Fish oil and fenofibrate inhibit pancreatic islet hypertrophy, and improve glucose and lipid metabolic dysfuntions with different ways in diabetic KK mice

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Summary

We examined the effects of fish oil and fenofibrate (FF) on the pancreatic islet hypertrophy, and on the modification of glucose and lipid metabolic dysfunctions in KK mice with insulin resistance. The mice were fed one of four diets [25 en% lard/safflower oil (LSO), 25 en% fish oil (FO), or each of these diets plus 0.1 wt% FF (LSO/FF, FO/FF)] for 9 weeks. FO group and both FF groups had significantly lower final body and adipose tissue weights than LSO group. Pancreatic islet hypertrophy was observed only in LSO group but not in the other groups with fish oil or FF. And, it is likely that fish oil has a stronger therapeutic effect on islet hypertrophy. Plasma adiponectin level was significantly higher in FO group but not in both FF groups. Expression of hepatic lipogenic enzyme genes such as fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 (SCD-1) was lower in FO groups with or without FF, whereas fatty acid oxidation-related mRNAs such as acyl-CoA oxidase (AOX) and uncoupling protein-2 (UCP-2) were more abundant in FF groups with or without fish oil.

Our results suggest that both fish oil and FF improve pancreatic islet hypertrophy with the amelioration of insulin resistance. Fish oil enhances insulin sensitivity by increasing plasma adiponectin; however, the beneficial effect of FF on insulin resistance seems to be independent of the plasma adiponectin level. These results mean that improvement of glucose and lipid metabolic dysfuctions in diabetic KK mice are independently approached by fish oil and FF.

Keywords: Fish oil, Fenofibrate; Islet hypertrophy; Insulin resistance

Introduction

High fat diets bring about lipid accumulation and *de novo* lipid synthesis, leading to obesity and increasing the risk of diabetes, hypertension, hyperlipidemia, metabolic syndrome, coronary heart disease, and stroke [1-3]. Conversely, diets rich in fish can reduce plasma triglyceride levels in patients with hyperlipidemia and decrease the risk of coronary heart disease [4, 5]. Fish oil contains n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), and inhibits lipogenesis and enhances fatty acid oxidation in the liver [6-8].

Fatty acid and cholesterol synthesis in the liver are mainly regulated by sterol regulatory element-binding proteins (SREBPs) [9-11]. Fish oil represses maturation of SREBP-1 and attenuates expression of genes encoding lipogenesis and cholesterol biosynthesis enzymes [8, 12]. On the other hand, fatty acid oxidation and triglyceride hydrolysis are modulated by peroxisome proliferator-activated receptor α (PPARα) [13-15]. Fish oil activates PPARα and enhances the expression of genes involved in fatty acid oxidation and triglyceride hydrolysis such as acyl-CoA oxidase (AOX), lipoprotein lipase, medium-chain acyl-CoA dehydrogenase, acyl-CoA synthetase and uncoupling protein-2 (UCP-2) [16, 17]. The clinical anti-hyperlipidemia drug fenofibrate, an agonist of PPARα, decreases triglyceride synthesis and increases hepatic fatty acid oxidation, reducing the amount of fatty acids available for triglyceride synthesis [18, 19]. And, fenofibrate treatment lowers plasma concentration of triglycerides and low density lipoprotein (LDL) cholesterol, and raises the high density lipoprotein (HDL) cholesterol level [20, 21].

Our previous study demonstrated that the combination of fish oil and fenofibrate decreased liver triglycerides and total cholesterol levels, white adipose tissue (WAT) weight, and final body weight of C57BL/6 mice [22]. And, in KK mice, which develop diabetes with moderate

obesity, fish oil inhibits the body weight gain and increases insulin sensitivity, whereas combination of fish oil and fenofibrate inhibits the body weight gain to a greater extent than does fish oil alone [23]. This study was designed to examine the beneficial effects of fish oil and fenofibrate on the pancreatic islet hypertrophy, and on the modification of glucose and lipid metabolic dysfunctions in KK mice with insulin resistance.

Materials and methods

Animals and diets

Female KK mice were obtained from Tokyo Laboratory Animals Science Co. (Tokyo, Japan) at 5 weeks of age and were fed a standard rodent diet (CE2; Clea, Tokyo, Japan) for 1 week for acclimatisation. The mice were maintained in a room with controlled temperature (23 ± 2°C) and humidity (55 ± 10%) with a 12h light/12h dark cycle at the Josai University Life Science Center. The mice were divided into four dietary groups (n = 5 in each group). All experimental diets contained 54 en% carbohydrate, 25 en% fat, and 21en% protein and some were supplemented with 0.1 wt% FF. The composition of experimental diets was modified on the basis of AIN-93G as described previously [24]. In this study, a 6:4 lard: safflower oil mixture (LSO) was used as the control dietary fat. The lard (Oriental Yeast, Tokyo, Japan) contained 44% of oleic acid (18:1 n-9), 24% of palmitic acid (16:0), and 14% of stearic acid (18:0) as the main fatty acids. Safflower oil (Benibana Foods, Tokyo, Japan) contained 78% of oleic acid. Fish oil (NOF Corporation, Tokyo, Japan) contained 7% of EPA (20:5 n-3), 25% of DHA (22:6 n-3), 20% of oleic acid, 18% of palmitic acid, and 5% of stearic acid. Fenofibate was purchased from Sigma–Aldrich (St. Louis, MO, USA). The mice

were fed one of four diets [25 en% lard/safflower oil (LSO), 25 en% fish oil (FO), or each of these diets plus 0.1 wt% FF (LSO/FF, FO/FF)] for 9 weeks. The mice were allowed free access to water and feed. The details of all the diets are presented in Table 1. The diet was changed every day, and the residual quantity was recorded. Food intake (g) was represented on per mouse daily basis. Body weight was recorded once every week. All animal experiments were performed in accordance with the "Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions" (Ministry of Education, Culture, Sports, Science and Technology, Japan, Notice No. 71, dated June 1, 2006) and approved by the Institutional Animal Care and Use Committee of the Josai University.

Table 1 Composition of the experimental diets

Ingredient (g)	LSO	FO	LSO/FF	FO /FF
Lard	4.20		4.20	
Safflower oil	6.30		6.30	
Fish oil		10.50		10.50
Casein	21.00	21.00	21.00	21.00
Sucrose	10.50	10.50	10.50	10.50
β-Starch	47.45	47.45	47.45	47.45
Vitamin mix[a]	1.31	1.31	1.31	1.31
Mineral mix[a]	3.68	3.68	3.68	3.68
Cellulose powder	5.25	5.25	5.25	5.25
L-Cystine	0.32	0.32	0.32	0.32
t-Butylhydroquinone	0.0021	0.0021	0.0021	0.0021
Fenofibrate			0.1	0.1
Total (g)	100.0	100.0	100.1	100.1
Total energy (kcal/100g)	386.6	385.8	386.4	385.6
Fat energy (kcal/100g)	97.4	96.6	97.4	96.5
Fat energy(%)	25.2	25.0	25 .2	25 .0

LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate. [a] Vitamin mix and mineral mix were prepared according to the American Institute of Nutrition (AIN)-93G formulation. Vitamin mix included 0.25% sucrose for chorine bitartrate supplementation.

Collection of blood and tissue samples

At the end of the experiment, mice fasted for 3 h were anesthetised with intraperitoneal injections of pentobarbital sodium (Dainippon Sumitomo Pharma, Osaka, Japan). Blood samples were drawn from the inferior vena cava and treated with EDTA-2Na, and were centrifuged (900 ×*g*, 4°C, 10 min) to separate plasma, which was then frozen at −80°C until analysis. The liver, white adipose tissue and pancreas were excised. Tissue samples were weighed, frozen in liquid nitrogen, and stored at −80°C until analysis.

Measurement of liver and plasma parameters

Hepatic lipids were extracted from approximately 100 mg of liver tissue per mouse by the method described by Folch et al. [25]. Hepatic triglycerides and total cholesterol, and plasma triglyceride, total cholesterol and nonesterified fatty acid (NEFA) levels were quantified by the enzymatic colorimetric method using commercial kits (Wako E-Test kits; Wako Pure Chemical Industries Ltd., Osaka, Japan). Plasma aspartate transaminase (AST) and alanine transaminase (ALT) levels were quantified using the Transaminase C-Ⅱ Test kit (Wako Pure Chemical Industries Ltd, Osaka, Japan). Plasma insulin (the mouse Insulin kit) and leptin (the mouse Leptin kit) levels were quantified by the enzyme-linked immunosorbent assay (ELISA) using commercial kits (Morinaga Institute of Biological Science, Tokyo, Japan). The plasma adiponectin level was measured using the mouse/rat Adiponectin ELISA kit (Otsuka Pharmaceutical, Tokyo, Japan). Insulin resistance was assessed using the homeostasis model of assessment-insulin resistance [HOMA-IR, fasting insulin (μU/mL) × fasting glucose (mmol/L) / 22.5].

Morphological analysis of the liver and pancreas

Liver and pancreatic tissue samples were collected from 4 to 5 mice in each group and fixed in 10% neutral buffered formalin (Wako Pure Chemical Industries Ltd, Osaka, Japan).

The tissue samples were then embedded in paraffin, cut into sections, and stained with haematoxylin and eosin (H&E) for morphological examination. In addition, the pancreatic sections were stained with insulin and glucagon antibodies (Takara Bio Inc., Shiga, Japan) for immunohistochemical analysis of islet structure by Kotobiken Medical Laboratories, Inc. (Tokyo, Japan). The core of the pancreatic islet consists of insulin-positive beta cells surrounded by glucagon-positive alpha cells (arrow).

Measurement of mRNA levels by real-time PCR

Portion of liver tissue was homogenised using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), and total RNA was isolated according to the manufacturer's protocol. The quantity and purity of total RNA were carefully verified on a spectrophotometer at A_{260} and A_{280}. mRNA amplification was performed using a QuantiTect SYBR Green Real-time PCR kit (Qiagen, Hilden, Germany), and relative mRNA expression levels were quantified by RT-PCR from the cycle number (C_t) for threshold signal detection on an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The thermal cycling conditions were as follows: reverse transcription at 50°C for 30 min, PCR initial activation at 95°C for 15 min, 40 cycles of denaturation at 94°C for 15 s, annealing at the optimum temperature of each primer for 30 s, and extension at 72°C for 1 min. A housekeeping transcript, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was used as an endogenous control gene. The details of all PCR primers used are shown in Table 2. Results were expressed as the ratio of the obtained value to that of LSO group.

Table 2 Primer sequences for real-time PCR amplification

Genes	Forward primer (5'-3')	Reverse primer (3'-5')
SREBP-1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
Insig-1	TCACAGTGACTGAGCTTCAGCA	TCATCTTCATCACACCCAGGAC
FAS	TCACCACTGTGGGCTCTGCAGAGAAGCGAG	TGTCATTGGCCTCCTCAAAAAGGGCGTCCA
SCD-1	CCGGAGACCCCTTAGATCGA	TAGCCTGTAAAAGATTTCTGCAAACC
MTP	GCTCCCTCAGCTGGTGGAT	CAGGATGGCTTCTAGCGAGTCT
AOX	TCAACAGCCCAACTGTGACTTCCATTA	TCAGGTAGCCATTATCCATCTCTTCA
UCP-2	GTTCCTCTGTCTCGTCTTGC	GGCCTTGAAACCAACCA
GAPDH	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGAT

SREBP-1c, sterol regulatory element binding protein 1; Insig-1, insulin induced gene 1; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase 1; MTP, microsomal triglyceride protein; AOX, acyl-coenzyme A oxidase; UCP-2, uncoupling protein 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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179

180 **Statistical analysis**

181 Values are presented as mean ± SD. Statistical analysis of most data was conducted using
182 two-way analysis of variance (ANOVA), followed by the Tukey–Kramer test (Statview 5.0;
183 SAS Institute Inc., USA). $P<0.05$ was considered significantly different. Statistical analysis
184 of the data for pancreas morphology and insulin positive area in islets was conducted one-
185 way analysis of variance (ANOVA), followed by the Tukey–Kramer test. Groups sharing
186 different letters significantly different.

187

188 **Results**

189 **Body and tissue weights**

190 The energy intakes (kcal/mouse/day) were lower tendency in FO and both FF groups. Final
191 body weight and parametrial WAT weight were lower in FO and both FF groups than in LSO
192 group (Table 3, final body weight: FO, $P = 0.0678$; FF, $P < 0.01$; FO×FF, $P < 0.01$,
193 parametrial WAT weight: FO, $P < 0.05$; FF, $P < 0.01$; FO×FF, $P < 0.01$). Body weight was
194 lower in FO and FO/FF groups than in LSO group as early as 3 weeks since the initiation of
195 the experimental diets (Fig. 1A).

196 Liver weight was decreased in FO group and increased in both FF groups compared to the
197 other groups (Table 3: FO, $P < 0.05$; FF, $P < 0.01$; FO×FF, $P < 0.01$). There was no main
198 effect of FF or FO×FF interaction, but there was a main effect of FO in the plasma aspartate
199 transaminase (AST) levels. And, there was no main effect of FO or FO×FF interaction, but
200 there was a main effect of FF in the alanine transaminase (ALT) levels (Table 3).
201

Table 3 Effects of fish oil and fenofibrate on body weights, tissue weights, and liver function makers of diabetic KK mice

	Groups				Two-way ANOVA *P*-values		
	LSO	FO	LSO/FF	FO/FF	FO	FF	FO × FF
Food intake (kcal/mouse/day)[a]	17.0	15.3	16.4	15.4	-	-	-
Body and tissue weights							
Initial body weight (g)	24.7±1.4	24.6±0.9	24.7±1.3	24.6±0.7	NS	NS	NS
Final body weight (g)	46.0±4.7	39.2±2.7	37.7±1.8	39.3±1.6	0.0678	<0.01	<0.01
Parametrial WAT weight (g)	4.62±0.67	3.34±0.43	2.78±0.49	2.88±0.31	<0.05	<0.01	<0.01
Perirenal WAT weight (g)	0.50±0.07	0.53±0.22	0.33±0.03	0.41±0.10	NS	<0.05	NS
Liver weight (g)	2.88±0.93	1.50±0.24	3.30±0.16	3.53±0.12	<0.05	<0.01	<0.01
Liver function makers							
AST activity (IU/L)	20.9±5.8	35.6±13.5	19.6±7.4	35.1±7.9	<0.01	NS	NS
ALT activity (IU/L)	6.7±2.1	6.2±3.9	10.9±5.7	11.3±2.2	NS	<0.05	NS

LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate; WAT, white adipose tissue; AST, aspartate aminotransferase; ALT, alanine aminotransferase. [a]Daily food energy intake calculated on the basis of total energy for each experimental diet. Values represent the mean ± S.D. (*n*=4-5). NS, not significant.

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203

204 **Plasma biochemical markers**

205 As shown in Table 4, although no differences were observed in blood glucose levels by fish
206 oil or fenofibrate (FF), plasma insulin and HOMA-IR levels were significantly lower in FO
207 and both FF groups than in LSO group. There was a main effect of FO and FF and FO×FF
208 interaction in the plasma insulin and HOMA-IR levels. Plasma adiponectin level was
209 significantly higher in FO group compare with the other groups, showing a main effect of FO
210 and FO×FF interaction, but no main effect of FF. However, plasma leptin levels did not
211 change among the groups.

No significant differences in plasma triglyceride levels were observed between all groups. Plasma total cholesterol levels were lower in FO and FO/FF groups and were higher in LSO/FF group than in LSO group. There was a main effect of FO and FF, but no main effect of FO×FF interaction. Plasma NEFA levels were also significantly lower in FO and both FF groups than in LSO group (Table 4: FO, $P < 0.01$; FF, $P < 0.01$; FO×FF, $P = 0.0561$).

Table 4 Effects of fish oil and fenofibrate on glucose homeostasis makers and plasma biochemical makers of diabetic KK mice

	Groups				Two-way ANOVA *P*-values		
	LSO	FO	LSO/FF	FO/FF	FO	FF	FO × FF
Glucose homeostasis makers							
Blood glucose (mmol/L)	10.6±2.6	10.6±2.5	13.1±1.8	11.5±1.3	NS	NS	NS
Plasma insulin (pmol/L)	1.52±0.62	0.47±0.34	0.31±0.22	0.18±0.06	<0.01	<0.01	<0.05
HOMA-IR	101±25	30±20	29±24	14±6	<0.01	<0.01	<0.01
Plasma biochemical makers							
Adiponectin (μg/mL)	17.4±4.4	31.1±7.8	21.6±1.5	21.8±2.6	<0.01	NS	<0.01
Leptin (ng/mL)	28.5±1.4	28.0±2.7	22.4±7.4	27.0±3.0	NS	NS	NS
Triglyceride (mmol/L)	1.29±0.53	1.25±0.13	1.26±0.18	0.87±0.25	NS	NS	NS
Total cholesterol (mmol/L)	4.56±0.81	1.94±0.09	5.35±0.45	3.54±0.37	<0.01	<0.01	NS
NEFA (mmol/L)	0.92±0.30	0.31±0.09	0.52±0.08	0.22±0.08	<0.01	<0.01	0.0561

LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate; HOMA-IR, homeostasis model assessment for insulin resistance; NEFA, non-esterified fatty acid. Values represent the mean ± S.D. (n=4-5). NS, not significant.

Liver morphology and hepatic lipids

To determine the effects of fish oil and FF on liver fat accumulation, liver tissue samples were compared using morphology analysis. Liver tissue in LSO group showed numerous fat droplets throughout the hepatocyte, whereas no fat droplets were detected in the hepatocytes of FO and both FF groups (Fig. 1B). Hepatic triglyceride and total cholesterol levels were significantly lower in FO and both FF groups than in LSO group. There was a main effect of FO and FF and FO×FF interaction in the hepatic triglyceride and total cholesterol levels (Fig. 1C and D).

Pancreas morphology and insulin positive area in islets

We examined whether fish oil and FF inhibit pancreatic islet hypertrophy. Haematoxylin and eosin (H&E) staining revealed a markedly greater islet size in LSO group than in FO and both FF groups (Fig. 2A). Statistical analysis of the data for pancreas morphology and insulin positive area in islets was conducted one-way ANOVA, because the number of islets among groups is too different to compare by two-way ANOVA. Pancreatic islets area was significantly lower in FO and FO/FF groups than in LSO group (Fig. 2A and D). Also, islet density (islet number /section) results were similar trends (15.3 ± 3.8, 7.3 ± 3.2, 4.8 ± 2.2 and 4.8 ± 1.3 in LSO, FO, LSO/FF and FO/FF, respectively). Insulin positive area in islets was significantly higher in FO and both FF groups than in LSO group (Fig. 2B and E). However, glucagon positive area did not differ significantly among the groups (Fig. 2C and F).

Hepatic expression of genes involved in lipid metabolism

To examine the effects of fish oil and FF on lipid metabolism in the liver, hepatic expression of mRNA encoding enzymes involved in lipid metabolism was measured. Only minor differences were observed in SREBP-1c mRNA levels among the groups. However, the mRNA levels of insulin induced gene 1 (Insig-1), stearoyl-CoA desaturase 1 (SCD-1) and fatty acid synthase (FAS) tended to be greatly lower in both FO groups, with or without FF. There was a main effect of FO, but no main effect of FF and FO×FF interaction in the mRNA levels of Insig-1, SCD-1 and FAS. The mRNA levels of microsomal triglyceride transfer protein were significantly higher in both FF groups than in FF-free groups, showing a main effect of FO and FF, but no main effect of FO×FF interaction (Fig. 3).

The mRNA levels of AOX and UCP-2, which are related to fatty acid oxidation and heat production, respectively, were significantly higher in both FF groups. There was a main effect

of FF, but no main effect of FO and FO×FF interaction in the mRNA levels of AOX and UCP-2 (Fig. 4).

Discussion

In this study, we evaluated the effects of fish oil and FF on the pancreatic islet hypertrophy and observed the modification of glucose and lipid dysfunction in KK mice with insulin resistance.

The diet containing fish oil significantly decreased final body weight and parametrial WAT weight in KK mice, which correlates with our previous results [23, 26].

FF treatment in the C57BL/6 mice has been resulted in liver weight increase that is caused by a peroxisome proliferation [27, 28]. In the present study also, the 0.1% FF treatment in the female KK mice increased the liver weights.

Fish oil consumption reduces the risk of developing a cardiovascular disease by decreasing the plasma triglyceride concentration [4, 5]. However, in the present study, the fish oil dose used in this study did not decrease the plasma triglyceride level. These results are consistent with our previous studies [23, 26]. We have been also observed that plasma triglyceride levels were markedly decreased in female KK mice fed 50 en% fish oil diet (data not shown). It appears that 25 en% fish oil is not sufficient to decrease plasma triglyceride level caused by the obese state of female KK mice.

Fish oil can induce AOX and UCP-2 mRNA expression through PPARα activation [16, 17]. In the present study, fish oil cannot induce AOX and UCP-2 mRNA expression in the liver, perhaps because 25 en% fish oil are insufficient for the induction of fatty acid β-oxidation. In contrast, 0.1% FF supplementation induced fatty acid oxidation through an increase in AOX and UCP-2 mRNA expression.

277 FF is known as an agonist of PPARα; it induces fatty acid β-oxidation and lowers hepatic
278 fat accumulation [20, 23]. The present study shows that FF-supplemented diets decreased
279 hepatic triglycerides and total cholesterol with or without fish oil. And, FF reduced both final
280 body weight and fat mass as well as plasma insulin levels. On the other hand, other studies
281 have shown that FF increases hepatic peroxisome proliferation and liver weight, resulting in
282 elevated AST and ALT levels [29, 30]. In the present study, although liver weights were
283 increased in both FF groups, and AST and ALT levels remained within the normal range.
284 These data suggest that 0.1% FF is sufficient for amelioration of genetic abnormalities in
285 lipid and glucose metabolism (regardless of the dietary fat source) without the risk of liver
286 damage.
287 The basal plasma insulin level is markedly higher in KK mice (2–3 pmol/L) than in
288 C57BL/6 mice (0.2–0.3 pmol/L), and obesity-prone KK mice develop hyperinsulinemia [23,
289 31]. In the present study, the plasma insulin level and the HOMA-IR index were extreamly
290 high in LSO group (fasting insulin 1.5 pmol/L, HOMA-IR 101). And, fish oil and/or FF
291 markedly decreased the plasma insulin level and HOMA-IR similar to those of non-obese,
292 non-diabetic C57BL/6 mice. Thus, we suggest that 25 en% fish oil and/or 0.1 wt% FF are
293 sufficient to protect obesity- and diabetes- prone KK mice from insulin resistance.
294 Many studies have linked obesity and insulin resistance with hypertrophy and hyperplasia
295 of pancreatic islet cells [32, 33]. According to our results, pancreatic islet hypertrophy and
296 islet density were markedly suppressed, and, insulin positive area was significantly increased
297 by both fish oil and FF. However, it is likely that fish oil has a stronger therapeutic effect on
298 islet hypertrophy. Further research is required to clarify the therapeutic effects of fish oil and
299 FF on pancreatic islet hypertrophy and insulin resistance.
300 Adiponectin released from adipose cells inhibits gluconeogenesis and increases glucose

uptake and fatty acid oxidation, resulting in enhanced insulin sensitivity [34, 35]. In the present study, plasma adiponectin levels were significantly higher in FO group but not in any FF-treated group. This result indicates that fish oil enhance insulin sensitivity by increasing plasma adiponectin; however, the beneficial effects of FF on body weight and insulin resistance are independent of the plasma adiponectin level. These results suggest that there is a diversity of relationships between the plasma adiponectin levels and insulin resistance.

Conclusions

Our results suggest that fish oil and FF improve pancreatic islet hypertrophy with the amelioration of insulin resistance, and positively modulate glucose and lipid metabolism in obesity and diabetes female KK mice. And, these good effects of fish oil and FF may be via a different way.

Abbreviations

ACC, acetyl-CoA carboxylase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AOX, acyl-CoA oxidase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAS, fatty acid synthase; FF, fenofibrate; FO, fish oil; H&E, haematoxylin eosin; HOMA-IR, homeostasis model assessment for insulin resistance; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase; SREBPs, sterol regulatory element-binding proteins; UCP, uncoupling protein; WAT, white adipose tissue.

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Conflict of interest

The authors have nothing to declare.

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Figure legends

Figure 1

Effects of fish oil and fenofibrate on body weight, hepatic fat accumulation, hepatic triglyceride and total cholesterol levels of diabetic KK mice. LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate. Mice were fed the indicated experimental diets for 9 weeks. (A) Body weights, (B) liver morphology, (C) hepatic triglyceride and (D) hepatic total cholesterol. Body weights were plotted until 9 weeks. Liver tissues from 4 to 5 mice in each group were embedded in paraffin, stained with H&E, and examined under a microscope (400×). Values represent mean ± S.D. (n = 4-5).Two-way ANOVA results are shown at the bottom of the each figure. NS, not significant.

Figure 2

Effects of fish oil and fenofibrate on morphology of pancreatic islet in diabetic KK mice. LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate. Mice were fed the indicated experimental diets for 9 weeks. (A) Islet stained with H&E, (B) islet marked with insulin antibodies, (C) islet marked with glucagon antibodies, (D) islets area, (E) insulin positive area and (F) glucagon positive area. Pancreatic tissues from 4 to 5 mice in each group were embedded in paraffin, and stained with H&E, insulin and glucagon antibodies. Then, the tissue section examined under a microscope (400×). The core of the pancreatic islet consists of insulin-positive beta cells surrounded by glucagon-positive alpha cells (arrow). Insulin and glucagon positive areas were represented as a percentage of the islets areas. (D, E) The data were compared by one-way ANOVA, followed by the Tukey-Kramer test. Groups sharing different letters significantly different ($P < 0.05$).

Figure 3

Effects of fish oil and fenofibrate on hepatic mRNA expression of genes involved in fatty acid synthesis in diabetic KK mice. LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate. Mice were fed the indicated experimental diets for 9 weeks. Panels show mRNA expression levels of (A) SREBP-1c, (B) Insig-1, (C) FAS, (D) SCD-1 and (E) MTP. mRNA levels were determined by real-time quantitative PCR. Results are expressed as the ratio of the obtained value to that of LSO group. Values represent mean ± S.D. (n = 4-5). Two-way ANOVA results are shown at the bottom of the each figure. NS, not significant.

Figure 4

Effects of fish oil and fenofibrate on hepatic mRNA expression of genes involved in fatty acid oxidation and cholesterol metabolism in diabetic KK mice. LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate. Mice were fed the indicated experimental diets for 9 weeks. Panels show mRNA expression levels of (A) AOX and (B) UCP-2. mRNA levels were determined by real-time quantitative PCR. Results are expressed as the ratio of the obtained value to that of LSO group. Values represent mean ± S.D. (n = 4-5). Two-way ANOVA results are shown at the bottom of the each figure. NS, not significant.

473

Table 1 Composition of the experimental diets

Ingredient (g)	LSO	FO	LSO/FF	FO /FF
Lard	4.20		4.20	
Safflower oil	6.30		6.30	
Fish oil		10.50		10.50
Casein	21.00	21.00	21.00	21.00
Sucrose	10.50	10.50	10.50	10.50
β-Starch	47.45	47.45	47.45	47.45
Vitamin mix[a]	1.31	1.31	1.31	1.31
Mineral mix[a]	3.68	3.68	3.68	3.68
Cellulose powder	5.25	5.25	5.25	5.25
L-Cystine	0.32	0.32	0.32	0.32
t-Butylhydroquinone	0.0021	0.0021	0.0021	0.0021
Fenofibrate			0.1	0.1
Total (g)	100.0	100.0	100.1	100.1
Total energy (kcal/100g)	386.6	385.8	386.4	385.6
Fat energy (kcal/100g)	97.4	96.6	97.4	96.5
Fat energy(%)	25.2	25.0	25 .2	25 .0

LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate. [a] Vitamin mix and mineral mix were prepared according to the American Institute of Nutrition (AIN)-93G formulation. Vitamin mix included 0.25% sucrose for chorine bitartrate supplementation.

Table 2 Primer sequences for real-time PCR amplification

Genes	Forward primer (5’-3’)	Reverse primer (3’-5’)
SREBP-1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
Insig-1	TCACAGTGACTGAGCTTCAGCA	TCATCTTCATCACACCCAGGAC
FAS	TCACCACTGTGGGCTCTGCAGAGAAGCGAG	TGTCATTGGCCTCCTCAAAAAGGGCGTCCA
SCD-1	CCGGAGACCCCTTAGATCGA	TAGCCTGTAAAAGATTTCTGCAAACC
MTP	GCTCCCTCAGCTGGTGGAT	CAGGATGGCTTCTAGCGAGTCT
AOX	TCAACAGCCCAACTGTGACTTCCATTA	TCAGGTAGCCATTATCCATCTCTTCA
UCP-2	GTTCCTCTGTCTCGTCTTGC	GGCCTTGAAACCAACCA
GAPDH	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGAT

SREBP-1c, sterol regulatory element binding protein 1; Insig-1, insulin induced gene 1; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase 1; MTP, microsomal triglyceride protein; AOX, acyl-coenzyme A oxidase; UCP-2, uncoupling protein 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 3 Effects of fish oil and fenofibrate on body weights, tissue weights, and liver function makers of diabetic KK mice

	Groups				Two-way ANOVA *P*-values		
	LSO	FO	LSO/FF	FO/FF	FO	FF	FO × FF
Food intake (kcal/mouse/day) [a]	17.0	15.3	16.4	15.4	-	-	-
Body and tissue weights							
Initial body weight (g)	24.7±1.4	24.6±0.9	24.7±1.3	24.6±0.7	NS	NS	NS
Final body weight (g)	46.0±4.7	39.2±2.7	37.7±1.8	39.3±1.6	0.0678	<0.01	<0.01
Parametrial WAT weight (g)	4.62±0.67	3.34±0.43	2.78±0.49	2.88±0.31	<0.05	<0.01	<0.01
Perirenal WAT weight (g)	0.50±0.07	0.53±0.22	0.33±0.03	0.41±0.10	NS	<0.05	NS
Liver weight (g)	2.88±0.93	1.50±0.24	3.30±0.16	3.53±0.12	<0.05	<0.01	<0.01
Liver function makers							
AST activity (IU/L)	20.9±5.8	35.6±13.5	19.6±7.4	35.1±7.9	<0.01	NS	NS
ALT activity (IU/L)	6.7±2.1	6.2±3.9	10.9±5.7	11.3±2.2	NS	<0.05	NS

LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate; WAT, white adipose tissue; AST, aspartate aminotransferase; ALT, alanine aminotransferase. [a]Daily food energy intake calculated on the basis of total energy for each experimental diet. Values represent the mean ± S.D. (*n*=4-5). NS, not significant.

Table 4 Effects of fish oil and fenofibrate on glucose homeostasis makers and plasma biochemical makers of diabetic KK mice

	Groups				Two-way ANOVA *P*-values		
	LSO	FO	LSO/FF	FO/FF	FO	FF	FO × FF
Glucose homeostasis makers							
Blood glucose (mmol/L)	10.6±2.6	10.6±2.5	13.1±1.8	11.5±1.3	NS	NS	NS
Plasma insulin (pmol/L)	1.52±0.62	0.47±0.34	0.31±0.22	0.18±0.06	<0.01	<0.01	<0.05
HOMA-IR	101±25	30±20	29±24	14±6	<0.01	<0.01	<0.01
Plasma biochemical makers							
Adiponectin (μg/mL)	17.4±4.4	31.1±7.8	21.6±1.5	21.8±2.6	<0.01	NS	<0.01
Leptin (ng/mL)	28.5±1.4	28.0±2.7	22.4±7.4	27.0±3.0	NS	NS	NS
Triglyceride (mmol/L)	1.29±0.53	1.25±0.13	1.26±0.18	0.87±0.25	NS	NS	NS
Total cholesterol (mmol/L)	4.56±0.81	1.94±0.09	5.35±0.45	3.54±0.37	<0.01	<0.01	NS
NEFA (mmol/L)	0.92±0.30	0.31±0.09	0.52±0.08	0.22±0.08	<0.01	<0.01	0.0561

LSO, lard/safflower oil; FO, fish oil; FF, fenofibrate; HOMA-IR, homeostasis model assessment for insulin resistance; NEFA, non-esterified fatty acid. Values represent the mean ± S.D. (*n*=4-5). NS, not significant.

Figure 1

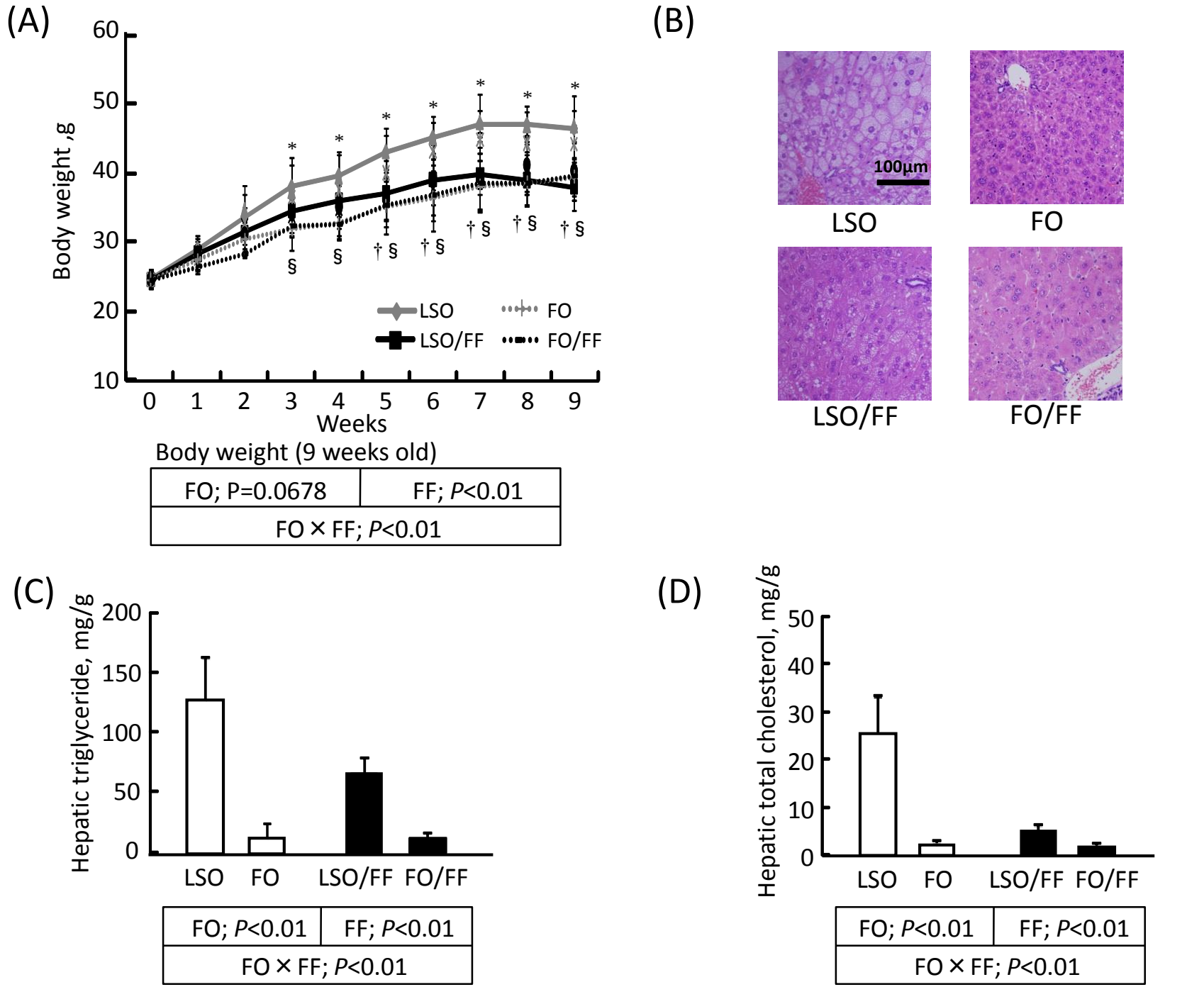
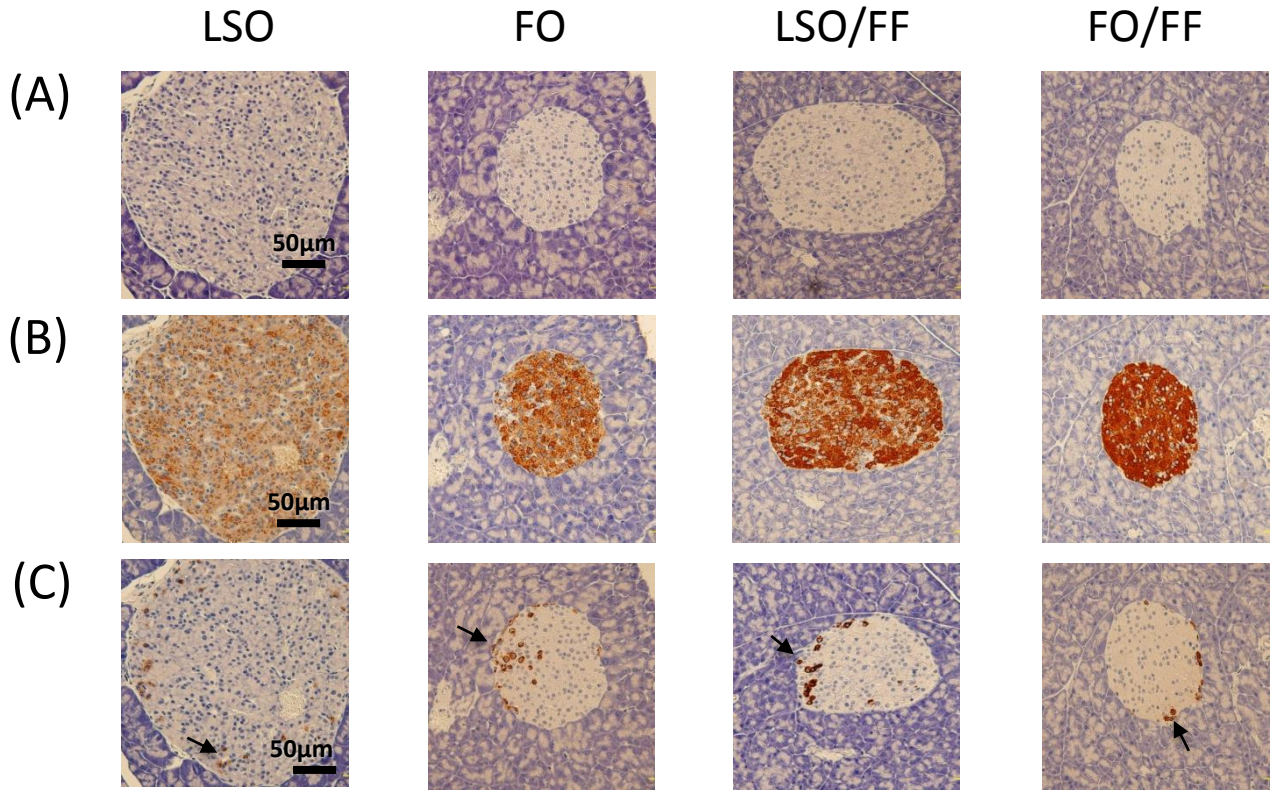
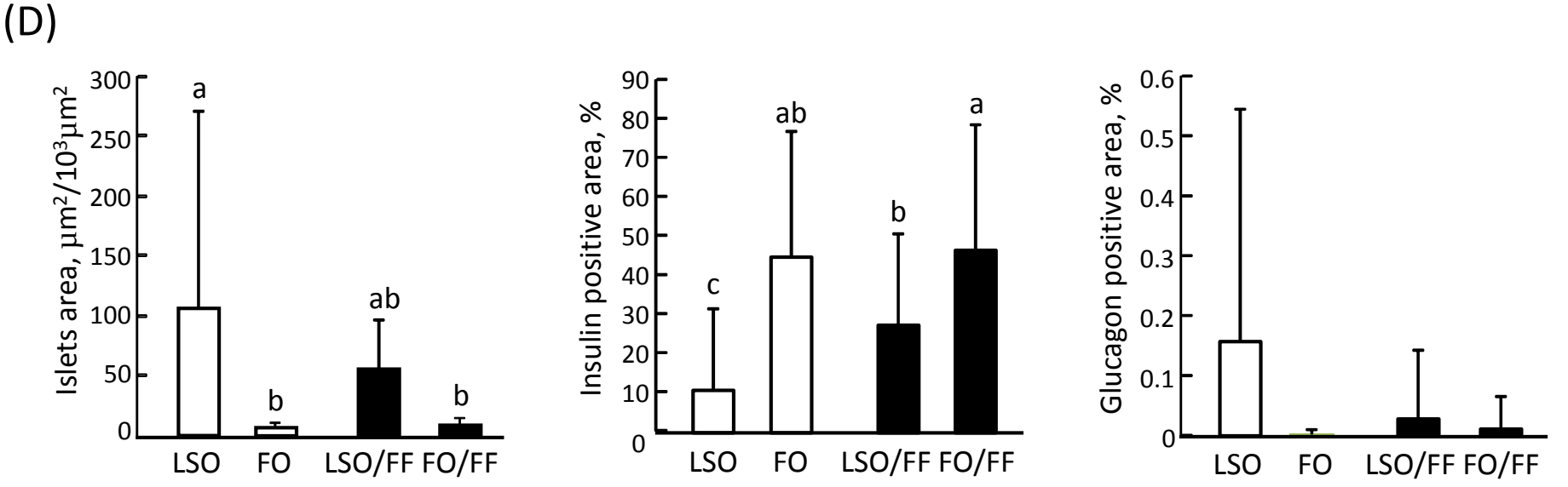

(A)
Body weight ,g
Weeks
LSO
FO
LSO/FF
FO/FF
Body weight (9 weeks old)
FO; P=0.0678
FF; P<0.01
FO × FF; P<0.01
(B)
100μm
LSO
FO
LSO/FF
FO/FF
(C)
Hepatic triglyceride, mg/g
LSO
FO
LSO/FF
FO/FF
FO; P<0.01
FF; P<0.01
FO × FF; P<0.01
(D)
Hepatic total cholesterol, mg/g
LSO
FO
LSO/FF
FO/FF
FO; P<0.01
FF; P<0.01
FO × FF; P<0.01

Figure 2


LSO
FO
LSO/FF
FO/FF
(A)
(B)
(C)
50µm
50µm
50µm


(D)
Islets area, µm²/10³µm²
300
250
200
150
100
50
0
a
b
ab
b
LSO
FO
LSO/FF
FO/FF
Insulin positive area, %
90
80
70
60
50
40
30
20
10
0
c
ab
b
a
LSO
FO
LSO/FF
FO/FF
Glucagon positive area, %
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0.5
0.4
0.3
0.2
0.1
0
LSO
FO
LSO/FF
FO/FF

Figure 3

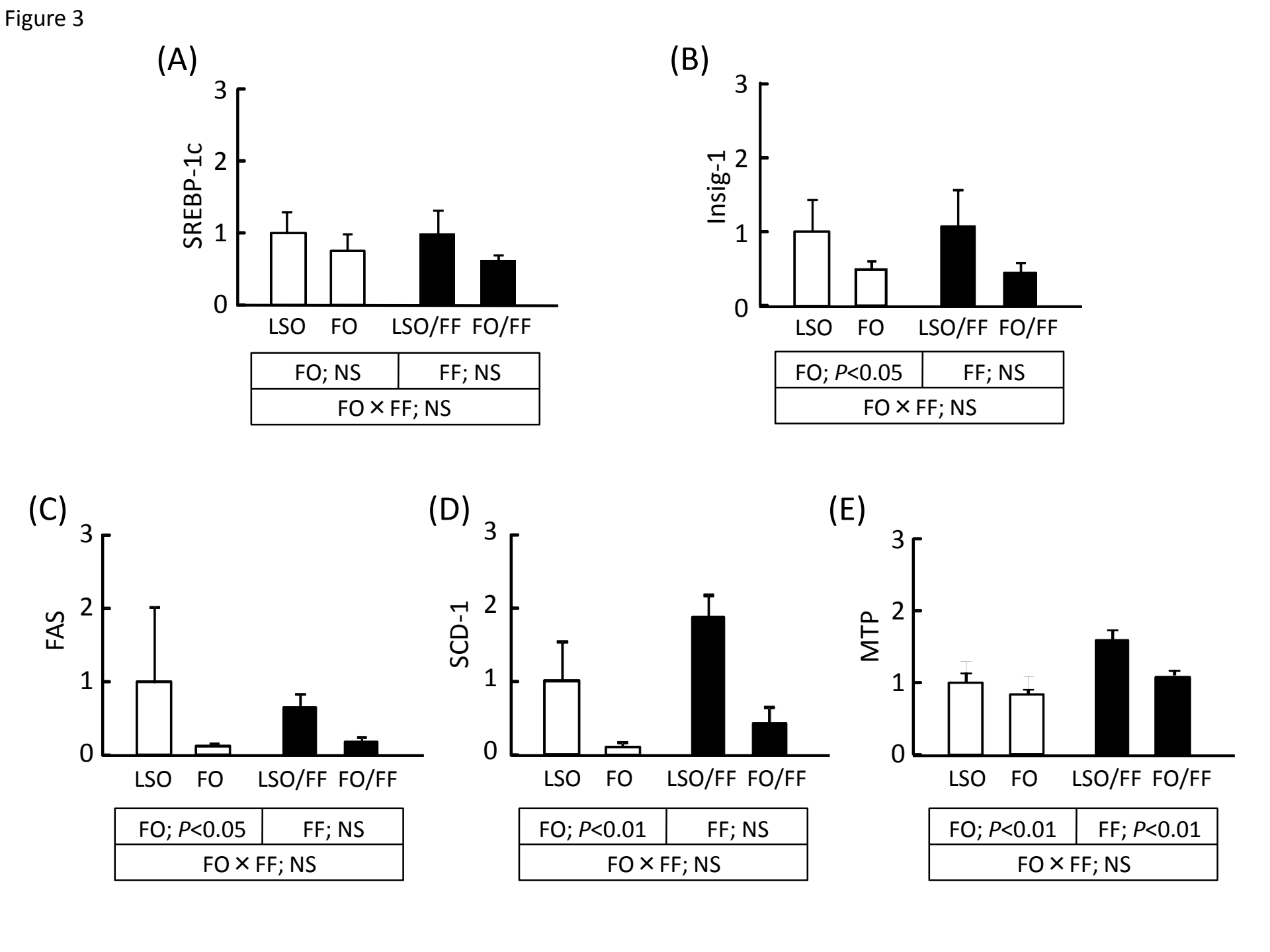
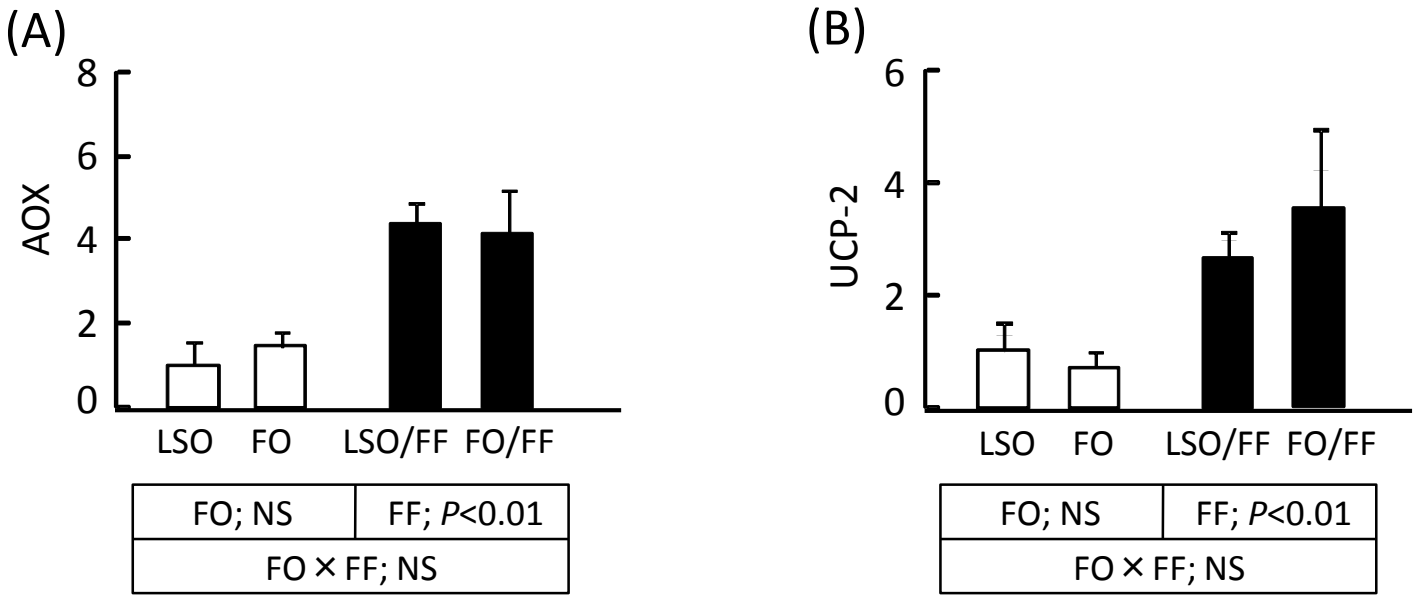

(A)
SREBP-1c
3
2
1
0
LSO
FO
LSO/FF
FO/FF
FO; NS
FF; NS
FO × FF; NS
(B)
Insig-1
3
2
1
0
LSO
FO
LSO/FF
FO/FF
FO; P<0.05
FF; NS
FO × FF; NS
(C)
FAS
3
2
1
0
LSO
FO
LSO/FF
FO/FF
FO; P<0.05
FF; NS
FO × FF; NS
(D)
SCD-1
3
2
1
0
LSO
FO
LSO/FF
FO/FF
FO; P<0.01
FF; NS
FO × FF; NS
(E)
MTP
3
2
1
0
LSO
FO
LSO/FF
FO/FF
FO; P<0.01
FF; P<0.01
FO × FF; NS

Figure 4


(A)
AOX
8
6
4
2
0
LSO
FO
LSO/FF
FO/FF
FO; NS
FF; P<0.01
FO × FF; NS
(B)
UCP-2
6
4
2
0
LSO
FO
LSO/FF
FO/FF
FO; NS
FF; P<0.01
FO × FF; NS