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Article

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Oral Administration of Apple Procyanidins Ameliorates Insulin Resistance via Suppression of Pro-inflammatory Cytokines Expression in Liver of Diabetic ob/ob Mice

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ABSTRACT

Procyanidins, the main ingredient of apple polyphenols, are known to possess anti-oxidative and anti-inflammatory effects associated closely with the pathophysiology of insulin resistance and type 2 diabetes. We investigated the effects of orally administered apple procyanidins (APCs) on glucose metabolism using diabetic ob/ob mice. We found no difference in body weight or body composition between APCs-treated and untreated mice. 4-week oral administration of APCs containing water (0.5% w/v) ameliorated glucose tolerance, insulin resistance, and hepatic gluconeogenesis in ob/ob mice. APCs also suppressed the increase of pancreatic β-cell. Insulin-stimulated Akt phosphorylation was significantly enhanced, pro-inflammatory cytokine expression levels were significantly decreased, and c-Jun N-terminal kinase (JNK) phosphorylation was down-regulated in the liver of those APCs-treated mice. In conclusion, APCs ameliorate insulin resistance by improving hepatic insulin signaling through suppression of hepatic inflammation in ob/ob mice, which may be a mechanism of possible beneficial health effects of APCs in disturbed glucose metabolism.

Keywords: apple procyanidins, insulin resistance, inflammation, ob/ob mouse

■INTRODUCTION

Insulin resistance induced by obesity readily develops into type 2 diabetes and leads to elevated risk of cardiovascular disease. The pathogenesis of type 2 diabetes is characterized by two major features, insulin resistance and impaired insulin secretion. If insulin demand due to insulin resistance is over the capacity of pancreatic β-cells, blood glucose homeostasis cannot be maintained, leading to chronic hyperglycemia. Reducing insulin resistance is therefore clinically important for the prevention and management of type 2 diabetes.

The mechanism of insulin resistance is still unclear. Insulin resistance is reported to be associated with a state of chronic and low-grade inflammation in insulin target tissues including adipose tissue, liver and skeletal muscle. Tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine produced from accumulated fat, activates various signaling cascades, including c-Jun N-terminal kinase (JNK). JNK leads to serine phosphorylation of insulin receptor substrate (IRS)-1 and 2, and consequently induces insulin resistance.[1] Oxidative stress also activates the JNK pathway and induces insulin resistance.[2]

Epidemiological studies suggest that consumption of fruits and vegetables reduces

the risk of developing type 2 diabetes.[3,4] The benefits of fruits and vegetables have been attributed to their dietary fiber and various phytochemicals, such as polyphenol. Apple is one of the most commonly consumed fruits in the world. Apple polyphenols are known to have various physiological effects including antioxidant activity, [5] anti-inflammation activity,[6] and anti-tumor activity.[7]

Apple procyanidins (APCs) are the main ingredient of apple polyphenols [8] and consist of flavanol units such as (+)-catechin and (–)-epicatechin, which are linked together through 4→8 and 4→6 interflavonoid bonds, and have many isomeric forms depending on the extent of polymerization and the nature of their constituent units (Figure S1). Recent research has indicated that APCs have various beneficial effects on health, including anti-aging effects in *Caenorhabditis elegans*,[9] an inhibitory effect on triglyceride absorption through inhibition of pancreatic lipase activity in mouse and human,[10] and anti-inflammatory and immunomodulatory effects on intestinal epithelial cells.[11]

However, there are few reports regarding ingestion of APCs and risk of type 2 diabetes. In this study, we investigated the effects of APCs on glucose metabolism using model mice for obesity and type 2 diabetes. Our findings may lead to a strategy for development of therapeutic agents for impaired glucose tolerance and type 2

diabetes.

■MATERIALS AND METHODS

Preparation of apple polyphenol extracts

The procyanidin fraction was prepared from apple (*Malus pumila* cv. Fuji) by preparative column chromatography with the method of previous study.[12] Briefly, the apple polyphenol fraction was prepared from apple juice using the preparative column with aromatic synthetic adsorbents**,** Sepabeads SP-850 (Mitsubishi Kasei Co., Ltd., Japan). Apple polyphenol extracts were lyophilized, and the powder obtained was dissolved in distilled water and adjusted to pH 6.5 with 5N NaOH. The sample was applied to a Diaion HP-20ss (Mitsubishi Kasei Co., Ltd., Japan) column, and after rinsing the column with distilled water, the procyanidin fraction was eluted with 25% ethanol. Finally, the eluate was concentrated by rotary evaporation at 45°C and lyophilized as the APCs fraction. APCs were analyzed using by reversed-phase HPLC equipped with an LC-10AD VP pump (Shimadzu, Kyoto, Japan), an SIL-10AD VP autosampler (Shimadzu), and a Inertsil ODS-3 (GL Sciences Inc., Tokyo, Japan) reversed-phase column (150 x 4.6 mm i.d.) at 40°C. Mixtures of 10 mM KH_2PO_4 solution (adjusted to pH 1.8 with H_3PO_4) and methanol was used as the mobile phase

91 [mobile phase A, 10 mM KH_2PO_4:MeOH (8:2) and mobile phase B, 10 mM
92 KH_2PO_4:MeOH (5:5)] were used as the mobile phases with a flow rate of 1.0 ml/min.
93 Detection was performed using a SPD-10A VP UV-vis detector (Shimadzu) at 280 nm.
94 For the first 10 min, the initial eluent used was 0% mobile phase B, followed by a
95 linear gradient from 100% mobile phase B for 40 min; subsequently the concentration
96 was held at 100% mobile phase B for 15 min and then returned to the initial conditions.
97 This fraction did not include phloretin glucoside (phlorizin) or chlorogenic acid
98 (Figure 1). The former has a blood glucose lowering effect by inhibiting sodium
99 glucose cotransporter (SGLT1 and SGLT2),[13] and the latter has several beneficial
100 biological properties including blood pressure lowering and anti-oxidative effects.[14,15]
101
102 **Mice**
103 5-week-old male B6.Cg-Lepob/J mice (C57BL/6J background) were purchased
104 from Charles River Japan Inc. (Kanagawa, Japan). The phenotype is obese and insulin
105 resistance but hyperglycemia is not so severe. They were divided into two groups: an
106 APCs-treated and an untreated group. The mice were housed in individual under
107 controlled environment at 23℃ and 12-hour light/dark cycle with free access to water
108 and a commercial nonpurified diet (MF, Oriental Yeast Co., Tokyo, Japan). All animal

109 experiments were approved by the Kyoto University Animal Care Committee.
110 Beginning at 8 weeks of age, mice were administered APCs dissolved in drinking
111 water (0.5%, w/v) ad libitum for 4 weeks. Body weight and food intake were measured
112 once every week and water intake was measured every day. During continuation of the
113 APCs administration to 16 weeks of age, insulin tolerance test (ITT), oral glucose
114 tolerance test (OGTT), and pyruvate tolerance test (PTT) were performed.
115
116 **Measurement of energy expenditure**
117 Energy expenditure of the mice at the age of 16 weeks was measured for 48 hours
118 using indirect calorimetry. Oxygen consumption and CO_2 production were determined
119 every 5 min in an open chamber with the mass spectrometry-based O_2 and
120 CO_2 analyzer ARCO-2000 (ARCO system, Chiba, Japan). Oxygen consumption was
121 normalized by lean body mass.
122
123 **Measurement of blood glucose, oral glucose tolerance test and insulin tolerance**
124 **test**
125 After 4 weeks administration of water with or without 0.5% APCs, APCs-treated
126 and untreated ob/ob mice were fasted overnight for 16 h, and received an oral dose of

1 g/kg glucose. Blood glucose levels and serum insulin concentrations were measured at 0, 15, 30, 60, and 120 min after oral injection by the glucose oxidase method (Sanwa Kagaku Kenkyusho, Nagoya, Japan) and an ELISA kit (Shibayagi Co. Ltd, Gunma, Japan), respectively. After 5 weeks administration of APCs, APCs-treated and untreated ob/ob mice were fasted for 16 h, and regular insulin (2 units/kg) was injected intraperitoneally. Blood glucose levels were measured at 0, 15, 30, 45, 60, 90, and 120 min after injection.

Pyruvate tolerance test

Pyruvate was dissolved with 0.9% (wt/vol) sterile saline. APCs-treated and untreated ob/ob mice were fasted overnight for 16 h, and pyruvate (1 g/kg) was injected intraperitoneally. Blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 min after injection.[16]

Calculation of homeostasis model assessment of insulin resistance

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using fasting blood glucose and insulin concentrations based on OGTT data. HOMA-IR is used to estimate insulin resistance in human and animals.[17,18] HOMA-IR

was calculated using the following formula:[19]

HOMA-IR = insulin (mU/L) x [glucose (mg/dL)/405]

Measurement of body fat composition

Body fat mass was measured by CT scan (LaTheta LCT-100, Aloka, Tokyo, Japan). The mice were anesthetized, and the images were analyzed using LaTheta software, version 1.00 and values of subcutaneous and visceral fat mass were quantified in grams (g).

Measurement of serum adiponectin

Mice at the age of 16 weeks were sacrificed and blood samples were taken. Serum adiponectin concentration was measured by ELISA kit (Otsuka Pharmaceutical Co. ltd, Tokyo, Japan) according to the instruction manuals.

Histomorphology and immunohistochemistry

The sections of paraffin embedding pancreas in mice at the age of 16 weeks were incubated with anti-glucagon mouse monoclonal antibody (cloneK79bB10, 1:2000

dilution; Abcam plc, Cambridge, UK) and polyclonal rabbit anti-insulin (H-86) antibody (1:100 dilution; Santa Cruz Biotechnology, Inc., Texas, U.S.A.). The sections were then incubated with goat anti-mouse IgG and goat anti-rabbit fluorescein-conjugated secondary antibody (1:200 dilution, Alexa Fluor 488; Alexa Fluor 546; Invitrogen/Life Technologies Japan, Tokyo, Japan). Two slides randomly selected from each pancreas were analyzed. After immunostaining, quantification of β-cell area was performed by immunofluorescent microscope using BZ-Ⅱ Analyzer software (Keyence Corp., Osaka, Japan). Results are expressed as percentage of total surveyed area containing cells positive for insulin. The insulin-positive cells were counted as the number of islets per area of pancreas.[20, 21]

Sections of liver tissues of mice at the age of 16 weeks were stained with Oil Red O and Hematoxylin and Eosin (H&E). For immunohistochemistry, the liver sections were incubated with anti-F4/80 rat-monoclonal antibody (1:100 dilution; Abcam plc, Cambridge, UK). The sections were then treated with anti-rat fluorescein-conjugated secondary antibody (Alexa Fluor 546; Invitrogen/Life Technologies Japan, Tokyo, Japan) and anti-rat horseradish peroxidase (HRP)-conjugated antibody (polyclonal rabbit anti-rat immunoglobulins/HRP; DakoCytomation, Glostrup, Denmark).[22]

Measurement of total cholesterol and lipid contents in liver

Hepatic lipids were extracted as described previously.[23] Total cholesterol and triglyceride were measured at Skylight Biotech, Inc. (Akita, Japan) using cholesterol and triglyceride assay kits (choletest-CHO and choletest-TG, Sekisui Medical Co., Ltd., Tokyo, Japan). Hepatic lipid content was defined as weight per gram of liver tissue.

Immunoblotting

Liver isolated from APCs-treated and untreated ob/ob mice were lysed in ice-cold lysis buffer (10 mmol/l Tris [pH 7.2], 100 mmol/l NaCl, 1 mmol/l EDTA, 1% Nonidet P-40, and 0.5% sodium deoxycholate) containing protease inhibitor cocktail (Complete; Roche, Mannheim, Germany), phosphatase inhibitor cocktail (Calbiochem, Darmstadt, Germany), and 5 mmol/l sodium pyrophosphate. Immunoblotting was performed. Primary antibodies used were rabbit anti-phospho-Akt (Ser473) and anti-Akt from Cell Signaling (Danvers, MA); mouse anti-phospho-JNK and anti-JNK were from Sigma (St. Louis, MO). Secondary antibodies used were horseradish peroxidase-conjugated anti-rabbit and mouse antibody (GE Healthcare). Band intensities were quantified with Multi Gauge software (Fujifilm, Tokyo, Japan).

197

198 **Isolation of total RNA and quantitative RT-PCR**

199 Total RNA was isolated from liver of APCs-treated mouse using Trizol (Invitrogen).
200 SYBR Green PCR Master Mix (Applied Biosystems, Foster, CA, USA) was prepared
201 for the quantitative RT-PCR run using TNF-α primer with the following sequence:
202 5'-AAATGGGCTTTCCGAATTCA-3' and 5'-CAGGGAAGAATCTGGAAAGGT-3',
203 IL-6 primer with the following sequence: 5'-GGAGGCTTAATTACACATGTT-3'
204 and 5'-TGATTTCAAGATGAATTGGAT-3', IL-1β primer with the following
205 sequence: 5'-ATCTTTGGGGTCCGTCAACTGAPDH-3' and
206 5'-GCAACTGTTCCTGAACTCAACT-3'. GAPDH mRNA was used as an internal
207 control. The sequences of GAPDH primer are as follows:
208 5'-AAATGGTGAAGGTCGG-3' and 5'-TCGTTGATGGCAACAA-3'. The thermal
209 cycling conditions were denaturation at 95 °C for 10 min followed by 50 cycles at
210 95 °C for 15 s and 60 °C for 1 min. mRNA levels were measured by real-time
211 quantitative RT-PCR using ABI PRISM 7000 Sequence Detection System (Applied
212 Biosystems/Life Technologies Japan, Tokyo, Japan).

213

214 **Statistical analysis**

215 The data are expressed as means ± SE. Statistical significance was determined by
216 unpaired Student's *t*-test. $P < 0.05$ was considered significant.

217

218 ■RESULTS

219 **Body weight, food intake, and energy expenditure**

220 We first evaluated the effect of APCs on body weight of ob/ob mice. There was no
221 difference between APCs-treated and untreated mice during the test period (Figure 2A).
222 Dietary food intake (Figure 2B) and water intake (APCs-treated mice: 6.24 ± 0.32 ml
223 of 0.5% APCs water per day, untreated mice: 7.11 ± 0.24 ml of water per day) were
224 also unchanged. Similarly, energy expenditure was not significantly different between
225 APCs-treated and untreated mice (Figure 2C).

226

227 **Glucose tolerance test and insulin tolerance test**

228 In OGTT, blood glucose levels were significantly lower at 15 min and 30 min in
229 APCs-treated ob/ob than those in untreated mice (Fig. 3A). The serum insulin levels
230 did not differ at these time points (Figure 3B). The value of HOMA-IR was

231 significantly lower in APCs-treated (27.3 ± 7.9) than that in untreated mice (76.0 ±
232 13.3). In ITT, blood glucose levels were significantly lower in APCs-treated ob/ob
233 (Figure 3C). These data suggest that APCs ameliorate insulin resistance in ob/ob mice.
234 Insulin resistance contributes to an adaptive change in pancreatic β-cell mass.[24] We
235 therefore observed pancreatic islets morphologically by immunohistochemistry using
236 anti-insulin and anti-glucagon antibodies (Figure 3D). β-cell area was decreased by
237 21 % in APCs-treated compared with that in untreated mice (Figure 3E). On the other
238 hand, there was no difference in the number of islets (Figure 3F). These data suggest
239 that hypertrophy of pancreatic islets was suppressed by treatment of APCs.
240
241 **Effects of APCs on body composition, adipocyte size, and serum adiponectin level**
242 We then examined the effects of APCs on adipose tissue, a target organ for insulin.
243 There was no difference in lean mass and fat composition between APCs-treated and
244 untreated mice (Figure 4A). In addition, the size of adipocytes and serum adiponectin
245 levels did not change (Figure 4B and C). It is therefore unlikely that APCs have an
246 effect on insulin resistance in adipose tissue.
247
248 **Effects of APCs on hepatic insulin signals and lipid content**

Hepatic gluconeogenesis is enhanced in the state of insulin resistance.[25] In PTT, APCs-treated ob/ob mice displayed lower blood glucose levels at 15 min and 30 min after pyruvate injection, indicating that APCs treatment suppresses hepatic gluconeogenesis (Figure 5A). It was reported that Akt phosphorylation of ob/ob mice was down-regulated, indicating impairment of the insulin signaling.[26] Insulin-stimulated Akt phosphorylation was elevated in APCs-treated compared with untreated mice (Figure 5B). Accumulation of fat in liver induces insulin resistance.[27] We therefore estimated lipid content in liver. Interestingly, total cholesterol and TG contents in liver did not differ between APCs-treated and untreated mice (Figure 5C and D). Similarly, change of lipid content was not observed by using other methods such as H&E staining and Oil Red O staining (Figure 5E). These data indicate that APCs suppress hepatic gluconeogenesis by improving the insulin signal without altering fat accumulation in liver.

Effects of APCs on inflammation in liver

We then considered whether APCs influence inflammation in liver to ameliorate insulin resistance, and evaluated macrophage infiltration into liver by immunostaining using anti-F4/80 anti-body, a marker for mature mouse macrophage.[28] The number of

macrophage in liver was decreased in APCs-treated ob/ob mice (Figure 6A). We examined the mRNA expression levels of pro-inflammatory cytokine in liver, and found that mRNAs of TNF-α and IL-6 were down-regulated by APCs treatment (Figure 6B). TNF-α activates TNF receptors on hepatocytes to induce JNK activation.[29] We therefore examined JNK activation by immunoblotting using anti-phospho-JNK and anti-JNK antibody. Phosphorylation of JNK was decreased by APCs treatment in liver (Figure 6C). These data suggest that APCs treatment attenuates inflammation in liver.

■DISSCUSSION

In this study, we show that oral administration of APCs ameliorates glucose intolerance in obese diabetic ob/ob mice.

Continuous but not single (Figure S2) oral administration of APCs ameliorated glucose intolerance, and suppressed the expression of inflammation-related genes and phosphorylation of JNK in liver, suggesting that APCs improve insulin sensitivity in liver through suppression of chronic inflammation. In our preliminary experiments, it was confirmed that the dosage of 0.5% APCs in drinking water is the appropriate concentration that has no effects on water intake, food intake or body weight.

Obesity is associated with chronic, low-grade, and systemic inflammation that may

286 contribute to the development of insulin resistance and type 2 diabetes.[30] Generally,
287 adipose tissue inflammation is considered to initiate adipocyte hypertrophy and
288 hyperplasia and to influence release of adipocytokines and pro-inflammatory signaling.
289 On the other hand, obesity also is associated with the regulation of adipocytokine
290 secretion, and causes adverse effects on inflammation and insulin sensitivity.[31]
291 Akiyama reported that apple proanthocyanidins do not affect body weight or food
292 intake in W/W^V and B10A mice.[32] There are several reports suggesting that
293 procyanidins improve glycemic control.[33] In accord with these data, while the body
294 weight, food intake, and fat mass of APCs-treated ob/ob mice were unchanged
295 compared with those of untreated mice, the insulin sensitivity was significantly
296 improved in our study.

297 The beneficial effects of procyanidins from various plants have been investigated.
298 Procyanidin from cinnamon was reported to lower levels of blood glucose, total
299 cholesterol, low-density cholesterol, and hemoglobin A1c in type 2 diabetes.[33]
300 Persimmon peel proanthocyanidins decrease blood glucose levels and glycosylated
301 protein concentrations and have a protective effect against diabetes-induced oxidative
302 stress in streptozotocin-induced diabetic rats.[34] Recently, tetrameric procyanidins from
303 cacao liquor were shown to increase the levels of plasma glucagon-like peptide-1

304 (GLP-1), an incretin hormone that potentiates insulin secretion.[35] Our data showing
305 that APCs ameliorate impaired hepatic insulin signaling in ob/ob mice may be useful in
306 clarifying the therapeutical actions of substances from vegetables and fruits.

307 Hepatic insulin resistance is a key feature of obesity-related type 2 diabetes.[36]
308 Kupffer cells, which are liver-resident macrophage-like cells, are activated by
309 inflammation, apoptosis, and necrosis of hepatocytes. Activation of Kupffer cells
310 causes the release of inflammatory cytokines such as TNF-α and IL-6 in liver.[37] It is
311 reported that accumulation of fat in hepatocyte induces inflammation in liver.[38]
312 However, APCs treatment decreased the number of macrophages and the expression
313 levels of TNF-α and IL-6 in liver without changing the lipid content in the liver of
314 ob/ob mice. These data suggest that the effect of APCs on liver inflammation is not due
315 to suppression of fat accumulation in hepatocytes.

316 It was suggested that intestinal bacteria may contribute to the pathogenesis of
317 inflammation in liver. Lipopolysaccharide (LPS), one of the gut-derived endotoxins,
318 was reported to cause liver damage via activation of Kupffer cells and release of
319 TNF-α and other cytokines.[39] Obesity alters the ecology of intestinal microbiota.[40] It is
320 reported that ob/ob mice have higher endotoxin levels in the portal blood than that in
321 wild-type mice.[41] Recent studies suggested that procyanidins can be degraded by some

322 kinds of intestinal microbiota[42] and also that administration of apple flavonoid alters
323 intestinal microbiota.[43] In addition to our preliminary experiment using ob/ob mouse
324 (data not shown), we have carried out an experiment whether APCs could change the
325 microbiota of high-fat/high-sucrose (HFHS)-fed C57BL/6J male mice.[44] We found the
326 chronic oral administration of high polymeric APCs prevent obesity associated with
327 gut microbial and metabolic changes. It is therefore possible that APCs suppress
328 inflammation in liver not by decreasing fat accumulation but through effects on
329 intestinal microbiota. Glycolysis, gluconeogenesis, glycogenolysis and glycogen
330 synthesis is involved in the maintenance of blood glucose levels.[45] Both
331 gluconeogenesis and glycogenolysis are important in glucose production in liver.
332 Pyruvate is one of well-known substances of gluconeogenesis. In this study, we
333 showed APCs are considered to be suppress pyruvate induced gluconeogenesis (Figure
334 5A). However, the effect of APCs on glycogen metabolism is not clear. Further studies
335 are needed to clarified the details of how APCs ameliorate glucose resistance in
336 diabetic state.

337 In conclusion, our data indicate that oral administration of APCs ameliorates insulin
338 resistance by improving hepatic insulin signaling through suppression of inflammation
339 in ob/ob mice. Moreover, further investigation of the mechanism of the effects of APCs

on glucose metabolism could shed light on the pathophysiology of insulin resistance and suggest new targets for type 2 diabetes therapy.

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Notes

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■ABBREVIATIONS USED

APCs (apple procyanidins), OGTT (oral glucose tolerance test), ITT (insulin tolerance test), PTT (pyruvate tolerance test), TNF (tumor necrosis factor), IL (interleukin), JNK (c-Jun N-teminal kinase), IRS (insulin receptor substrate), SGLT (sodium glucose cotransporter), HOMA-IR (homeostasis model assessment of insulin resistance), GLP (glucagon-like peptide)

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Figure Legends

Figure 1

Reversed phase HPLC profile of apple polyphenol (upper) and apple procyanidins (lower). These panels show that our apple procyanidins do not include chlorogen acid or phloretin glucoside (Phlorizin) fractions, which are included as apple polyphenols.

Figure 2

Effects of APCs administration on body weight, food intake, and energy expenditure in ob/ob mice. (A) Body weight (n=12) and (B) food intake (n=12) were measured once every week. (C) Energy expenditure (n=4) was measured for 48 h. APCs (-) and APCs (+) indicate APCs-untreated and APCs-treated ob/ob mice, respectively. Results are presented as mean ± SE. $^{*}P<0.05$

Figure 3

Effects of APCs administration on OGTT, ITT, and pancreatic islet size in ob/ob mice. (A) Blood glucose levels and (B) serum insulin levels were measured during OGTT (load of glucose 1g/kg body weight). (C) Blood glucose levels measured during ITT (load of insulin 2U/kg body weight) (D) immunocytochemistry of pancreatic islets. (E) β-cell area in the total pancreatic area (n=4) and (F) the number of islets per area of pancreas (n=4). APCs (-) and APCs (+) indicate APCs-untreated and APCs-treated ob/ob mice, respectively. Results are presented as mean ± SE.$^{*}P<0.05$

Figure 4

Effects of APCs administration on adipose tissue adipocyte size and serum adiponectin level in ob/ob mice. (A) Visceral, subcutaneous, and total fat mass in APCs-treated and untreated mice were measured using CT images of transverse abdominal sections (n=4). (B) Representative images of HE staining of adipose tissue section. (C) Serum adiponectin concentrations (n=8). APCs (-) and APCs (+) indicate APCs-untreated and treated ob/ob mice, respectively. Results are presented as mean ± SE.

Figure 5

Effects of APCs administration on liver tissue in ob/ob mice. (A) Glucose levels measured during PTT (load of pyruvate 1g/kg body weight, n=8) (B) The ratio of phosphorylated Akt to total Akt (t-Akt) in liver tissues (n=3). (C) Total cholesterol (n=4) level and (D) triglycerides level in liver were measured (n=4). (E) HE and Oil red O staining of the liver. APCs (-) and APCs (+) indicate APCs-untreated and treated ob/ob mice, respectively. Results are presented as mean ± SE. $^{*}P<0.05$

Figure 6

Effects of APCs administration on liver inflammation in ob/ob mice.

(A) Immunohistochemistry with anti-F4/80 antibody in liver of APCs-treated and untreated mice. Arrows indicate representative macrophages in liver. (B) mRNA levels of TNF-α, IL-6, and IL-1β in the liver (n=8). (C) Effects of APCs administration on the ratio of phosphorylated JNK to total JNK (t-JNK) in liver in ob/ob mice (n=3). APCs (-) and APCs (+) indicate APCs-treated and untreated ob/ob mice, respectively. Results are presented as mean ± SE.

FIGURE 1

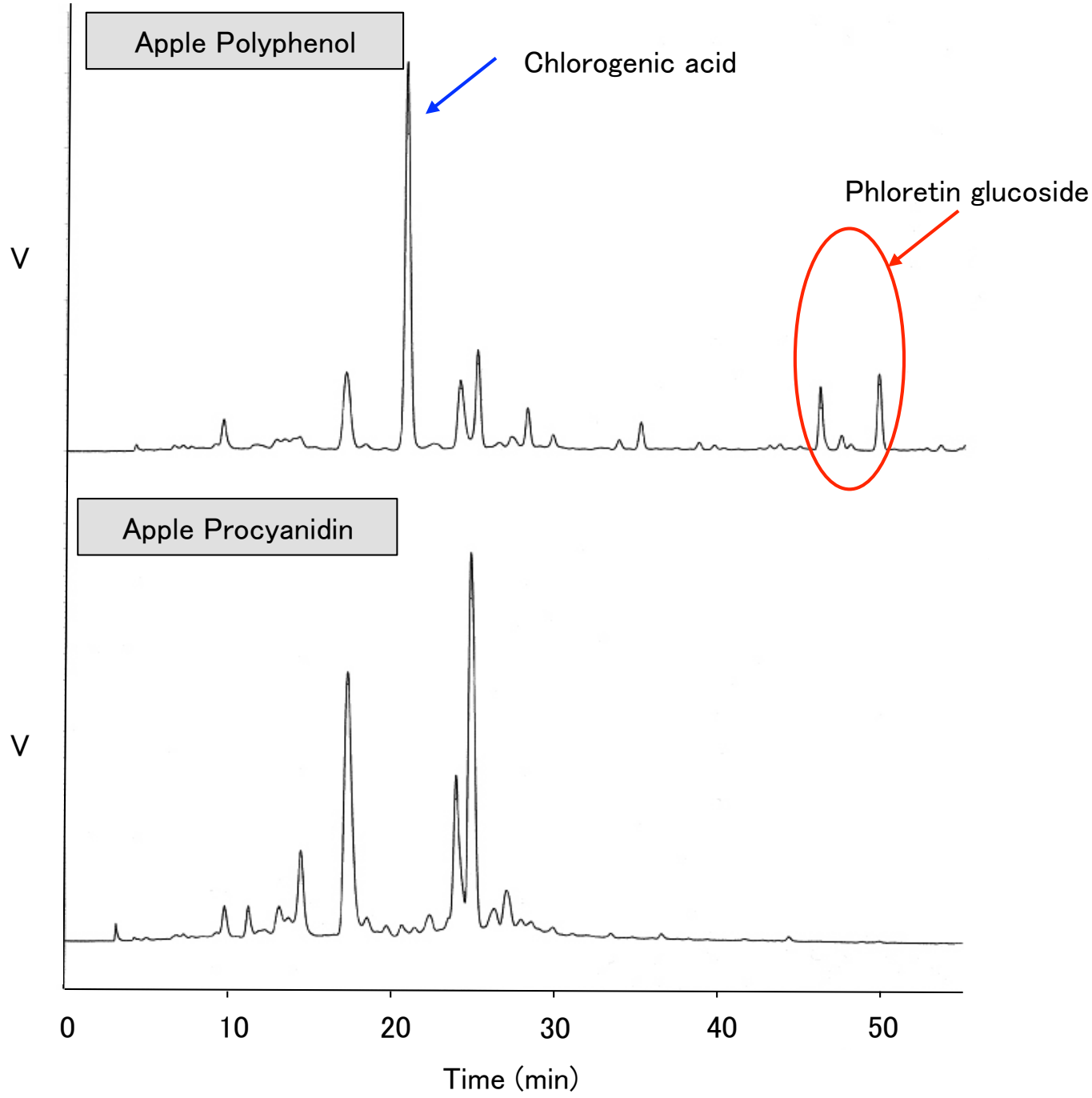

Apple Polyphenol
Chlorogenic acid
Phloretin glucoside
V
Apple Procyanidin
V
0
10
20
30
40
50
Time (min)

FIGURE 2

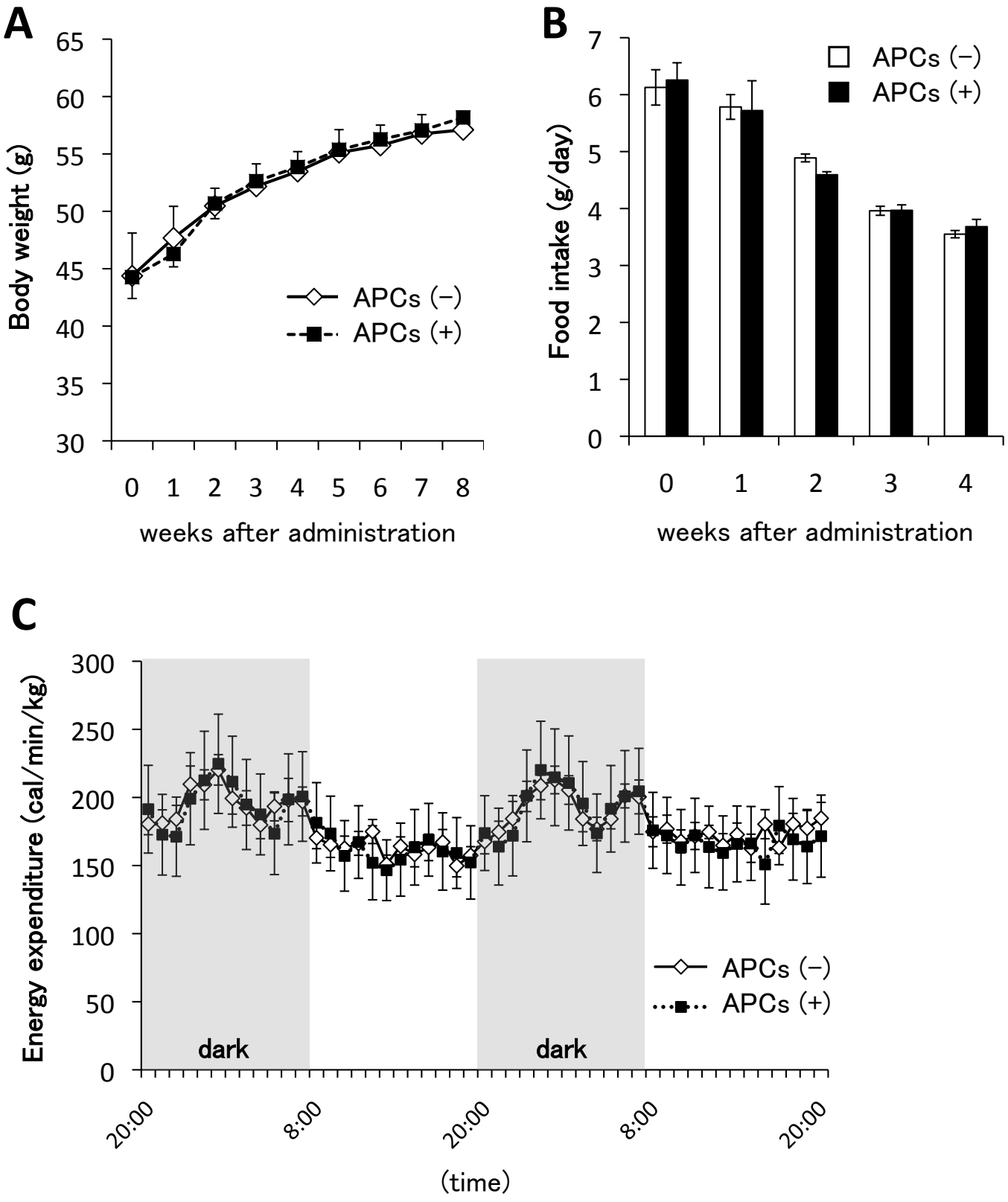

A
Body weight (g)
65
60
55
50
45
40
35
30
0 1 2 3 4 5 6 7 8
weeks after administration
APCs (−)
APCs (+)
B
Food intake (g/day)
7
6
5
4
3
2
1
0
0 1 2 3 4
weeks after administration
APCs (−)
APCs (+)
C
Energy expenditure (cal/min/kg)
300
250
200
150
100
50
0
dark
dark
20:00
8:00
20:00
8:00
20:00
(time)
APCs (−)
APCs (+)

FIGURE 3

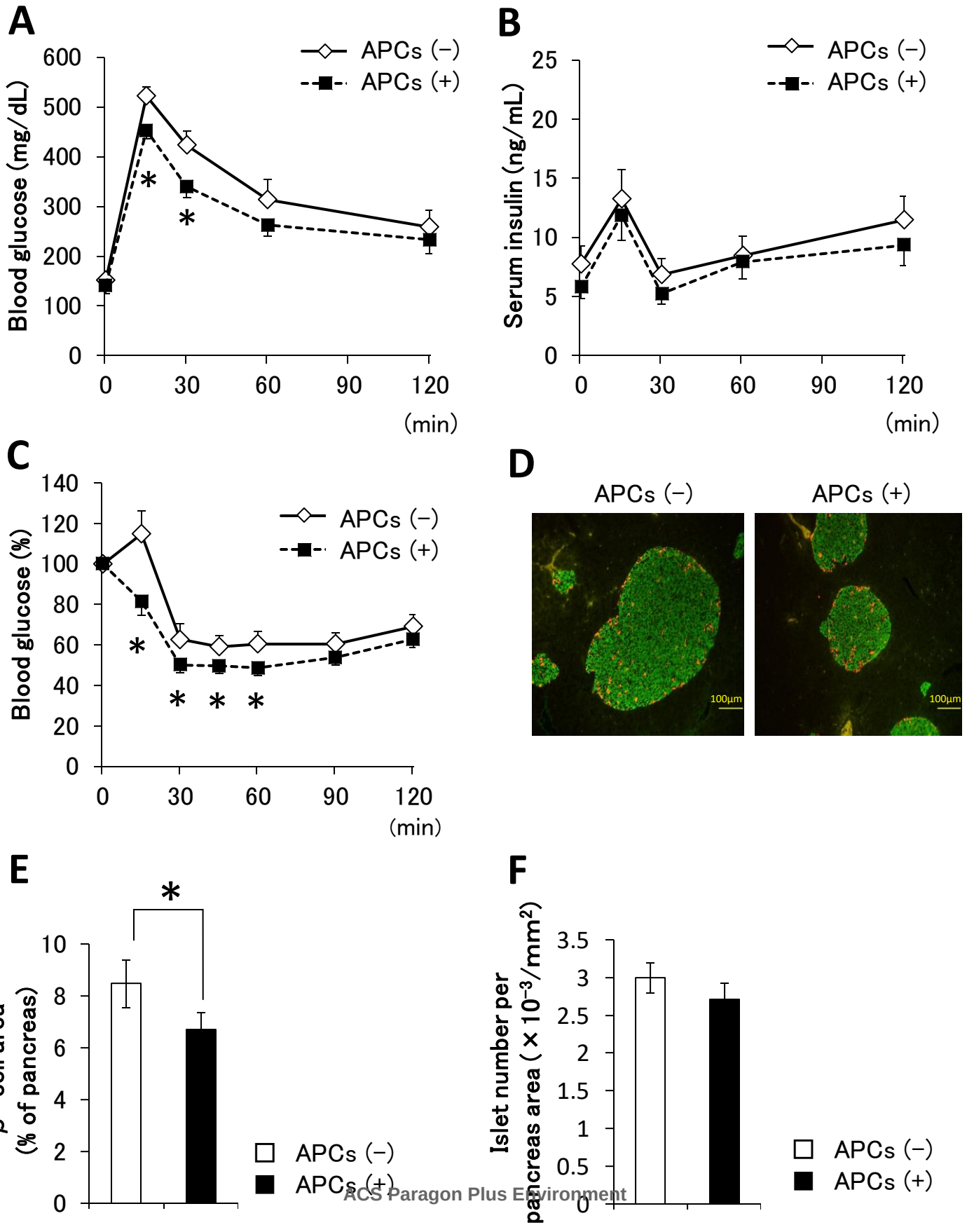


FIGURE 4

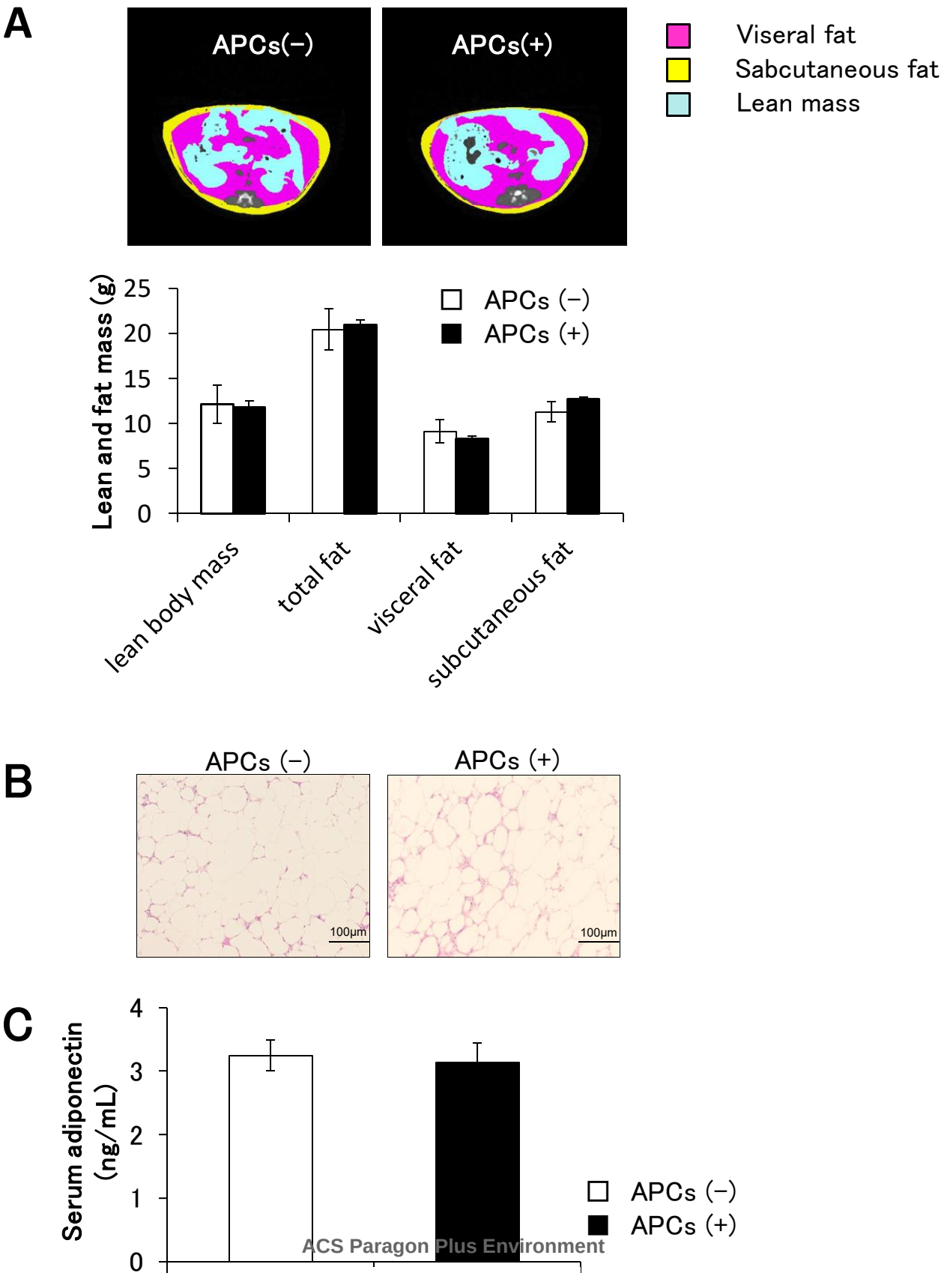


FIGURE 5

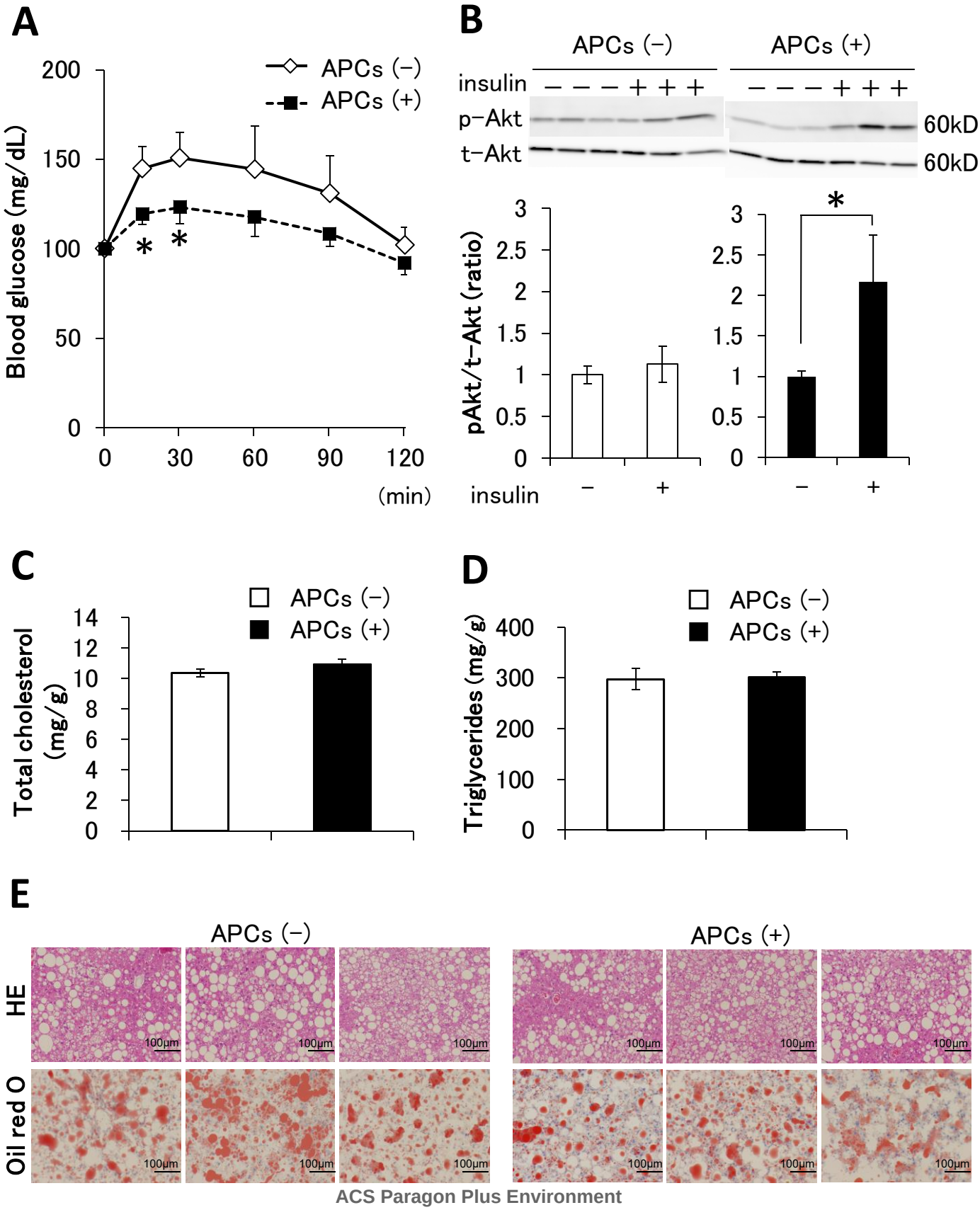
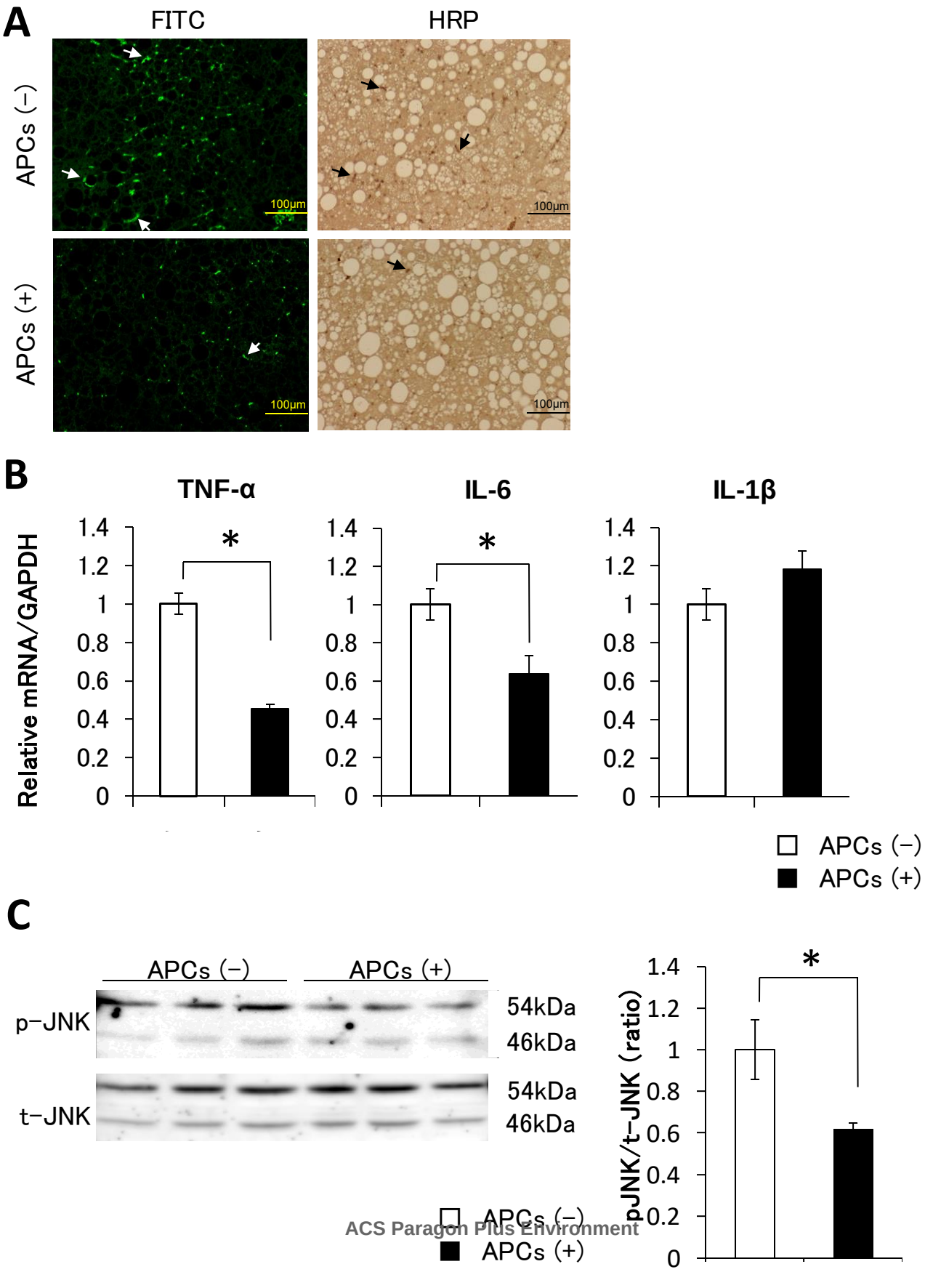
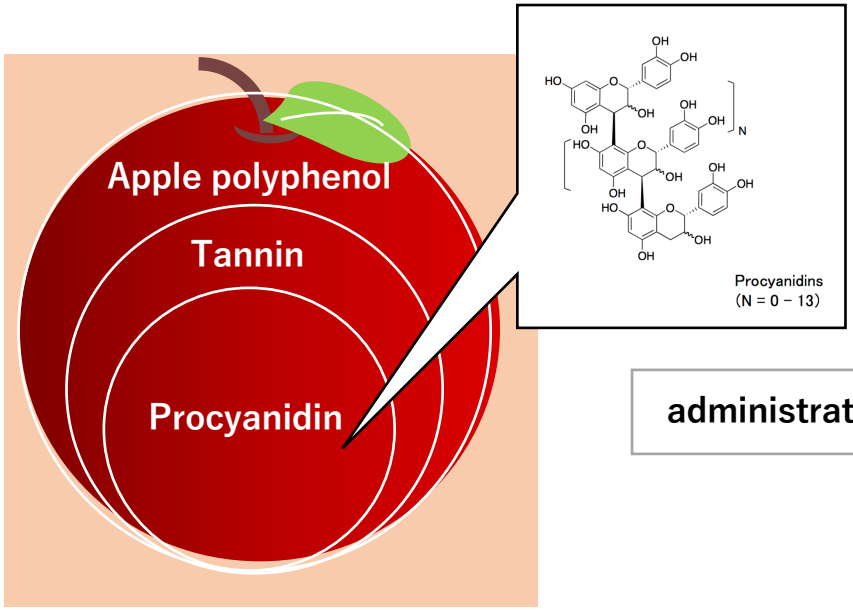

A
Blood glucose (mg/dL)
200
150
100
50
0
0
30
60
90
120
(min)
APCs (−)
APCs (+)
*
*
B
APCs (−)
APCs (+)
insulin
− − − + + +
− − − + + +
p−Akt
t−Akt
60kD
60kD
pAkt/t−Akt (ratio)
3
2.5
2
1.5
1
0.5
0
*
insulin
−
+
−
+
C
Total cholesterol (mg/g)
14
12
10
8
6
4
2
0
APCs (−)
APCs (+)
D
Triglycerides (mg/g)
400
300
200
100
0
APCs (−)
APCs (+)
E
APCs (−)
APCs (+)
HE
Oil red O
100μm

FIGURE 6

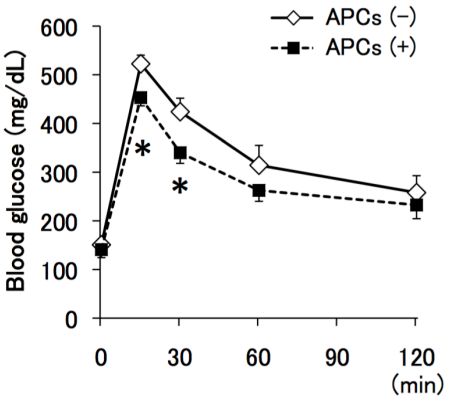




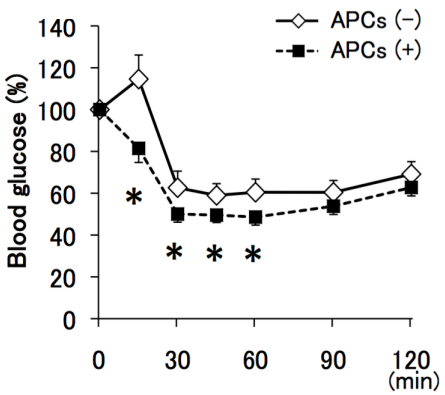
administration

Ameliorate insulin resistance via suppression of pro-inflammatory cytokines expression in liver

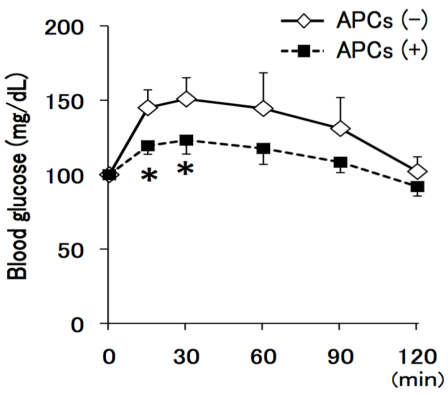
OGTT



ITT



PTT



Immunohistochemistry with anti-F4/80 antibody in liver