European Journal of Nutrition

Dietary conjugated α-linolenic acid (CLNA) did not improve glucose tolerance in a neonatal pig model

--Manuscript Draft--

Manuscript Number:	
Full Title:	Dietary conjugated α-linolenic acid (CLNA) did not improve glucose tolerance in a neonatal pig model
Article Type:	Original Contribution
Keywords:	conjugated linolenic acid; n-3 fatty acid; insulin resistance; pig
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Title: Dietary conjugated α-linolenic acid (CLNA) did not improve glucose tolerance in a neonatal pig model

Running title: CLNA and glucose tolerance in neonatal pigs

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Abstract

Purpose: There is an increased interest in the benefits of conjugated α-linolenic acid (CLNA) on obesity-related complications such as insulin resistance and diabetes. The aim of the study was to investigate whether a 1% dietary supplementation of mono-CLNA isomers (*c9-t11-c15*-18:3 + *c9-t13-c15*-18:3) improved glucose and lipid metabolism in neonatal pigs. **Methods:** Since mono-CLNA isomers combine one conjugated two-double bond system with an n-3 polyunsaturated fatty acid (PUFA) structure, the experimental protocol was designed to isolate the dietary structural characteristics of the molecules by comparing a CLNA diet with three other dietary fats: 1) conjugated linoleic acid (*c9-t11*-18:2 + *t10-c12*-18:2; CLA), 2) non-conjugated n-3 PUFA and 3) n-6 PUFA. Thirty-two piglets weaned at 3 weeks of age were distributed into the four dietary groups. Diets were isoenergetic and food intake was controlled by a gastric tube. After 2 weeks of supplementation, gastro-enteral (OGTT) and parenteral (IVGTT) glucose tolerance tests were conducted. **Results:** Dietary supplementation with mono-CLNA did not modify body weight/fat or blood lipid profiles ($p>0.82$ and $p>0.57$, respectively) compared with other dietary groups. Plasma glucose, insulin and C-peptide responses to OGTT and IVGTT in the CLNA group was not different from the three other dietary groups ($p>0.18$ and $p>0.15$, respectively). Compared to the non-conjugated n-3 PUFA diet, CLNA-fed animals had decreased liver composition in three n-3 fatty acids (18:3n-3; 20:3n-3; 22:5n-3) ($p<0.001$). **Conclusions:** These results suggest that providing 1% mono-CLNA is not effective in improving insulin sensitivity in neonatal pigs.

Key words: conjugated linolenic acid: n-3 fatty acid: insulin resistance: pig

Introduction

Conjugated fatty acids refer to a set of positional and geometric isomers of polyunsaturated fatty acids (PUFA) with conjugated double bonds. Conjugated linoleic acids (CLA) were first identified in the late eighties by Pariza et *al*. [1]. Since then, consumption of CLA was associated to weight loss [2] and improving of insulin sensitivity [3]. Another group of conjugated fatty acids recently received more attention because it combined an n-3 and a conjugated double bond: conjugated α-linolenic acids (CLNA) [4]. CLNA isomers are naturally present in plant seeds (di-CLNA) and in dairy products (mono-CLNA). Mono- and di-CLNA differ by their conjugated double-bond system: mono-CLNA have a single conjugated double-bond system at the n-5 or n-7 carbon, *i.e* rumelenic acid, *c9-t11-c15*-18:3, whereas di-CLNA have a double conjugated double-bond system at the n-5/n-7 or n-8/n-10 carbons, *i.e* α-eleostaric acid *c9-t11-t13*-18:3. Mono-CLNA isomers are produced by biohydrogenation of α-linolenic acid by rumen bacteria [5, 6].

Because of the worldwide problem of obesity in children and the related health problems such as hypertension and diabetes mellitus [7], there is an increased interest in the development of preventive and therapeutic strategies for improving insulin resistance [8]. Indeed, using antidiabetic drugs is not appropriate for treating diabetes in children unless there is severe glucose intolerance, thereby finding natural strategies such as CLNA isomers is an attractive approach which deserves to be studied. Di-CLNA are able to decrease body weight [9] and fat[10, 11], as well as increase insulin sensitivity in rodents [9, 12, 13]. Some studies also reported that either CLA [14] or n-3 PUFA [15] or the combination of the two [16] could improve glucose tolerance. Since a mono-CLNA such as *c9-t11-c15*-18:3 isomer combines the conjugated double bond system of CLA and the n-3 double bond of α-linolenic acid, it is reasonable to speculate that this original fatty acid structure may provide similar or even enhanced glucose tolerance than the conjugated or n-3 double bond structure.

Bioavailability of mono-CLNA was reported to be high in rodents. The metabolism of mono-CLNA has already been studied in different animal models excluding pigs [5, 6, 17].

The objective of the present study was to investigate whether dietary supplementation with mono-CLNA (*c9-t11-c15*-18:3 + *c9-t13-c15*-18:3) improves glucose metabolism in neonatal piglets. In order to isolate the role of the conjugated double-bond in combination with the n-3 PUFA structure, mono-CLNA group will be compared with three other dietary treatments: CLA isomers, non-conjugated n-3 and non-conjugated n-6 PUFAs.

Methods and materials

Animals and diets

Thirty two Yorkshire × Landrace × Duroc piglets (females and castrated males) weaned at 3 weeks of age were separated into eight groups of four animals. To control for food intake, an oesophageal gastric tube was installed into all animals as previously described by Cortamira et *al.* [18]. Individual adjoining metabolism cages with plastic floors allowed free movement and room temperature was kept at 27°C. At baseline, average body weight was 7.6 ± 0.4 kg. Within each group the piglets (from the same litter) were assigned to one of four dietary treatment groups, fed entirely with a commercial diet (barley (25%), maize (20%), dried whey (20%), soybean meal (10%), extruded soybean (8%) and plasma protein (5%); Table 1) plus either 1% of the caloric intake of the basal diet in the form of one the following lipid emulsion of specific fatty

acids: (1) synthetic mixture of two mono-conjugated α-linolenic acids (*c9-t11-c15*-18:3 + *c9-t13-c15*-18:3; CLNA); (2) mixture of two conjugated linoleic acids (*c9-t11*-18:2 + *t10-c12*-18:2; CLA); (3) n-3 fatty acids (W3); or (4) n-6 fatty acids (W6).

Mono-CLNA isomers were synthesized by alkali isomerization of α-linolenic acid. Thereafter, CLNA isomers were purified by preparative chromatography using a reverse phase column, as previously described by Trottier [19]. The fatty acid profile of the four lipid emulsions is shown in Table 2.

All diets were isoenergetic. The feeding regime was based on daily increment of 7.1 g feed per $kg^{0.75}$ body weight ($g/kg^{0.75}$) up to the target maximum daily value of 56 $g/kg^{0.75}$. The daily intake was adjusted three times per week according to changes in body weight in order to maintain the weight stable. Basal diet was mixed with water (1:2) and infused with a syringe into the stomach via the gastric tube. Daily meals were given at 08:00, 11:30 and 16:00 hours; each representing 45%, 20% and 35% of the diet caloric intake, respectively. Dietary treatments were given during the morning meal. Before the morning meal on day 0 (before attribution of treatments) and on day 15 post-weaning, body weight was measured and blood samples were collected via jugular venipuncture as previously described by Matte et *al.* [20].

On day 9 of the experimental protocol, a jugular catheter was installed by a non-surgical technique described by Matte et *al.* [21]. All animals were tested for insulin resistance by two glucose tolerance tests: a gastro-enteral (OGTT) and a parenteral (IVGTT) test. Briefly, after fasting for 18h, piglets (n=32) were given either an oral (OGTT) or an IV (IVGTT) dose of glucose (1.0g/kg BW) over a period of 120 min. Blood samples were collected every 30 min for 240 min (0, 30, 60, 90, 120, 150, 180, 210 and 240 min) starting after the initial glucose infusion. Blood samples were centrifuged at 3000 rpm for 10 min at 4°C and plasma was stored at -20°C until glucose, insulin and C-peptide analyses were performed. Two days after the first glucose test, the protocol was repeated with the other glucose test using the same animal. All animals were sacrificed on day 17. The liver was removed, weighed and samples were stored at -20°C for further analysis. The digestive tract, brain, lungs and heart were removed and stored at –20°C until lipid quantification was performed.

Throughout the experimental protocol animals were cared for according to the recommended code of practice of Agriculture Canada [22] and the procedure was approved by the local Animal Care Committee following the guidelines of the Canadian Council on Animal Care [23].

Biochemical analyses

Plasma glucose was measured by an enzymatic colorimetric assay (GLU GOD-PAP; Roche Diagnostics, Indianapolis, IN, USA) whereas insulin (Porcine Insulin RIA Kit PI-12K; Linco Research Inc., St Charles, MI USA) and C-peptide (Porcine C-peptide RIA kit PCP-22k; Linco Research Inc.) were assayed by commercial RIA kits. The homeostatic model assessment (HOMA2), described by Levy et *al.* [24] was used to estimate insulin sensitivity (HOMA2-%S) and secretion (HOMA2-%B) from baseline plasma parameters measured during OGTT and IVGTT. The area under the curve (AUC) of glucose, insulin and C-peptide were calculated using the trapezoidal method [25] between 0 and 210 min. Matsuda's insulin sensitivity whole-body index (ISI) was also calculated from the data generated during the OGTT [26]. An insulin sensitivity index (SI) derived from the IVGTT was calculated according to a modified method described by Bergman and colleagues [27, 28].

SI was determined from 120 to 210 min of the IVGTT, *i.e.* after the end of the infusion to assess the deconvolution of glucose with regards to insulin after the glucose peak. Total cholesterol, triglyceride and non-esterified fatty acid (NEFA) concentration in plasma at day 1 and day 15 were measured by the service diagnostic of the Faculty of Veterinary Medicine at the Université de Montréal (Montreal, QC, Canada). The fatty acid composition of the four diets and liver was determined by gas chromatography as previously described by Castellano et *al.* [29].

Statistical Analysis

According to results obtained from similar porcine studies [15], the calculated sample size per group (n = 8) was sufficient to detect a difference in insulin sensitivity of at least 15% with a power of 80% and a level of significance of 0.05.

The data were analyzed by using the MIXED procedure implemented in Statistical Analysis Systems software (version 6.11 of SAS, Cary, NC, USA) [30] according to a completely randomised design with four treatments (CLNA, CLA, W3, and W6) as the main factor. The piglet was considered as the experimental unit. The following model was used:

$Y_{ij} = \mu + F_i + e_{ij}$

where Y_{ij} is the dependent variable, μ is the overall mean, F_i is the treatment effect and e_{ij} is the residual error. Comparisons among treatments were done using the following *a priori* contrasts (CLNA *vs.* W3 for CLA properties; CLNA *vs.* W6 for both CLA and n-3 PUFA properties; CLNA *vs.* CLA for n-3 PUFA properties) using a Dunnett's correction. All values are presented as mean ± SEM and differences are considered significant at $p<0.05$.

Results

Anthropometry and blood lipid parameters

Body weight of piglets pre-treatment was 7.6 ± 0.4 kg. After 2 weeks of supplementation (day 15), there was no difference ($p>0.82$) between treatments for either body weight (10.2 ± 0.4 kg) or fat content (10.0 ± 0.8 %). Total cholesterol, triglyceride and NEFA concentrations in blood plasma for day 1 *vs.* day 15 were, 5.9 ± 0.8 *vs.* 2.0 ± 0.1 mmol/l, 0.7 ± 0.1 *vs.* 0.3 ± 0.1 mmol/l and 708.3 ± 146.7 *vs.* 769.6 ± 101.8 µmol/l, respectively. There was no difference ($p>0.34$) according to dietary treatments for these parameters.

Liver fatty acid profile

There was no significant difference ($p=0.67$) in liver weight between dietary treatments. The overall organ weight was 248 ± 14 g. Evaluation of liver fatty acid composition was used as an indicator of whole body fatty acid status modification from dietary treatments. Mono-CLNA (*c9-t11-c15*-18:3 + *c9-t13-c15*-18:3) liver content, was higher ($p<0.001$) in the CLNA diet than the other diets (0.25 *vs.* 0.01 g/100g fatty acids, respectively). With regards to n-3 fatty acids, 18:3n-3, 20:3n-3 and 22:5n-3 were 20 to 40% lower ($p<0.001$) in CLNA diets compared to the W3 diet. Total n-3 PUFA was also significantly lower ($p<0.001$) in the CLNA diet than the W3 diet (9.27 *vs.* 10.77 g/100g fatty acids, respectively). In contrast, the proportion of arachidonic acid (20:4n-6) was 8% higher in the CLNA group compared to the W3 group, resulting in a significantly higher total n-6 PUFA level (CLNA *vs.* W3, 40.61 *vs.* 39.52 g/100g fatty acids, respectively).

Basal plasma glucose, insulin and C-peptide concentration

Baseline plasma glucose, insulin and C-peptide concentrations were evaluated before the OGTT or IVGTT load of glucose (Table 3). A significant treatment effect was detected for fasting insulin concentration on the OGTT day (p=0.03) and on calculated insulin sensitivity HOMA-%S but the specific contrast test did not allow discrimination between the CLNA group and the other dietary groups (p>0.22). There was no other treatment difference for OGTT and IVGTT (p>0.14; Table 3).

Monitoring of glucose, insulin and C-peptide during OGTT and IVGTT

AUC for glucose, insulin and C-peptide monitored between 0 and 210 min are reported in Table 4. Among the four dietary groups, there was no significant difference in glucose, insulin and C-peptide monitoring over the OGTT and the IVGTT. Dietary intake did not improve the Matsuda's ISI (p=0.71) calculated from the OGTT nor the minimal model-derived insulin sensitivity calculated from the IVGTT (SI; p=0.51).

Discussion

The present study aimed to investigate whether dietary supplementation with mono-CLNA improves body composition and glucose tolerance in neonatal piglets.

This model was chosen because piglets represent 1) an accelerated model of postnatal development to study human neonatal nutrition and development [31], 2) a relevant model for insulin resistance [32] and 3) a suitable model for evaluating nutritional strategies to enhance glucose tolerance and prevent type 2 diabetes and cardiovascular diseases later in life [33].

Body composition and blood lipids

Mono-CLNA might combine anti-obesity properties of CLA, along with those of the α-linolenic acid. There is evidence suggesting that CLA decreases body weight, fat accumulation and improves serum lipids in mice [34], rats [35], hamsters [36] and humans [37]. Similarly, α-linolenic acid was reported to improve the same biomarkers in hamsters [38] and humans [39]. Many studies in rodents [10-12, 40-42] showed that dietary di-CLNA supplementation decreases body weight, body fat as well as plasma triglycerides and cholesterol. This study speculates that because mono-CLNA has an original structure compared to di-CLNA, this conjugated fatty acid will have improved or equally effective glucose tolerance than CLA or α-linolenic acid alone. None of them combined an n-3 PUFA and a CLA structure. However, dietary supplementation of piglets with mono-CLNA for 14 days did not improve body weight, body fat nor blood lipid profiles. Our findings extend previous studies in rodents which reported that dietary mono-CLNA did not lower body weight [17, 43]. By analogy, dietary di-CLNA isomers did not lower adipose tissue weight as well as total cholesterol and triglycerides in the plasma of animals [12, 17, 42, 44, 45] and humans [46].

Liver fatty acid composition

CLNA and CLA concentrations in liver reflected the dietary intake of these fatty acids. This response suggests that a 2-week supplementation was sufficient for stabilization of PUFA status within the piglet's body. Our results are in the line

with Chartrand et *al.* [47] who reported that dietary fatty acid content consumed for at least 14 days was proportional to plasma fatty acid profiles and remained constant up to study completion at 36 days. Our results also showed that giving mono-CLNA to piglets changed the n-3 and n-6 fatty acid balance. More specifically, compared to the W3 diet, feeding mono-CLNA for 2 weeks decreased the proportions of 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3 together with an increase in 20:4n-6 and total n-6 PUFA content. Since a previous study in mice reported that n-3 PUFA chronic depletion in the liver led to the development of hepatic insulin resistance over a 3 month period [48], further studies need to be carried out for an extended period of time in pigs fed CLNA with additional health indicators (blood biochemical, other tissue lipid profile, etc) in order to better assess the safety aspects of consuming dietary mono-CLNA isomers.

Glucose tolerance

One of our hypotheses was that combining a conjugated and an n-3 PUFA structure in one fatty acid, like mono-CLNA, would improve insulin sensitivity considering that dietary CLA seems to lower insulinemia in rats [49] and α-linolenic acid intake seems to reduce insulin resistance in rats [50] and in humans [51].

Our results suggest a significant treatment effect for fasting insulin concentration and insulin sensitivity on the OGTT day (p=0.03). However, we consider that this result is unlikely of biological significance because specific *a priori* contrasts indicate that there is no treatment effect of mono-CLNA diet compared to the three other dietary groups. Moreover, these differences were not confirmed at the day of IVGTT or during the two glucose tolerance tests. One possible explanation could be a higher insulin secretion generated by environmental/psychological stresses during the first experiment, *i.e.* human presence, handling, noise, etc [52, 53]. Also, no treatment effect was seen on C-peptide, a good indicator of endogenous insulin secretion [54], either for OGTT or IVGTT.

In regards to the different indexes of insulin sensitivity (HOMA2-%S, $ISI_{Matsuda}$ and SI) and insulin secretion (Insulin and C-peptide levels, AUCs and HOMA2-%B), none were improved by ingestion of mono-CLNA for 2 weeks.

Results on a closer structural analog to mono-CLNA such as di-CLNA showed some inconsistency. Indeed, although several studies reported that dietary supplementation with di-CLNA isomers can decrease type 2 diabetes risk [12] and improve glucose tolerance [9, 55] in mice, others reported an increase in insulin resistance (HOMA-IR index) in rats [44] similar to what is reported in mice [56, 57], pigs [58], and humans [59]. Moreover, most of the studies using n-3 PUFA supplement in humans failed to improve insulin sensitivity [60, 61].

Limitations of the present study

The present study extends previous findings [9, 40, 44, 55] using a neonatal pig model and randomised experimental design to compare mono-CLNA diet *vs.* three other dietary treatments (CLA, W3, and W6). Nevertheless, it has some limitations including the duration of the supplementation and the composition of the CLA diet, since we used a mixture of two isomers: *c9-t11*-18:3 + *t10-c12*-18:3. Even if most previous studies have used a CLA isomer mixture, recent findings show that purified CLA isomers could have opposite actions on glucose tolerance, with *t10-c12*-18:3 reducing insulin sensitivity and *c9-t11*-18:3 enhancing insulin tolerance [3]. Mono-CLNA was also a mixture of two isomers and this is mostly because it is not possible to cost effectively separate the two CLNA isomers for generating high doses of single CLNA isomers for

feeding animals. Therefore, a direct comparison between CLA and CLNA diets based only on the chemical structure is limited.

Conclusions

This study showed that mono-CLNA, combining conjugated and an n-3 double-bound structure, did not provide additive improvement for body composition, glucose tolerance or blood lipid profile in the neonatal piglet model supplemented for a period of 2 weeks. Conversely, mono-CLNA decreased total n-3 PUFA in liver, a finding which merits consideration in regards to neonatal development and safety.

Acknowledgments

The authors are grateful to M. Guillette for her invaluable technical assistance; to Mélanie Turcotte and the animal care team under supervision of D. Morrissette; and to S. Methot for his help with statistical analysis. The authors are also grateful to Fonds Québécois de Recherche sur la Nature et les Technologies (FQRNT) and Naturia Inc (Sherbrooke, Canada) for a Ph.D scholarship to M. P at the time where the experiment was carried-out and to Fonds de recherche Québec-santé for a current Junior 1 salary award. Mono-CLNA were generously provided by Naturia Inc. (Sherbrooke, QC, Canada). The financial support was provided by Naturia Inc and the Mathing Investment Initiative of Agriculture and Agri-Food Canada. None of the authors has any financial conflict of interest.

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395 Table 1: Composition of basal diet

Ingredients	Calculated concentration
Digestible energy (MJ/kg)	14.98
Total protein (%)	19.94
Crude fibre (%)	1.90
Fat (%)	7.49
Lysine (%)	1.40
Methionine (%)	0.43
Tryptophan (%)	0.24
Calcium (%)	0.80
Phosphorus (%)	0.70

Provided (per kg basal diet): Mn, 40 mg; Zn, 2935 mg; Fe, 299 mg; Cu, 19 mg; I, 2 mg; Se 297 μg; vitamin A, 4.9 mg; vitamin D, 37.5 μg; vitamin E, 66.8 mg; menadione, ; thiamin, 2.7 mg; riboflavin, 8.7 mg; niacin, 31 mg; panthothenic acid 21.2 mg; folic acid, 0.7 mg; pyridoxine, 2.6 mg; biotin, 120 μg; vitamin B12, 25.1 μg; choline, 303 mg

396

397 Table 2: Analytical fatty acid composition (g/100 g fatty acids) of lipid emulsions added to the dietary treatments

Fatty acid	CLNA	CLA	W3	W6
16:0	6.37	6.04	6.10	5.97
16:1n-7	0.05	0.04	0.07	0.08
18:0	3.23	3.39	3.60	3.63
18:1n-9	22.40	21.61	23.39	22.89
18:1n-7	0.01	0.04	0.06	0.05
18:2n-6	15.72	19.49	18.58	53.08
18:3n-3	12.65	13.24	46.99	12.88
18:3n-6	0.19	0.08	0.21	0.10
20:0	0.06	0.14	0.14	0.23
20:1n-9	0.02	0.01	0.02	0.08
20:2n-6	1.72	1.50	0.06	0.07
20:3n-3	0.40	0.04	0.08	0.03
20:3n-6	1.13	0.03	0.04	0.03
20:4n-6	0.10	0.01	0.01	0.01
20:5-n-3	0.62	0.02	0.01	0.01
22:1n-9	0.66	0.04	0.08	0.03
22:5n-3	0.11	0.03	0.12	0.03
22:6n-3	0.05	0.04	0.03	0.03
24:0	0.84	0.07	0.06	0.12
24:1n-9	0.59	0.12	0.01	0.03
c9-t11-18:2	0.87	16.82	0.03	0.05
t10-c12-18:2	1.70	16.77	0.02	0.02
c9-t11-c15-18:3 + *c9-t13-c15*-18:3	30.33	0.25	0.16	0.46
Total conjugated	32.61	33.50	0.24	0.53
SFA	10.24	9.63	9.92	9.95
MUFA	23.86	21.92	23.90	23.34
PUFA	65.87	68.45	66.23	66.81
n-3:n-6 ratio	2.06	0.25	2.50	0.25

SFA = total saturated fatty acids; MUFA = total monounsaturated fatty acid; PUFA = total polyunsaturated fatty acids; n-3:n-6 ratio = n-3 PUFA to n-6 PUFA ratio

398

399 Table 3: Basal plasma concentrations of glucose, insulin and C-peptide during OGTT or IVGTT according to the dietary
400 treatments

	Diet					
Index	CLNA	CLA	W3	W6	SEM	*P-value*
OGTT						
Glucose (mmol/l)	5.6	5.4	5.5	5.8	0.2	0.74
Insulin (pmol/l)	58.8	50.3	63.9	62.6	5.9	0.03
C-peptide (pmol/l)	53.0	64.4	68.3	49.9	7.9	0.27
HOMA2-%S[a]	94.6	106.6	87.0	89.8	9.0	0.05*
HOMA2-%B[a]	82.0	79.1	89.3	80.7	6.7	0.57
IVGTT						
Glucose (mmol/l)	5.8	5.3	5.3	5.6	0.3	0.26
Insulin (pmol/l)	57.3	64.3	55.2	58.9	6.9	0.62
C-peptide (pmol/l)	43.9	58.8	57.3	66.9	9.0	0.31
HOMA2-%S[a]	88.5	102.3	98.8	92.7	12.5	0.76
HOMA2-%B[a]	82.8	96.6	88.2	81.5	7.4	0.14

W3 = omega-3 fatty acids diet; W6 = omega-6 fatty acids diet; CLNA = conjugated alpha-linolenic acids diet; CLA = linoleic acids diet; OGTT=oral glucose tolerance test; IVGTT = intravenous glucose tolerance test.

[a]Calculated insulin sensitivity (HOMA2-%S) and β-cell function (HOMA2-%B) based on homeostatic model assessment [24].

*Specific contrasts *p*-values for CLNA *vs.* CLA, CLNA *vs.* W3 and CLNA *vs.* W6 were 0.22, 0.53 and 0.81, respectively.

401

402 Table 4: Plasma glucose, insulin and C-peptide responses in OGTT and IVGTT

	Diet					
Index	CLNA	CLA	W3	W6	SEM	*P-value*
OGTT						
Glucose (mmol × min/l)[a]	23.3	22.6	22.2	22.9	0.6	0.56
Insuline (nmol × min/l)[a]	478.3	441.1	453.0	426.4	36.4	0.70
C-peptide (nmol × min/l)[a]	618.5	550.5	573.8	563.8	52.6	0.68
ISI (0, 210 min)[b]	7.7	8.7	7.7	7.9	0.6	0.21
IVGTT						
Glucose (mmol × min/l)[a]	28.5	27.6	28.2	27.5	0.9	0.81
Insuline (nmol × min/l)[a]	517.5	565.9	530.9	484.6	36.8	0.42
C-peptide (nmol × min/l)[a]	706.1	791.8	744.3	697.8	54.58	0.55
SI (120, 210 min)[c]	5.0	4.5	4.7	5.4	0.68	0.51

W3 = omega-3 fatty acids diet; W6 = omega-6 fatty acids diet; CLNA = conjugated alpha-linolenic acids diet; CLA = linoleic acids diet; OGTT = oral glucose tolerance test; IVGTT = intravenous glucose tolerance test;

[a]Values are AUC from 0 to 210 min during OGTT or IVGTT.

[b]Insulin sensitivity index (ISI) [26] calculated as follow: $ISI = (Glu_{basal} \times Ins_{basal} \times Glu_{mean} \times Ins_{mean})^{0.5}$.

[c]Minimal model-derived insulin sensitivity index (SI) based on MINMOD Millennium [28].

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