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Fish oil diets do not improve insulin sensitivity and secretion in healthy adult male pigs

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The effects of long-term dietary supplementation of fish oil (*n*-3 PUFA-rich) in adult male pigs on body condition as well as insulin sensitivity and secretion were examined. Fifteen Duroc boars aged 204·5 (SD 9·4) d (body weight 145·8 (SD 16·8) kg) received daily 2·5 kg basal diet with a supplement of: (1) 62 g hydrogenated animal fat (*n* 5); (2) 60 g menhaden oil containing 10·8 g DHA and 9·0 g EPA (*n* 6); (3) 60 g tuna oil containing 19·8 g DHA and 3·9 g EPA (*n* 4). Rations were balanced to be isoenergetic. After 7 months of treatments, oral glucose and meal tolerance tests were conducted after insertion of a catheter into the jugular vein. Dietary supplementation with *n*-3 PUFA altered the blood plasma profile: DHA and EPA increased whereas arachidonic acid decreased ($P<0·01$). Plasma glucose, insulin and C-peptide responses to oral glucose and the test meal were not affected by treatments ($P>0·34$). For all animals, total body fat estimated from body weight and back fat thickness was correlated with both β-cell function (by homeostasis model assessment (HOMA); $r+0·63$) and insulin sensitivity (index of whole-body insulin sensitivity and by HOMA; $r-0·63$ and $r+0·66$, respectively). In conclusion, long-term supplementation with dietary *n*-3 PUFA did not affect insulin metabolism in healthy adult male pigs. The relationship between body fat and insulin sensitivity, well documented in human subjects, suggests that the adult male pig could be a promising animal model for studies on insulin metabolism.

Fish oil: *n*-3: Insulin: Glucose: Pigs

n-3 PUFA play several prominent roles in energy metabolism, membrane structure, gene expression, visual and neuronal development as well as PG synthesis[1]. Particularly abundant in seafood, mainly in fish fat[2], *n*-3 long-chain PUFA especially EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) also affect several chronic diseases in man such as CVD, dyslipidaemia and diabetes[3]. Type 2 diabetes is commonly associated with a defect of insulin secretion and sensitivity[4], the latter being related to body fat[5]. *In vitro*, it has been shown that insulin action is modulated by the chain length and the degree of saturation of fatty acids[6]. *In vivo*, high-fat diets rich in SFA can impair insulin sensitivity[7,8]. By contrast, fish oil improved insulin action on glucose metabolism[9,10] and prevented the development of insulin resistance[3]. Several animal models were used to support this evidence. In rats[7], miniature pigs[11] and poultry[12], the presence of fish oil enhanced insulin sensitivity. On the other hand, in dogs[13] and horses[14], *n*-3 PUFA diets did not improve insulin sensitivity. In humans, the impact of long-chain PUFA on insulin metabolism is still controversial. In healthy subjects, Delarue *et al.* [15] showed that fish oil could improve insulin sensitivity while Vessby *et al.* [16], as other recent studies[17,18], reported no significant effects. These conflicting studies are possibly related to variations in experimental conditions such as fish oil composition (DHA:EPA ratio), the daily amount of *n*-3 intake and the duration of the dietary supplementation. For the first two factors, it has been shown that insulin resistance is inversely correlated with the amount of long-chain fatty acids in muscle[19] while for the last factor, the complete incorporation of EPA and DHA into membranes has been reported to be as long as 18 weeks[20].

The present study aimed to further study the effects of long-term (7 months) dietary supplementation of saturated fat as the control treatment *v.* long-chain PUFA (fish oils) on parameters related to lipid and glucose metabolism in response to an oral glucose tolerance test or to a standard meal test in adult male pigs.

Experimental methods

Animals and diets

A total of fifteen Duroc boars were selected at age 204·5 (SD 9·4) d and individually housed in pens on partially slatted floors. The average body weight was 145·8 (SD 16·8) kg at

Abbreviations: AF, animal fat; AUC, area under the curve; BW, body weight; HOMA2, homeostasis model assessment; HOMA2-%B, β-cell function by HOMA2; HOMA2-IR, insulin sensitivity by HOMA2; ISI, index of whole-body insulin sensitivity; MO, menhaden oil; P2, back fat thickness; TO, tuna oil.

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initiation of treatments. Animals were fed for 7 months (30 weeks) 2·5 kg of a basal diet daily (Table 1) top-dressed with 0·3 kg of the treatment mixture per d (containing 79 % of maize and 20–21 % of fat). Boars were distributed in three groups, each group being assigned to one treatment. The first group (n 5) was fed with 62 g animal fat (AF) rich in hydrogenated SFA (Table 2). The second group (n 6) was supplemented with 60 g menhaden oil (MO) containing 10·8 g DHA and 9·0 g EPA. The third group (n 4) received 60 g tuna oil (TO) containing 19·8 g DHA and 3·9 g EPA. All diets were balanced to be isoenergetic and provided an equivalent of 989 mg vitamin E per d. Fish oils were stabilised with an antioxidant (500 parts per million of Ethoxyquin, E8260; Sigma-Aldrich, St Louis, USA). Measures of body weight (BW; kg) and back fat thickness (P2; mm) were recorded before the beginning of treatments and during the 7 months of the diet. P2 was ultrasonically measured 65 mm from the midline at the level of the last rib with an Ultrascan 50 (Alliance Medical Products Inc., Irvine, CA, USA) as described by Dourmad *et al.* [21]. Blood samples (for fatty acid analysis) were collected at the same time as body measurements by jugular venepuncture as described by Matte *et al.* [22] before the morning meal. At the end of the experimental period, an indwelling catheter was placed in the jugular vein, 24 h before the glucose tolerance test, using a non-surgical technique described by Matte[23]. Food was withheld overnight. On the morning of the oral glucose tolerance test, a dose of dextrose (1 g per kg BW) was mixed with 250 ml apple sauce (no sugar added) and given in a bolus, which the animals consumed voluntarily and rapidly (less than 10 min). Blood samples were taken at 30 min intervals for 240 min following the glucose slop and thereafter at 60 min intervals for another 2 h. Samples were immediately centrifuged at 3000 rpm for 10 min at 4°C; plasma was divided into samples and stored at −20°C until glucose, insulin or C-peptide analyses. The same protocol was used 1 week after with the same animals receiving this time their respective treatment diet. All animals were cared for according to the recommended code of practice of Agriculture and Agri-food Canada[24] and the procedure was approved by the local Animal Care Committee following the guidelines of the Canadian Council on Animal Care[25].

Table 1. Composition of the basal diet*

Ingredients	g/kg
Maize	294·4
Barley	150·0
Wheat	300·0
Soyabean meal	122·0
Gluten feed 21 %	75·0
Vitamin–mineral premix†	25·0
Limestone	10·0
Dicalcium phosphate	8·3
Salt	5·0
Animal and vegetable fat	5·0
Lysine	3·0
Methionine	1·9
MycoCURB® S powder‡	0·5

*The calculated composition for digestible energy, total protein, fat, crude fibre, lysine, methionine, tryptophan, Ca and total P of the basal diet (as-fed basis) was: 12 706·8 kJ/kg, 16·35, 3·38, 6·63, 0·93, 0·45, 0·45, 0·19, 0·82 and 0·79 %.
†Provided (per kg basal diet): Mn, 74·8 mg; Zn, 131·5 mg; Fe, 307·2 mg; Cu, 23·6 mg; I, 220 μg; Se, 300 μg; vitamin A, 2·4 mg; vitamin D, 30 μg; vitamin E, 60 μg; menadione, 0·8 mg; thiamin, 0·8 mg; riboflavin, 4 mg; niacin, 20 mg; pantothenic acid, 16 mg; folic acid, 1·9 mg; pyridoxine, 2·4 mg; biotin, 200 μg; vitamin B_{12}, 20 μg; choline, 160 mg.
‡Kemin Industries Inc. (Des Moines, IA, USA).

Table 2. Fatty acid composition (g/100 g fatty acids) of the basal diet and sources of supplemented fat

Fatty acid	Basal diet	AF*	MO†	TO‡
12 : 0	ND	0·09	0·13	0·04
14 : 0	0·22	3·83	9·27	2·95
14 : 1*n*-5	ND	0·03	0·05	0·06
15 : 0	ND	0·59	1·02	1·10
16 : 0	15·83	26·62	19·03	19·10
16 : 1*n*-7	0·38	0·14	11·10	3·76
17 : 0	ND	6·81	2·71	4·11
18 : 0	3·01	47·32	3·49	5·19
18 : 1*n*-9	24·64	1·66	5·71	12·60
18 : 1*n*-11	1·69	0·35	3·23	2·03
18 : 2*n*-6	50·30	0·04	1·69	1·21
20 : 0	0·56	0·63	0·27	0·88
20 : 1*n*-9	0·32	ND	0·96	1·44
18 : 3*n*-3	3·06	0·02	1·81	0·38
20 : 4*n*-6	ND	ND	1·37	2·17
20 : 5*n*-3	ND	ND	15·60	6·52
22 : 5*n*-6	ND	ND	0·78	2·01
22 : 5*n*-3	ND	ND	3·39	1·39
22 : 6*n*-3	ND	ND	18·41	33·06
SFA	19·62	78·40	32·06	28·12
MUFA	27·03	2·15	21·00	19·83
PUFA	53·36	0·06	43·05	46·74
n-3:*n*-6 ratio	0·06	0·50	10·21	7·67

AF, animal fat; MO, menhaden oil; TO, tuna oil; ND, not detected.
*Fat Pak 100 (MSC, Dundee, IL, USA distributed by JEFO Import Export Inc.).
†Virginia prime gold menhaden fish oil (Omega Protein Inc., Reedville, VA, USA).
‡Tuna oil (Bluecina Inc., Calgary, AB, Canada).

Analyses

Plasma glucose was measured by an enzymic 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. The C-peptide:insulin ratio and the glucose:insulin ratio were estimated as an indicator of hepatic insulin extraction and clearance[26] and insulin resistance[27]. The homeostatic model assessment (HOMA2), described by Levy *et al.* [28], was used to estimate insulin sensitivity (HOMA2-IR) and β-cell function (HOMA2-%B) from basal plasma insulin and glucose concentrations. An index of whole-body insulin sensitivity (ISI), proposed by Matsuda & DeFronzo[29], was also calculated from the oral glucose tolerance test (0, 120 min) and the meal test. Plasma fatty acids composition was evaluated according to a modified method described by Park & Goins[30]. All chemicals and fatty acid methyl ester standards were purchased from Sigma-Aldrich (St Louis, MO, USA) except when otherwise specified. The transesterification was done on 50 μl plasma, on 50 μl supplemented fat and 50 mg finely ground basal diet (1 mm). Each sample was mixed with 20 μl methylene chloride (CH_2Cl_2) and 200 μl sodium methoxide (MeOH/NaOH 0·5 M) and incubated in a 100°C

water-bath for 10 min. After cooling in a cold water-bath, 500 μl boron trifluoride solution (BF3/MeOH 14 %) was added and samples were incubated again at 100°C during 10 min. After cooling, the required amounts of fatty acid methyl esters from plasma, fats and basal diet were extracted into hexane (HPLC-grade hexane; Fisher Scientific, Fairlawn, NJ, USA). The supernatant fraction layer was decanted, after centrifugation, in a GC vial directly for GLC analysis. Fatty acid methyl ester profiles were measured on a Hewlett-Packard 6890 chromatograph fitted with a 60 m × 0·25 mm × 0·20 μm Supelco SP2380 fused silica capillary column (Supelco Inc., Bellefonte, PA, USA). The gas carrier was He at 6 ml/min. Inlet and detector temperatures were 260 and 300°C, respectively. After an initial period of 2·87 min at 140°C, the temperature was programmed to 235°C, rising by 10·35°C per min with a hold of 2·31 min at that temperature and then programmed to 258°C at 15·52°C per min with a final hold of 2·31 min at that temperature. The H_2 flow was 40 ml/min and the airflow was 450 ml/min. The split:splitless ratio was 1:1 and the injection volume was 1 μl. The data were processed with Agilent GC Chemstation software version B.01.01 (Agilent Technologies, Inc., Santa Clara. CA, UK). Peaks were identified by retention times relative to individual fatty acid standards.

Statistics

The area under the curve (AUC), calculated according to the trapezoidal method[31], was computed for glucose, insulin and C-peptide with the zero value as the baseline to integrate the overall response. The data were analysed using the Mixed procedure of SAS (SAS Institute Inc., Cary, NC, USA)[32] according to a completely randomised design with treatment (three levels: AF, MO and TO) as the main factor. The boar 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.

A Tukey–Kramer correction was applied for all pairwise comparisons. Pearson correlation coefficients were calculated to relate body condition and parameters of insulin metabolism. Differences are considered significant at $P<0·05$ and all results are expressed as adjusted mean values with their standard errors.

Results

Body condition (body weight, back fat thickness and total fat)

Although there were no differences among treatments for BW and P2 ($P=0·07$ and $P=0·20$, respectively), the calculated total fat[21] of animals receiving fish oils was higher than for the control diet after 7 months of the experiment ($P=0·03$) (Table 3). However, there were no differences in fat gain ($P=0·7$) among the three diets (AF, 36·8 (SEM 2·3) kg; MO, 39·4 (SEM 2·1) kg; TO, 40·2 (SEM 2·6) kg). These apparent effects on BW and total fat are unlikely to be of biological relevance because the present animals represented a subpopulation of a larger group (eight animal in the AF group, nine animals in the FO group and eleven animals in the TO group) where body condition was similar among treatments ($P=0·22$).

Lipid profile of blood plasma

There were no significant differences ($P>0·29$) in blood plasma lipid profile among animals before attribution of treatments. The average profile is shown in Table 4. Ingestion of different dietary fats modified the plasma concentration of specific fatty acids. EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) contents in blood plasma were significantly enhanced after supplying boars with fish oils for 7 months. For DHA, there was a significant increase ($P<0·01$) of 104 % in the MO group and 121 % in the TO group compared with the AF group. Furthermore, on these diets, the EPA content was increased ($P<0·01$) 20- to 30-fold compared with the AF group and was higher ($P<0·05$) in the MO than in the TO group, whereas arachidonic acid (20 : 4*n*-6) significantly decreased ($P<0·01$) by 47 and 52 % in the MO and TO groups, respectively. The *n*-3:*n*-6 ratio was also affected by the treatment: compared with the AF diet, the *n*-3:*n*-6 ratio was increased ($P<0·01$) in both the MO and TO diets. However, although the two fish oils have different dietary DHA:EPA ratios, 1:1 and 5:1 for MO and TO respectively, this was not reflected in blood plasma.

Table 3. Age and body condition (back fat level, body weight and calculated total fat) before and at the end of the experiment

(Adjusted mean values with their pooled standard errors)

	Initial					After 7 months of treatment				
	AF	MO	TO	SEM	*P*	AF	MO	TO	SEM	*P*
Age (d)	205·4	203·2	201·7	5·5	0·80	415·4	413·2	411·7	5·5	0·88
P2 (mm)	8·7	9·8	10·5	0·6	0·10	14·9	18·7	19·5	2·0	0·20
Body weight (kg)	136·5	150·7	154·9	7·7	0·20	267·1	276·8	283·8	4·8	0·07
Total fat (kg)*	14·5	19·1	20·9	2·2	0·12	51·3[a]	58·5[b]	61·2[b]	2·5	0·03
Fat gain (kg)†	–	–	–	–	–	36·8	39·5	40·2	2·6	0·58

AF, animal fat; MO, menhaden oil; TO, tuna oil; P2, back fat thickness; EBW, empty body weight.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* Total fat from EBW and P2[21] $= -26·4 + 0·221$ EBW (kg) $+ 1·331$ P2 (mm), where EBW $= 0·912$ weight$^{1·013}$.
† Fat gain = total fat (after 7 months) − total fat (initial).

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Table 4. Plasma fatty acid composition (g/100 g fatty acids) before and at the end of the experiment

(Adjusted mean values with their standard errors or pooled standard errors)

	Initial		After 7 months of treatment				
Fatty acid	Mean*	SEM	AF	MO	TO	SEM	*P*
16 : 0	20·09	0·15	20·46	20·11	22·57	0·75	0·82
16 : 1*n*-7	0·56	0·03	0·33[a]	0·53[b]	0·34[a]	0·05	0·02
17 : 0	0·54	0·01	0·35	0·35	0·48	0·05	0·18
18 : 0	26·50	0·28	29·26	28·51	28·06	1·19	0·75
18 : 1*n*-9	13·24	0·25	10·90	9·08	10·19	0·61	0·08
18 : 2*n*-6	24·34	0·50	26·25	24·25	25·27	1·10	0·90
18 : 3*n*-3	0·39	0·04	0·15	0·12	0·13	0·03	0·66
18 : 3*n*-6	0·52	0·02	0·20	0·24	0·21	0·05	0·79
20 : 0	0·73	0·03	0·36	0·49	0·36	0·07	0·25
20 : 1*n*-9	0·27	0·03	0·41[a]	0·76[b]	0·41[a]	0·09	0·01
20 : 2	1·59	0·26	0·52	0·61	0·50	0·14	0·82
20 : 3*n*-6	0·97	0·09	0·72	1·26	0·51	0·18	0·47
20 : 4*n*-6	4·87	0·28	4·36[a]	2·08[b]	2·23[b]	0·32	<0·001
20 : 5*n*-3	0·06	0·01	0·10[a]	2·67[b]	1·74[c]	0·21	<0·001
21 : 0	0·16	0·03	0·08	0·15	0·15	0·02	0·07
22 : 0	1·06	0·15	0·51	0·74	0·44	0·40	0·75
22 : 5*n*-3	0·64	0·03	0·61	0·79	0·78	0·15	0·57
22 : 6*n*-3	0·38	0·03	0·97[a]	1·98[b]	2·14[b]	0·21	0·002
Total *n*-3 PUFA	1·47	0·05	1·49[a]	5·12[b]	4·36[b]	0·42	<0·001
Total *n*-6 PUFA	31·20	0·50	31·66[a]	28·89[b]	28·23[b]	0·66	0·004
n-3:*n*-6 ratio	0·05	0·01	0·05[a]	0·18[b]	0·15[b]	0·02	*<0·001*

AF, animal fat; MO, menhaden oil; TO, tuna oil.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* Values are means of the fifteen animals selected for the oral glucose tolerance test.

Basal plasma glucose, insulin and C-peptide concentration

Fasting plasma glucose and insulin as well as C-peptide concentrations were evaluated before the oral glucose load and the typical meal. Glucose, insulin and C-peptide basal concentrations in the AF, MO and TO groups did not significantly differ ($P>0·09$) from each other (Table 5). The homeostatic model[28] used to estimate HOMA2-IR as well as HOMA2-%B did not differ ($P>0·12$) among treatments (Table 5).

Responses to oral glucose tolerance test

Glucose, insulin and C-peptide profiles in response to the oral glucose tolerance test are illustrated in Fig. 1 and their respective AUC calculated from 0 to 360 min after the bolus of glucose are reported in Table 6. There was no significant difference in glucose, insulin and C-peptide plasma levels ($P=0·46$, $P=0·35$ and $P=0·67$, respectively) among the three dietary groups. MO induced a significant increase ($P=0·02$) of the C-peptide:insulin ratio compared with AF and with TO.

Responses after the meal

Glucose, insulin and C-peptide responses (AUC from 0 to 360 min) after the meal are reported in Table 6. There were no significant differences in glucose, insulin and C-peptide plasma levels ($P=0·21$, $P=0·37$ and $P=0·44$, respectively) among the three dietary groups, nor for any of the indexes calculated (C-peptide:insulin ratio, glucose:insulin ratio and ISI) during the meal test ($P>0·40$).

Correlations between body condition and insulin secretion and sensitivity

Relationships between body fat and variables of insulin secretion (insulin, C-peptide and HOMA2-%B) and insulin sensitivity and resistance (ISI, HOMA2-IR) after the oral glucose tolerance test or meal are both reported in Table 7. A positive correlation was found between insulin secretion

Table 5. Basal plasma concentrations of glucose, insulin and C-peptide

(Adjusted mean values with their pooled standard errors)

Index	AF	MO	TO	SEM	*P*
Glucose (Glu_0; mmol/l)					
OGTT	4·6	4·9	5·0	0·2	0·20
Meal	4·6	4·9	4·7	0·3	0·64
Insulin (Ins_0; pmol/l)					
OGTT	41·2	82·5	79·8	22·3	0·13
Meal	49·4	50·2	52·4	9·3	0·96
C-peptide ($Cpep_0$; pmol/l)					
OGTT	149·4	226·6	256·9	52·2	0·09
Meal	168·0	265·2	280·6	56·7	0·20
HOMA2-%B*					
OGTT	94·8	133·2	119·7	19·0	0·20
Meal	102·2	94·2	108·0	11·8	0·55
HOMA2-IR*					
OGTT	0·8	1·5	1·5	0·4	0·12
Meal	0·9	0·9	1·0	0·2	0·96

AF, animal fat; MO, menhaden oil; TO, tuna oil; OGTT, oral glucose tolerance test; HOMA, homeostatic model assessment; HOMA2-%B, β-cell function by HOMA; HOMA2-IR, insulin sensitivity by HOMA.
* Calculated β-cell function (HOMA2-%B) and insulin sensitivity (HOMA2-IR) based on HOMA by computer model[52].

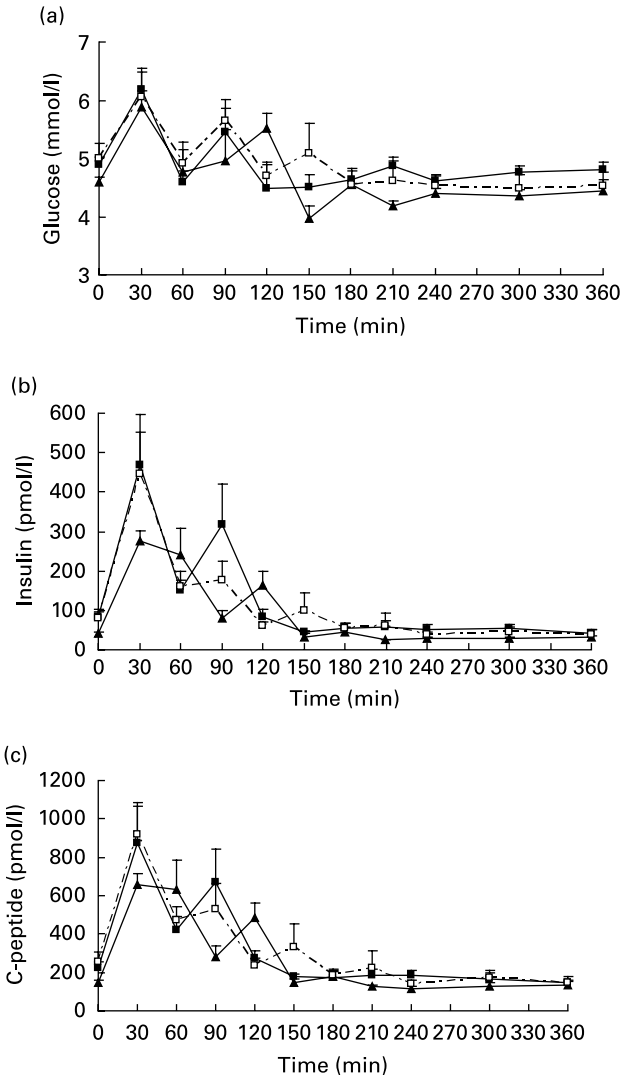

Fig. 1. Plasma glucose (a), insulin (b) and C-peptide (c) profiles obtained from oral glucose tolerance tests in male pigs fed diets supplemented with animal fat (–▲–; *n* 5), menhaden oil (–■–; *n* 6) or tuna oil (-·-□-·-; *n* 4). Values are means, with standard errors represented by vertical bars.

($AUC_{insulin}$, $AUC_{C\text{-}peptide}$ and HOMA2-%B) and back fat depth ($r + 0{\cdot}51$, $r + 0{\cdot}48$ and $r + 0{\cdot}50$, respectively). The same association was observed with total fat ($r + 0{\cdot}53$, $r + 0{\cdot}50$ and $r + 0{\cdot}63$, respectively). Insulin sensitivity, as estimated by ISI, was also correlated with both P2 and total fat. A negative correlation was found between ISI and P2 ($r - 0{\cdot}54$) as well as ISI and total fat ($r - 0{\cdot}63$). HOMA2-IR was also positively correlated with both P2 ($r + 0{\cdot}55$) and total fat ($r + 0{\cdot}66$).

Discussion

The present study investigated the effects of dietary supplementation with fish oils (MO and TO) during 7 months on insulin secretion and sensitivity in healthy male adult pigs. Furthermore, two different sources of fish oils allowed us to evaluate the eventual effects of variations in the DHA:EPA ratio on insulin sensitivity.

Plasma fatty acid composition

As shown previously[2], fatty acid profiles in blood plasma reflect the fatty acid composition of the dietary intake. As expected, supplementation of the diet with fish oils (rich in *n*-3 PUFA) significantly increased levels of EPA and DHA (*n*-3) and was associated with a decrease of arachidonic acid (*n*-6). By extension, the *n*-3:*n*-6 ratio was higher for the fish oil diets than the hydrogenated AF diet and clearly indicated a modification of the *n*-3:*n*-6 PUFA balance. Differences were also observed between the two fish oil treatments. Feeding MO increased 16 : 1*n*-7, 20 : 1*n*-9 and 20 : 5*n*-3 when compared with TO. The present study showed different responses of plasma *n*-3 fatty acids to their dietary provisions. In fact, whereas EPA concentration in blood plasma reflected the dietary intake of this fatty acid, the response of plasma DHA concentrations suggests the presence of a saturable dose-dependent process to the fish oil supplementation. Similar observations have been previously reported[33] in human subjects where DHA concentrations in plasma were maximised by a dietary provision of 2 g DHA/d which represents 28 mg/kg BW (for an average human BW of 70 kg). In the present study, the proportions of DHA provided by MO and TO represent 39 and 72 mg/kg BW, respectively (for an average pig weight of 275 kg). Furthermore, despite the large amounts of *n*-3 PUFA provided by the diet, the content of EPA and DHA never exceeded 5 % of total fatty acids of blood plasma and remained at a steady state throughout the 7 months of supplementation.

Table 6. Plasma glucose, insulin and C-peptide responses in oral glucose and meal tests

(Mean values with their pooled standard errors)

Index	AF	MO	TO	SEM	*P*
Glucose (mmol × min/l)*					
OGTT	1675·4	1753·4	1750·0	54·4	0·46
Meal	1679·4	1742·9	1812·3	52·0	0·21
Insulin (nmol × min/l)*					
OGTT	30·6	43·4	38·4	6·9	0·35
Meal	58·8	87·7	76·5	16·3	0·37
C-peptide (nmol × min/l)*					
OGTT	94·4	109·2	112·1	16·3	0·67
Meal	149·1	195·8	204·5	34·8	0·44
C-peptide:insulin ratio†					
OGTT	3·1[b]	2·5[a]	3·0[b]	0·14	0·02
Meal	2·7	2·4	2·6	0·2	0·40
Glucose:insulin ratio‡					
OGTT	56·6	45·9	50·2	7·5	0·51
Meal	38·6	27·6	24·4	11·4	0·61
$ISI_{Matsuda}$ (0, 120 min)§					
OGTT	0·99	0·65	0·71	0·11	0·08
Meal	0·98	0·78	0·85	0·16	0·61

AF, animal fat; MO, menhaden oil; TO, tuna oil; OGTT, oral glucose tolerance test; ISI, index of whole-body insulin sensitivity; AUC, area under the curve.

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

* Values are AUC from 0 to 360 min after the bolus or the meal.

† C-peptide:insulin ratio = $AUC_{C\text{-}peptide}/AUC_{insulin}$.

‡ Glucose:insulin ratio = $AUC_{glucose}/AUC_{insulin}$.

§ $ISI_{Matsuda}$ = 10 000/square root of $((Glu_0 \times Ins_0) \times$ (mean glucose × mean insulin during OGTT)) (Matsuda & DeFronzo[29]).

Table 7. Correlations between body fat (back fat thickness and total fat) and insulin secretion and sensitivity

	Back fat thickness		Total fat	
	r	*P*	*r*	*P*
Insulin secretion				
$AUC_{insulin}$	+0·51	0·052	+0·53	0·04
$AUC_{C\text{-}peptide}$	+0·48	0·067	+0·50	0·059
HOMA2-%B	+0·50	0·055	+0·63	0·01
Insulin sensitivity				
HOMA2-IR	+0·50	0·059	+0·66	0·01
Glucose:insulin ratio	−0·54	0·037	−0·52	0·04
$ISI_{Matsuda}$*	−0·54	0·038	−0·63	0·01

AUC, area under the curve; HOMA2-%B, β-cell function by homeostasis model assessment; HOMA2-IR, insulin sensitivity by homeostasis model assessment; ISI, index of whole-body insulin sensitivity.
*Matsuda & DeFronzo[29] (see Table 6).

Insulin sensitivity

Although the oral glucose and meal tolerance tests, as used in the present experiment, offer unique information about glucose tolerance, they did not directly evaluate insulin sensitivity[34]. The glucose:insulin ratio has been used as an indicator of insulin sensitivity but its relevance is controversial[27,29]. In fact, indexes of insulin sensitivity, such as HOMA2-IR and $ISI_{Matsuda}$, are considered more reliable for insulin sensitivity and were calculated to incorporate the results from oral glucose and meal tests[34]. Epidemiological studies with animal models have suggested an association between *n*-3 PUFA intake and insulin sensitivity[35] but, according to Toft *et al.* [36], such a positive effect in human subjects has not been demonstrated in spite of 16 weeks of fish oil supplementation. The present study is in accordance with the lack of influence of fish oil on any indices of insulin sensitivity. There was no difference between the diets for the glucose:insulin ratio, $ISI_{Matsuda}$ and HOMA2-IR considering oral glucose or meal tests. Furthermore, no positive effect of a change in the *n*-3:*n*-6 ratio or of the specific fatty acids EPA and DHA were reported on insulin sensitivity[17,37].

In his review, Heine[38] associated the lack of effect of fish oils on insulin sensitivity to the various ways in which fatty acids are incorporated in phospholipids and their influence on several other metabolic pathways. In pigs, a study on whole-body deposition reported that only 40 % of the digestible preformed *n*-3 fatty acids were recovered intact in the body[39]. Vessby *et al.* [16] also suggested that it could take years rather than months to see any possible effect of *n*-3 fatty acids on insulin action. Another possible explanation of the present lack of treatment effects on insulin sensitivity could be related to the composition of the basal diet, in particular, its relatively high proportion of crude fibre (6·6 %). Such a high-fibre diet could have potentially influenced basal glucose metabolism[40,41] and masked eventual effects of fish oil treatments.

Insulin secretion

In vitro, Stein *et al.* [42] have noted that insulin secretion increased with chain length and degree of saturation. *In vivo*, it has been recently reported that insulin secretion is directly related to the proportion of MUFA in the diet[43]. By contrast, although many of the human studies showed that *n*-3 PUFA or the *n*-3:*n*-6 ratio did not alter the insulin response[17,44], Lardinois *et al.* [45] reported a stimulation of insulin secretion with a meal rich in EPA and DHA. C-peptide concentration was measured in the present experiment because it is recognised to give a better estimate of the secretion rate of insulin than do peripheral insulin measurements. Insulin and C-peptide are secreted from the β-cells in blood in equimolar amounts[46] and cleared from the plasma by different mechanisms. Insulin is degraded in the liver[47] whereas C-peptide catabolism and excretion take place in the kidneys[48]. Results from the present study show that the fish oil diets did not significantly change the insulin response (insulin and C-peptide levels, HOMA2-%B) compared with the control diet.

Calculation of the C-peptide:insulin ratio has been used to provide indication of hepatic insulin extraction and clearance[49]. In the present study, the C-peptide:insulin ratio with the MO diet was significantly lower than with the other two treatments. The decrease in the C-peptide:insulin ratio suggests a decrease in hepatic insulin extraction. To the best of our knowledge, only one study in human subjects[50] has already reported a greater effect of PUFA (safflower-seed oil composed of 78 % of linoleic acid, 18 : 2*n*-6) on insulin clearance. This effect was also associated with a reduction in insulin resistance and a decrease in C-peptide secretion in contrast to the present study. In regard of this finding, further studies need to be carried out with more animals and a reference method used to assess insulin sensitivity[18] such as the euglycaemic clamp technique or the intravascular glucose tolerance test with minimal model analysis.

Body fat and insulin

As reviewed by Lombardo & Chicco[3], it is clearly established that supplementation with *n*-3 PUFA has an effect on body fat accumulation. In healthy men, it is accepted that body fat distribution is an important determinant factor of insulin resistance[5]. In the present study, although diets did not significantly change the P2 or calculated fat gain of the animals, the individual variation within the whole group of animals allows to estimate reliable correlations between variables. In fact, as for human subjects, positive correlations were found between both insulin secretion and resistance, and body fat (P2 and total fat). Those correlations suggest that the adult male pig can be a reliable model for insulin metabolism. The relationship between insulin resistance and insulin secretion is well documented; an enhancement of insulin resistance is followed by a compensatory increase in insulin secretion from pancreatic β-cells to maintain the overall disposal of glucose[51].

Conclusion

A supplementation for 7 months of fish oil (rich in *n*-3 long-chain PUFA) did not alter insulin metabolism in healthy adult male pigs. According to previous human studies, the results do support the lack of effect of *n*-3 long-chain PUFA on insulin sensitivity (HOMA2-IR, ISI) and insulin secretion (C-peptide, HOMA2-%B). The relationship between body fat and insulin sensitivity also suggests that the adult male pig could be a promising animal model for studies on insulin metabolism.

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All authors contributed to the writing of the manuscript and interpretation of the results, reviewed its content and approved the final version submitted for publication. J. M. was the principal investigator for the design of the study and supervision of data collection; C.-A. C. and I. A. contributed to the design of the study, data collection and laboratory analysis; C.-A. C. carried out the statistical analysis; Y. C. contributed to laboratory analysis.

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