The PUFA dose is low compared to earlier studies, which would suggest that the effects of CLA/PUFA observed in this study depend mainly on the CLA components of the dietary supplementation combination. However, it should be emphasized that we used a PUFA preparation enriched in *n-3* fatty acids, and comparisons with previous studies using fish oil with a lower proportion of *n-3* fatty acids are difficult. Therefore, further studies are required to distinguish between the effects of CLAs and PUFAs regarding islet effects.

We found that dietary supplementation with CLA/PUFA reduced adipose tissue mass in both normal and obese mice, as evidenced by DEXA. The reduction in body fat was not due to reduced food intake or increased metabolic efficiency, but rather to the direct lipodystrophic action of CLA/PUFA. It has been reported that, in mice, 1% CLAs in a normal diet resulted in a 10-fold increase in the accumulation of triglycerides in the liver, and that fish oil could prevent this adverse effect (14). In the present study, no increased accumulation of triglycerides was seen in the liver of mice after 12 weeks of normal diet with CLA/PUFA supplementation, while in the high-fat fed mice, there was a 2-fold increase in liver triglyceride content. Thus, the amount of *n-3* PUFA used in this study was sufficient to block, or in high-fat-fed mice at least partly inhibit triglyceride accumulation in the liver.



Furthermore, plasma triglyceride and free fatty acid levels were similar in all dietary groups, except in the mice fed the normal diet with CLA/PUFA, where both triglyceride and fatty acid levels were reduced, which is in agreement with an earlier study (30). It is thus possible that CLA/PUFA addition induces increased uptake and augmented lipid metabolism in peripheral tissues in mice.

We did not find any improvement in insulin sensitivity in high-fat-fed mice given CLA/PUFA. This is at variance with the improved insulin resistance observed in other rodent models of insulin resistance after CLA feeding (8, 11, 41). This can probably be explained by the different models used; particularly in regard to leptin signaling. The high-fat-fed mouse model used in this study has intact leptin signaling, whereas the severely insulin-resistant rodent models (ZDF rats, ob/ob and db/db mice) used in the studies where dietary CLAs had positive effects on glucose tolerance and insulin sensitivity, have defective leptin or leptin signaling. Although the investigation of the mechanism underlying this was beyond the scope of this study, we observed an interesting correlation between insulin sensitivity and plasma adiponectin levels. The reduction in body fat content after CLA/PUFA supplementation caused a reduction in circulating levels of adiponectin, and the significant correlation between adiponectin levels and insulin sensitivity indicates that the insulin resistance may be due to the low adiponectin levels; in fact, association between insulin resistance and low adiponectin has been demonstrated in both animals and humans (9, 12).

We conclude that dietary supplementation of CLA/PUFA augments insulin secretion in mice fed the normal diet, possibly due to elevated glucose oxidation, but that this augmentation is insufficient to counterbalance the induction of insulin resistance, which is simultaneously observed, resulting in glucose intolerance. In insulin-resistant high-fat-fed mice, CLA/PUFA addition did not improve the glucose intolerance and neither did it affect

islet function, suggesting that dietary fat prevents the deleterious effects of the CLA/PUFA supplement.

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## Figure legends

**Fig. 1.** Body weight (A), energy intake (B) and metabolic efficiency (C) in female C57BL/6J mice fed the normal diet (ND) or the high-fat diet (HFD) supplemented with 1% CLAs and 1% PUFAs or 2% control fat for 12 weeks. Metabolic efficiency was calculated by dividing the accumulated energy intake by body weight gain. The data are means $\pm$ SE from two independent 12-week experiments with 20-23 mice in each dietary group.

**Fig. 2.** Plasma levels of glucose and insulin after intravenous glucose administration: 1 g/kg glucose was injected into the tail vein in normal diet (ND) fed (A and B) and high-fat diet (HFD) fed (C and D) mice supplemented with 1% CLAs and 1% PUFAs or 2% control fat. The IVGTT was performed 8 weeks after starting the diets. The data are means $\pm$ SE from two independent experiments, n=20-23 in each dietary group. Asterisks indicate the probability of random differences between the groups: \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

**Fig. 3.** Plasma levels of glucose and insulin after oral glucose administration: 150 mg glucose was administered through a gastric tube to normal diet (ND) fed (A and B) and high-fat diet (HFD) fed (C and D) mice with 1% CLAs and 1% PUFAs or 2% control fat. The OGTT was performed 9 weeks after starting the diets. The data are means $\pm$ SE from two independent experiments, n=20-23 in each dietary group. Asterisks indicate the probability of random differences between the groups: \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

**Fig. 4.** Insulin secretion from freshly isolated islets incubated for 1 h with different glucose concentrations (3.3, 5.6, 8.3, 11.1, 16.7 and 22.2 mM glucose). Islets were isolated from A) normal diet (ND) or B) high-fat diet (HFD) fed mice with and without 1% CLAs and 1% PUFAs. The studies were performed after 12 weeks on the different diets. The results are

expressed as mean±SE of three independent experiments with n=8 for each incubation condition. Asterisks indicate the probability of random differences between the groups: \*\*P<0.01.

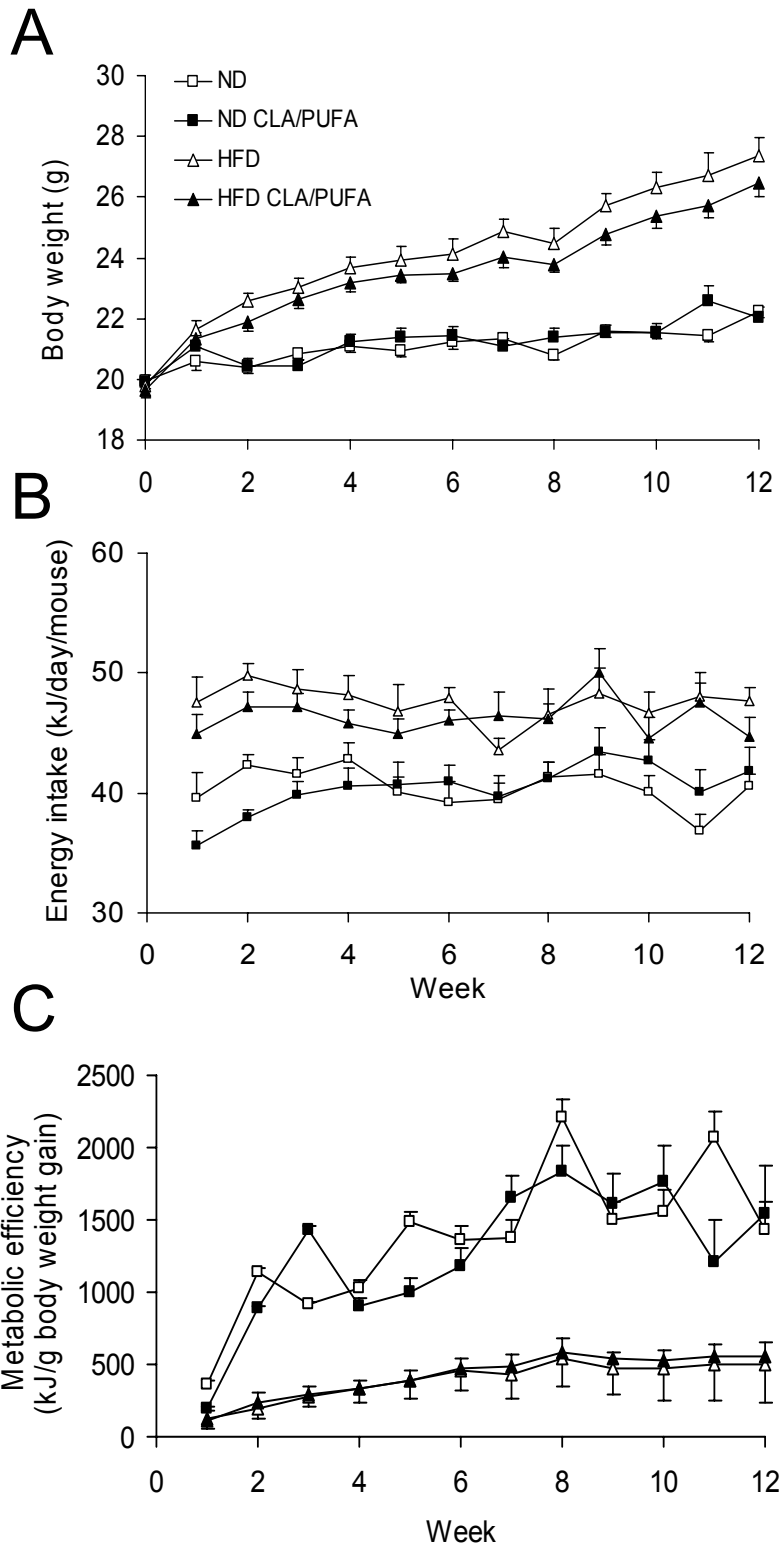
**Fig. 5.** Glucose oxidation (A) and palmitate oxidation (B) in freshly isolated islets. Batches of 30 islets were incubated for 2 h in 2.8 mM or 16.7 mM glucose together with <sup>14</sup>C-glucose for glucose oxidation determination or <sup>14</sup>C-palmitate for fat oxidation determination. Fuel oxidation is expressed as mean±SE of three independent experiments where each condition was run in quadruplicate. The studies were performed after 12 weeks on the different diets. Asterisks indicate the probability of random differences between the groups: \*P<0.05, \*\*P<0.01.

Table 1. *Effect of CLA and n-3 PUFA supplementation to normal diet (ND) fed or high-fat diet (HFD) fed mice on growth, plasma components, and islet insulin and triglyceride content. DEXA was performed after 10 weeks and acute insulin response (AIR), glucose elimination ( $K_G$ ) and insulin sensitivity ( $S_I$ ) were calculated from IVGTT performed after 8 weeks on the diets.*

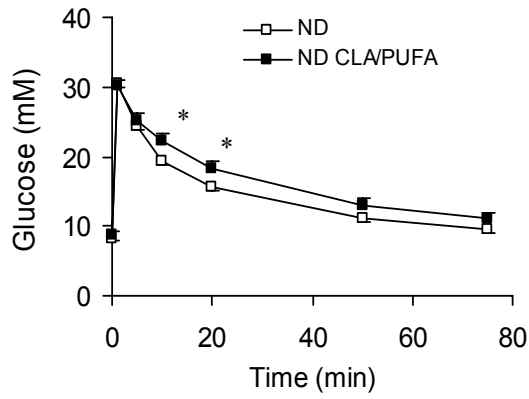
	ND	ND-CLA/PUFA	HFD	HFD-CLA/PUFA
Fat weight (g)	4.3±1.1	2.8±0.4***	7.3±2.7	6.2±1.9*
Lean weight (g)	18±1	20±2***	20±1	20±1
Total weight (g)	23±1	23±2	27±3	27±3
Body fat (%)	19±4	12±2***	26±6	23±5**
Triglyceride (mM)	0.25±0.01	0.18±0.01***	0.22±0.01	0.23±0.01
NEFA (mM)	0.54±0.04	0.43±0.03*	0.47±0.02	0.49±0.03
Glucose (mM)	9.3±0.3	8.7±0.2	9.2±0.2	10.2±0.3**
Insulin (pM)	121±22	257±62*	130±24	273±57*
Leptin (nM)	8.4±0.6	6.4±0.6*	13.0±3	13.2±1
Adiponectin (µg/ml)	16.3±1.0	1.7±0.1***	14.1±0.6	9.4±0.3***
AIR (pM)	539±53	1033±220*	495±50	333±69
$K_G$ (%/min)	3.6±0.2	2.7±0.1***	2.0±0.1	2.0±0.1
$S_I$ ( $10^{-4}$ min <sup>-1</sup> /pM)	1.5±0.2	0.48±0.08***	0.68±0.12	0.52±0.08
Islet insulin (ng/islet)	64±9	64±7	77±9	70±9
Islet TG (µg/mg prot.)	27±7	25±5	26±6	22±3

Values are means±SE, n=32-38/group for DEXA and basal blood parameters, n=19-22/group for the IVGTT results, and n=5 for islet insulin and triglyceride (TG) measurements. Two

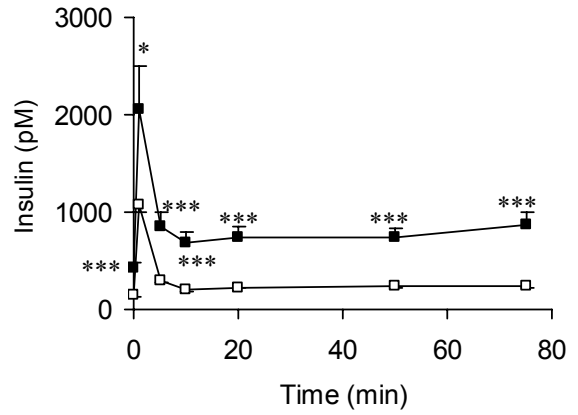
groups of mice were fed the normal diet supplemented with either 2% control fat (ND) or 1%CLAs and 1%PUFAs (ND-CLA/PUFA). The other two groups of mice were fed the high-fat diet with control fat (HFD) or CLA/PUFA supplementation (HFD-CLA/PUFA). The mice were fed the different diets for 12 weeks. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  indicate statistical differences between the CLA/PUFA group and their respective dietary control group.



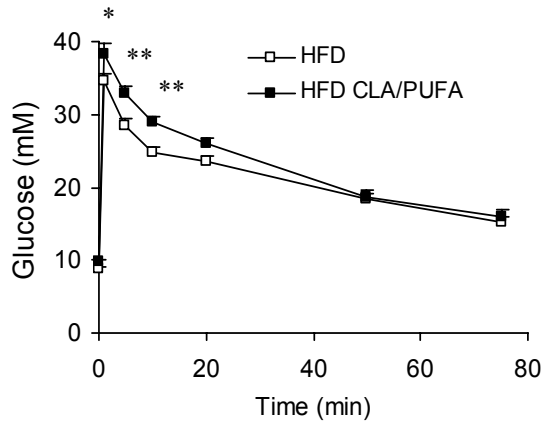
**A**



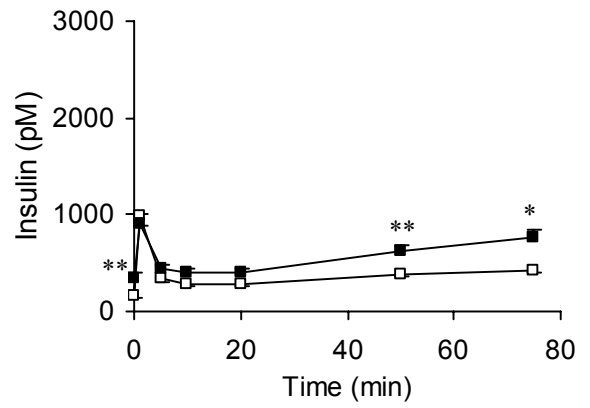
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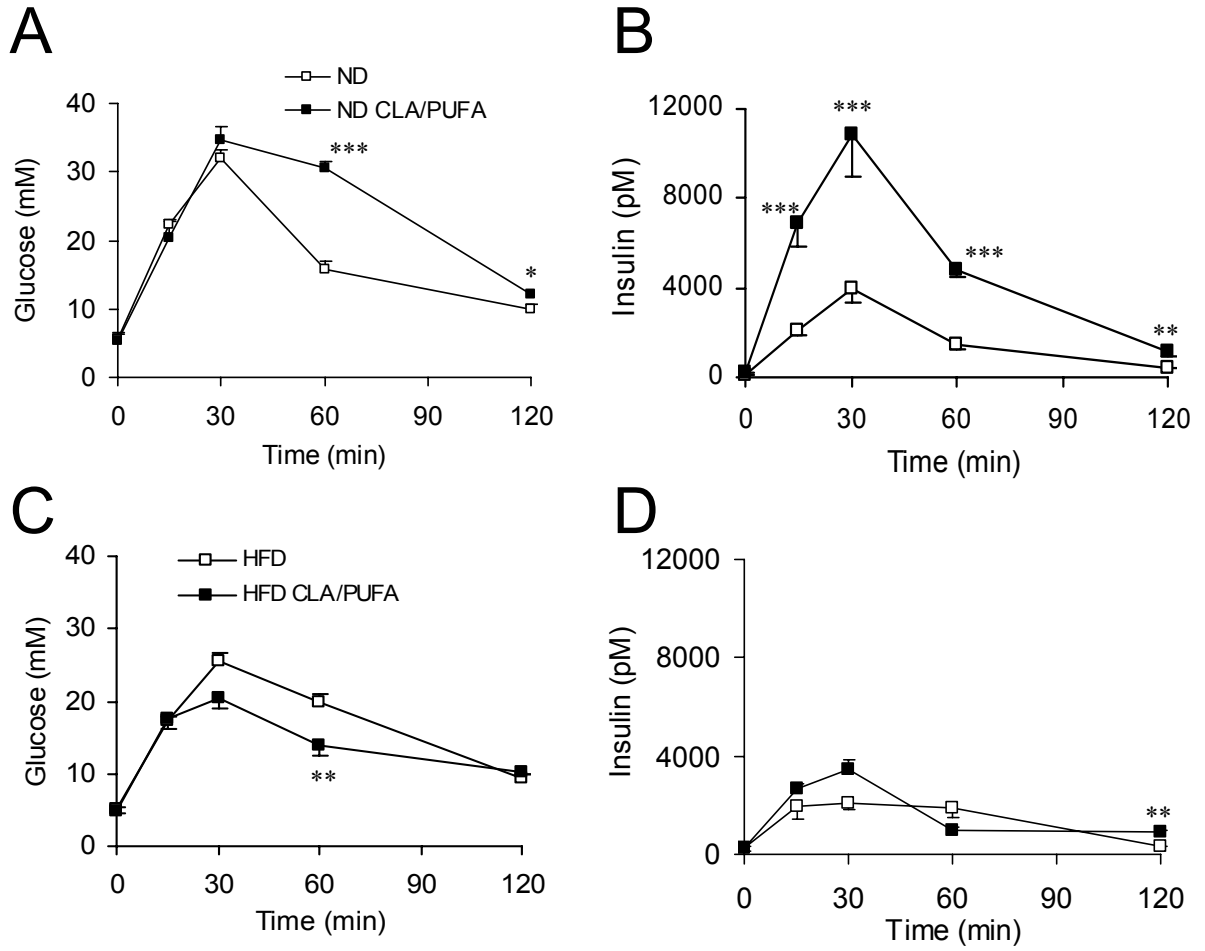


**C**

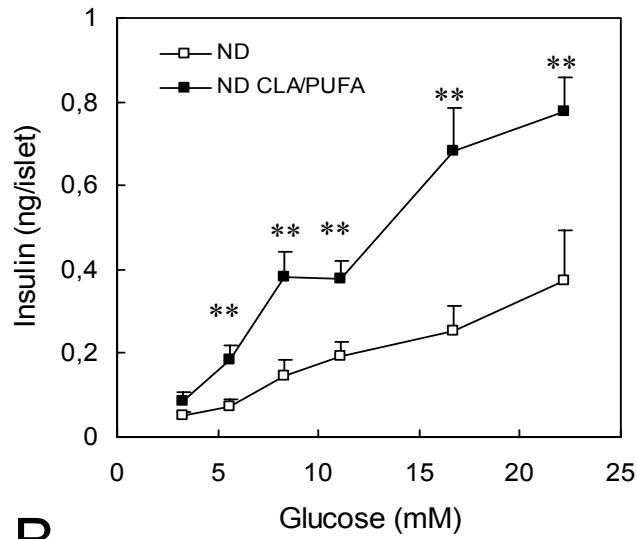
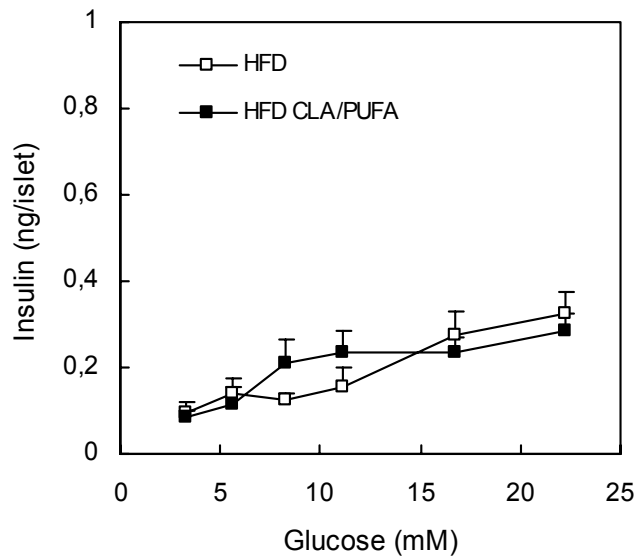


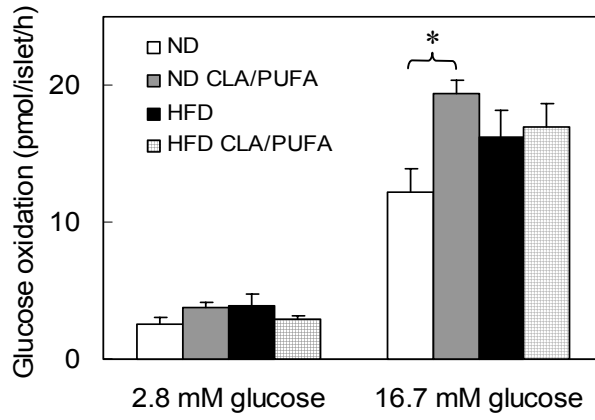
**D**







**A****B**

**A****B**