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4 # Evaluating the appropriate oral lipid tolerance test model for investigating
5 # plasma triglyceride elevation in mice

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14 # Abstract

15 The oral lipid tolerance test (OLTT) has been known to assess intestinal fat metabolism and
16 whole-body lipid metabolism, but rodent models for OLTT are not yet established. Differences
17 in OLTT methodology preclude the generation of definitive results, which may cause some
18 confusion about the anti-hypertriglyceridemia effects of the test materials. To standardize and
19 generate more appropriate methodology for the OLTT, we examined the effects of mice strain,
20 dietary lipid sources, fasting period, and gender on lipid-induced hypertriglyceridemia in mice.
21 First, lipid-induced hypertriglyceridemia was more strongly observed in male ddY mice than in
22 C57BL/6N or ICR mice. Second, the administration of olive and soybean oils remarkably
23 repressed lipid-induced hypertriglyceridemia in male ddY mice. Third, fasting period before the
24 OLTT largely affected the plasma triglyceride elevation. Fasting for 12 h, but less than 48 h,
25 provoked lipid-induced hypertriglyceridemia in male ddY mice. Fourth, we explored the
26 suppressive effects of epigallocatechin gallate (EGCG), a green tea polyphenol, on lipid-induced
27 hypertriglyceridemia. The administration of 100 mg/kg of EGCG suppressed lipid-induced
28 hypertriglyceridemia and intestinal lipase activity in male ddY mice after 12 h fasting. Fifth,
29 EGCG-induced suppressive effects were observed after lipid-induced hypertriglyceridemia was
30 triggered in male mice. Lastly, lipid-induced hypertriglyceridemia could be more effectively
31 induced in mice fed a high-fat diet for 1 week before the OLTT. These findings indicate that
32 male ddY mice after 12 h fasting displayed marked lipid-induced hypertriglyceridemia in
33 response to soybean oil and, hence, may be a more appropriate OLTT model.

34 **Keywords:** oral lipids tolerance test**,** hypertriglyceridemia, soybean oil, ddY mice, fasting
35 period, gender, epigallocatechin gallate

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36 # Introduction

37 According to 2017 data from the World Health Organization, about 56 million people die per
38 year worldwide [1]. Cardiovascular diseases have been identified as one of the most common
39 causes of death globally. Postprandial hyperlipidemia and postprandial hyperglycemia are
40 independent risk factors for cardiovascular diseases according to epidemiological evidence [2–
41 9]. Coronary heart disease, type 2 diabetes, insulin resistance, and obesity are all associated with
42 elevated postprandial plasma triglyceride (TG) levels [10, 11]. Recently, it was also suggested
43 that a short-term high-fat feeding regimen in mice exacerbated postprandial plasma TG levels
44 without altering fasting plasma TG levels [12]. Therefore, the improvement of postprandial
45 hypertriglyceridemia is supposedly a more valuable approach in lowering the risk of
46 cardiovascular diseases than the improvement of fasting TG levels.

47 Postprandial plasma TG levels are strongly correlated with fasting triglyceride levels.
48 However, a difference in fasting TG levels only partially accounts for the interindividual
49 variation in the magnitude of postprandial hyperlipidemia. The postprandial plasma TG response
50 can be affected by genetic background, diet, physical activity, age, gender, and health conditions
51 [13, 14]. In general, researchers have focused on fasting plasma TG levels, but not on
52 postprandial levels in both human and rodent studies because they want to exclude the proximate
53 effects of food materials on lipid metabolism, since health check programs in humans are often
54 carried out under fasting conditions.

55 To examine the factors affecting postprandial hypertriglyceridemia and their mechanisms, an
56 appropriate mice model of hypertriglyceridemia in response to dietary lipids is required.
57 Information about the intestinal digestion and absorption of dietary fats is presented in Fig 1 (Fig
58 1). The oral lipid tolerance test (OLTT) can assess not only intestinal lipid metabolism but also

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59 whole-body lipid metabolism. The ability to quickly normalize hyperlipidemia following the
60 administration of lipids provides integrated information about intestinal lipid absorption, lipid
61 transport via lipoproteins, and tissue-specific lipid metabolism. Thus, the OLTT is an essential
62 and useful method to examine lipid metabolism. Importantly, data from the OLTT can be used to
63 access information on the pancreas lipase inhibitory activity and intestinal lipid absorption when
64 investigating the suppressive effects of food materials on postprandial hypertriglyceridemia.
65 However, rodent models for postprandial hypertriglyceridemia have not yet been standardized.
66 As shown in Table 1, many researchers have used various models to examine lipid-induced
67 hypertriglyceridemia; specifically, the mice model, lipid dosage, lipid sources (lipids or lipids
68 emulsions), and the fasting period before the OLTT often vary among studies, making direct
69 comparisons difficult (Table 1). Various experimental protocols cannot generate the determinate
70 results, which can cause confusion about the pharmaceutical effects of food materials. Fatty
71 acids and dietary lipid composition and fasting period can largely affect lipid metabolism [15–
72 17]. For example, when the anti-hypertriglyceridemia effects of diacylglycerol acyltransferase-1
73 inhibitors were investigated in mice, lipid sources, dosage, and the fasting period before the
74 OLTT were not uniform [18–20] (Table 1). Furthermore, postprandial hyperglycemia has been
75 identified as another well-known risk factor for coronary diseases, but its evaluation in mice and
76 human models has been generally standardized [21], unlike in postprandial hyperlipidemia
77 testing. In humans, the glucose tolerance test is performed with the individual drinking a 75 g
78 glucose solution following overnight fasting; in mice, glucose tolerance is assessed through the
79 oral administration of 2 g/kg glucose following 6 h of fasting [21].
80
81 **Fig 1. Model of dietary lipid metabolism in small intestine.** Dietary lipids in digestive tract are

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82 emulsified and hydrolyzed by pancreas lipase to free fatty acids and monoglycerides. After the
83 hydrolyzation, lipids are absorbed into small-intestinal epithelial cells, and followed by re-
84 synthesized to TG. Chylomicrons are formed with synthesized TG and apolipoprotein B-48 and
85 are transferred to blood via lymph. Some functional materials (e.g. EGCG) inhibit the
86 emulsification and hydrolyzation of TG in diets, resulting to the suppression of lipids absorption.
87 On the other hand, some functional materials activate the fatty acids β-oxidation through PPARα
88 and AMPK activations.

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89 **Table 1. List of OLTT models using mice or rats for evaluation of plasma TG levels.**

Animal					Lipids used for OLTT			Fasting period	Sample	Ref.
Sp.	Strain	Diet	Gender	Age	Lipids	Emulsion	Dose			
M	C57BL/6J	HFD (2 w)	male	10 w	Olive	-	0.3 mL/head	16 h	DHA	31
M	C57BL/6J	HFD (1 w)	male	8-10 w	Coconut	-	0.15 mL/head	4 h	-	12
M	C57BL/6J	HFD (1 w)	male	10 w	Olive	-	0.3 mL/head	-	Bezafibrate	46
M	C57BL/6J	HFD (6 w)	male	15 w	Olive	-	10 mL/kg	16 h	Inulin	41
M	C57BL/6J	chow	male	6 w	Olive	-	5 mL/kg	o.n.	Oolong tea and green tea	47
M	C57BL/6J	HFD (4 w)	male	12 w	Olive	-	17 mL/kg	o.n.	Pemafibrate	48
M	ICR	chow	male	8 w	Olive and lard	-	5 mL/kg	20 h	Cocoa tea	24
M	ICR	-	-	8 w	Corn	-	5 mL/kg	16 h	DGAT-1 inhibitor	20
M	ddY	-	male	6 w	Olive	-	10 mL/kg	-	Aged garlic	49
M	ICR	chow	male		-	20% soybean oil	10 mL/kg	14 h	AZD7687 (DGAT-1 inhibitor)	18
R	Wistar	chow	male		Corn	-	5 mL/kg	14 h		
M	C57BL/6J, C57BL/6N, ddY, etc.	chow	male	8 w	Safflower	-	0.4 mL/head	24 h	-	29
M	C57BL/6, ICR etc.		-	5-9 w	Corn	-	6 mL/kg	16 h	A-922500 (DGAT-1 inhibitor)	19
R	SD, JCR		-	6-9 w		-				
R	SD	chow	male	5 w	Corn	-	5 mL/kg	o.n.	Resistant starch	50
R	Wistar	chow	male	9 w	-	Soybean oil + lecithin + glycerol	10 mL/kg	o.n.	Black tea polyphenol	36
R	Wistar	chow	male	8 w	-			o.n.	Green tea catechin	37
R	Wistar	chow	male	9 w	-			o.n.	Green tea catechin	23
R	SD	chow	-	8 w	-			18 h	Epsilon-polylysine	38
R	SD	chow	-	9 w	-	High-oleic safflower oil + lecithin + glycerol	10 mL/kg	o.n.	*Lactobacillus pentosus*	*39*
R	SD	chow	-	5 w	-	10% soybean oil	15 mL/kg	o.n.	Tea leaves	40

91 Sp., Species; M, Mice; R, Rat; -, not shown or unclear; o.n., overnight

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93 The objective of this study was to determine the optimal protocols for the OLTT. We looked
94 at variables such as mice strains, lipid sources, fasting period, and gender in mice.
95

96 Materials and Methods

97 The animal experimentation protocol was approved by the President of Kitasato University
98 through the judgment of the Institutional Animal Care and Use Committee of Kitasato University
99 (Approval No. 19-194).
100

101 Effect of mice model on lipids-induced hypertriglyceridemia (Exp 1)

102 I selected mice strains that are commonly used for OLTT studies. Male ICR, ddY, and
103 C57BL/6N mice were purchased from Japan SLC (Hamamatsu, Japan) at 7 weeks of age. Mice
104 (n = 10) were housed at 23 ± 2°C with lights on between 08:00 and 20:00. Food and water were
105 accessed *ad libitum* (CE-2; Japan Clea, Tokyo) during a 2-week acclimation period. Mice were
106 fasted for 12 h before the OLTT. Fasted mice were orally administered soybean oil [5 mL/kg;
107 FUJIFILM Wako Pure Chemicals Corporation (Wako), Osaka, Japan]. Blood (30 μL/mice) was
108 then collected from the tail vein and centrifuged (6,200×*g*, 4°C, 5 min) to obtain plasma prior to
109 and at 60, 120, 180, 240, and 360 min after the administration of the oil. Plasma TG levels were
110 immediately measured using a commercial kit (Wako). The area under the curve (AUC) values
111 of plasma TG levels were calculated hourly using the trapezoidal rule.
112

113 Effect of lipids species on lipids-induced hypertriglyceridemia in
114 mice (Exp 2)

115 Male ddY mice were obtained from Japan SLC at 6 weeks of age. Mice were acclimated for

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116 2 weeks as described above. The ddY mice were fasted for 12 h before the OLTT. Mice were
117 then divided into five groups (n = 8–10). Different oils were orally administered to each group of
118 fasted mice. The dietary oils used in this study were olive oil, soybean oil, perilla oil, fish oil,
119 and beef tallow (Wako). The fatty acid composition [C14:0, C16:0, C18:0, C18:1, C18:2, C18:3,
120 C20:5 (EPA), and C22:6 (DHA)] of the test oils was determined using a gas chromatography
121 (GC) system (GC2014, Shimadzu Co. Ltd., Kyoto, Japan) with a 60 m capillary column (TC70,
122 GL Science Inc., Tokyo) and pure helium carrier gas, as described in the previous study [22].
123 The injector and detector temperatures of the GC equipment were 250 °C and 260 °C,
124 respectively; the column oven temperature was constant at 180 °C for 40 minutes and was later
125 increased by 20 °C/min from 180°C to 260°C. Before GC analysis, the fatty acids from the test
126 oils were methyl-esterified using a commercial kit (Nacalai Tesque, Inc., Kyoto). The peak
127 components were identified by comparing each retention time with that of a fatty acid methyl
128 ester (GLC-211, Funakoshi Co., Tokyo). The sum of the fatty acid percentages was estimated to
129 be at 100 %. The fatty acid composition of the oils is shown in Fig 2 (Fig 2). Blood collection
130 and the measurement of plasma TG levels and AUC values were carried out as described above
131 (Exp 1). After completing Exp 2, mice were maintained on CE-2 for 4 weeks and were later used
132 for Exp 3.
133
134 **Fig 2. Fatty acids composition of the test oils (Exp 2).**
135

136 Effect of fasting period on lipids-induced hypertriglyceridemia in 137 mice (Exp 3)

138 Male ddY mice used in Exp 2 were used for another OLTT at 10 weeks of age. Mice were

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139 divided into five groups (n = 8–10). Before the OLTT, ddY mice were fasted for 0 (non-fasted),
140 3, 6, 12, and 48 h. Soybean oil (5 mL/kg) was orally administered. Blood collection and the
141 measurement of plasma TG levels and AUC values were carried out as described above (Exp 1).
142 After completing Exp 3, mice were maintained on CE-2 for 2 weeks and then used for Exp 4.
143

144 Effect of green tea polyphenol, EGCG on lipids-induced 145 hypertriglyceridemia in mice (Exp 4)

146 EGCG, a main polyphenol compound in green tea (*Camellia sinensis*) leaves, is an
147 established food-derived pancreas lipase inhibitor known to suppress diet-induced
148 hypertriglyceridemia in rodents [23, 24]. In Exp 4a, the suppressive effect of EGCG on lipid-
149 induced hypertriglyceridemia was confirmed under the experimental conditions previously
150 described in Exps 1–3.
151 The male ddY mice used in Exp 3 were used in Exp 4a at 12 weeks of age. Before the OLTT,
152 the ddY mice were fasted for 12 h. Mice were divided into two groups (n = 8). Fasted mice were
153 orally administered soybean oil (5 mL/kg) and water (5 mL/kg) (control group) or soybean oil (5
154 mL/kg) and 100 mg/kg of EGCG (purity ≧90.0 %, Wako) (EGCG group). Blood collection and
155 the measurement of plasma TG levels and AUC values were carried out as described above (Exp
156 1). After completing the OLTT, the mice were maintained on CE-2 for 1 week before they were
157 used for biochemical analyses of lipid metabolism in the liver and small intestine (Exp 4b). Here,
158 male ddY mice, age 13 weeks, were divided into two groups, with five mice per group. The five
159 mice in each group were selected out of the eight mice on the basis of the lipid-induced elevation
160 of plasma TG levels at 2 h during Exp 4a. Fasted mice were orally administered soybean oil (5
161 mL/kg) and water (5 mL/kg) (control group) or soybean oil and EGCG (100 mg/kg) (EGCG

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162 group). Two hours after administration, mice were euthanized under isoflurane anesthesia. The
163 collected blood was centrifuged (6,200 g, 4°C, 15 min) to obtain plasma. The liver and small
164 intestine were quickly removed and stored at −80°C until analyses were performed. The total
165 lipids in the liver and the contents of the small intestine were extracted with a mixture of
166 chloroform and methanol (2:1, v/v), according to the Folch method [25]. The liver TG content
167 within the crude lipid extract was determined using a commercial kit (Wako). Extracted lipids in
168 the small intestine were separated on a high-performance thin-layer chromatography plate
169 (HPTLC; silica gel 60 plates, Merck, Germany) [26]. The plate was developed with a mixture of
170 hexane/diethyl ether/acetic acid (60:40:1, v/v). The spots of each lipid (particularly TG,
171 diacylglycerides, monoglycerides, and fatty acids) were visualized using iodine. The activity of
172 fatty acid synthase (FAS) in the liver was spectrophotometrically determined as described by
173 Nepokroeff et al. [27]. For the extraction and crude purification of the enzymes, a small part of
174 the liver was powdered in liquid nitrogen and homogenized in ice-cold Tris-HCL buffer
175 containing 0.25 mol/L sucrose (pH 7.4). The total cytosol fraction was separated by
176 centrifugation at 500 × *g* for 10 min, followed by 9,000 × *g* for 10 min and 12,000 × g for 120
177 min. The enzymatic activity was measured at 30 °C and expressed as units per mg of wet tissue
178 weight.
179

180 Effect of gender on lipids-induced hypertriglyceridemia in mice 181 (Exp 5)

182 Male and female ddY mice were similarly acclimated for a week as described above (Exp 1),
183 and then, it was used for an OLTT at 7 weeks of age. The mice were divided by gender into
184 groups: eight males and nine females. The mice were fasted for 12 h before the OLTT. Fasted

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185 mice were orally administered soybean oil (5 mL/kg) (control group) or soybean oil (5 mL/kg)
186 with EGCG (100 mg/kg) (EGCG group). Blood collection and the measurement of plasma TG
187 levels and AUC values were carried out as described above (Exp 1). After the completion of
188 Exp. 5, the male mice were maintained on CE-2 for 1 week and then used for Exp 6.
189

190 Effect of 1 week-feeding of high-fat and high-sucrose diet on lipids-191 induced hypertriglyceridemia in mice (Exp 6)

192 Male ddY mice used in Exp 5 were also used for Exp 6 at 8 weeks of age. The mice were fed
193 with either low fat (LF) AIN-93G diet [7 wt% fat; 10 wt% sucrose] or the AIN-93G-based high-
194 fat (HF) and high-sucrose diet [30 wt% fat; 20 wt% sucrose; F2HFHSD diet, Oriental Yeast Co.,
195 Ltd., Tokyo]. After feeding LF or HF diet, the mice in each diet group were divided into two
196 groups (n = 8–9) for OLTT. The mice were then fasted for 12 h before the OLTT. Fasted mice
197 were orally administered soybean oil (5 mL/kg) and water (5 mL/kg) (control group) or soybean
198 oil (5 mL/kg) and EGCG (100 mg/kg) (EGCG group). Blood collection and the measurement of
199 plasma TG levels and AUC values were carried out as described above (Exp 1).
200

201 Statistical analyses

202 All statistical analyses were performed using Excel Statistics 2015 (SSRI, Tokyo, Japan). A
203 difference where $p < 0.05$ was considered statistically significant. In Exp 1, the data were
204 expressed as mean ± SE (n = 8–10). The statistical analysis of differences among the three mice
205 strains was performed using a one-way analysis of variance (ANOVA) and Tukey-Kramer test.
206 In Exp 2, data are expressed as mean ± SE (n = 8–10). The statistical analysis of differences
207 among the five dietary oil groups was performed using a one-way ANOVA and Tukey-Kramer

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208 test. In Exp 3, data are expressed as mean ± SE (n = 8–10). Statistical analysis of differences
209 among the five fasting period groups was performed using a one-way ANOVA and Dunnett test,
210 in which the fasting groups were compared to the non-fasting group. In Exp 4, the data were
211 expressed as mean ± SE (n = 8). The statistical analysis of differences between the two groups
212 was performed using the Student's t-test. In Exp 5, the data are expressed as mean ± SE (n = 8–
213 9). Statistical analysis of differences among the groups was performed using a two-way ANOVA
214 (gender and EGCG) and the Tukey-Kramer test. In Exp 6, the data are expressed as mean ± SE
215 (n = 8−9). Statistical analysis of differences among the groups was performed using a two-way
216 ANOVA (HF diet and EGCG) and the Tukey-Kramer test.
217

218 Results

219 An increase in lipids-induced hypertriglyceridemia in ddY mice 220 (Exp 1)

221 The fasting plasma TG levels were found to be significantly higher in the ddY mice than in
222 the other two strains (ICR, 78 ± 11 mg/dL; ddY, 142 ± 13 mg/dL; C57BL/6N, 60 ± 3 mg/dL).
223 Plasma TG levels during the OLTT were also largely higher in ddY mice than in C57BL/6N and
224 ICR mice. The AUC values of the plasma TG levels during the OLTT were also higher in the
225 ddY mice than in C57BL/6N and ICR mice (Fig 3).
226

227 **Fig 3. Effect of mice strain on plasma TG levels and AUC values during the OLTT.** Values
228 are means ± SE (n = 8−10). The data were analyzed with one-way ANOVA, followed by the
229 post-hoc Tukey-Kramer test. Different letters are significantly different at $p<0.05$.
230

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231 ## Olive and soybean oils leads to lipids-induced hypertriglyceridemia
232 ## (Exp 2)

233 The AUC values of the plasma TG levels during the OLTT were found to be largely higher
234 in ddY mice that were administered perilla oil, fish oil, and beef tallow than in ddY mice that
235 were administered olive and soybean oils. The elevation of plasma TG levels at 60 and 120 min
236 after oil administration was higher in mice administered olive and soybean oils (Fig 4).
237
238 **Fig 4. Effect of lipid sources on plasma TG levels and AUC values during the OLTT in ddY**
239 **mice.** Values are means ± SE (n = 8–10). The data were analyzed with one-way ANOVA,
240 followed by the post-hoc Tukey-Kramer test. *: Asterisks are significantly different at $p < 0.05$
241 between the groups as below; *1, soybean - beef tarrow; *2, soybean, fish - beef tarrow; *3,
242 perilla, fish, beef tarrow - olive; *4, perilla - soybean; *5, perilla, fish, beef tarrow - olive; *6,
243 fish - soybean. Different letters in the AUC values are significantly different at $p < 0.05$.
244

245 ## A longer, but not too long, fasting leads to lipids-induced
246 ## hypertriglyceridemia (Exp 3)

247 The elevation of plasma TG levels at 60 and 120 min after oil administration during the
248 OLTT was significantly higher in ddY mice, particularly after 12 h fasting. Plasma TG levels at
249 180 min after oil administration during the OLTT were significantly higher in mice, which were
250 fasted for 12 h or 48 h. The AUC values of the plasma TG level during the OLTT were higher in
251 a fasting period-dependent manner (Fig 5).
252
253 **Fig 5. Effect of fasting period on plasma TG levels and AUC values during the OLTT in**

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254 **ddY mice.** Values are means ± SE (n = 8–10). The data were analyzed with one-way ANOVA,
255 followed by the post-hoc Dunnett test. *: asterisks show significant differences ($p < 0.05$) in
256 compared to 0 h group. (*): (asterisks) show slight differences ($p < 0.1$) in compared to 0 h
257 group.
258

259 EGCG suppresses lipids-induced hypertriglyceridemia in 12 h 260 fasted ddY mice (Exp 4)

261 Plasma TG levels and AUC values during the OLTT were significantly lower in ddY mice
262 that were administered 100 mg/kg EGCG (Fig 6). The HPTLC data showed that spots of TG and
263 diacylglycerides were clearly observed in the EGCG group, whereas those spots were not
264 observed in the control group (Fig 7). The plasma TG levels at 120 min after oil administration
265 were significantly lower in the EGCG group, but the liver TG levels and FAS activity remained
266 unchanged (Fig 8).
267

268 **Fig 6. Effect of EGCG on plasma TG levels and AUC values during the OLTT in ddY mice.**
269 Values are means ± SE (n = 8). The data were analyzed with Student-t test. *: asterisks show
270 significant differences ($p < 0.05$). (*): (asterisks) show slight differences ($p < 0.1$).

271 **Fig 7. Effect of EGCG on intestinal lipids source following the lipids administration in ddY**
272 **mice.** STD contained a mixture of triolein, diolein, monoolein, and oleic acid. Extracted lipids in
273 the small intestine were separated on a HPTLC. The plate was developed with hexane/diethyl
274 ether/acetic acid (60:40:1, v/v). The spots of each lipid were visualized using iodine.

275 **Fig 8. Effect of EGCG on plasma and liver TG levels and liver FAS activity in ddY mice.**
276 Values are means ± SE (n = 8). The data were analyzed with Student-t test. **: asterisks show

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277 significant differences ($p < 0.01$).

278

279 Lipids-induced hypertriglyceridemia is not fully induced in female 280 ddY mice (Exp 5)

281 In male mice, the plasma TG levels and the AUC values observed during the OLTT were 282 lower in the EGCG group (Fig. 5). Conversely in female mice, the elevation of the plasma TG 283 levels at 60 min after oil administration was lower in the EGCG group, but the maximal TG 284 levels after oil administration and the corresponding AUC values were not changed by the 285 presence of EGCG (Fig 9).

286

287 **Fig 9. Effect of gender on plasma TG levels during the OLTT in ddY mice.** Values are 288 means ± SE (n = 8–9). The data were analyzed with two-way ANOVA, followed by the post-hoc 289 Tukey-Kramer test.

290

291 A short-term HF diet treatment induced lipids-induced 292 hypertriglyceridemia (Exp 6)

293 The elevation of plasma TG levels after oil administration during the OLTT was significantly 294 higher in ddY mice treated with HF diet for 1 week prior to testing than those fed with the AIN-295 93G control diet. Treatment with EGCG (100 mg/kg) suppressed lipid-induced plasma TG 296 elevation and AUC values (Fig 10).

297

298 **Fig 10. Effect of short-term HFD on plasma TG levels during the OLTT in ddY mice.** 299 Values are means ± SE (n = 8). The data were analyzed with two-way ANOVA, followed by the

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300 post-hoc Tukey-Kramer test.

301

302 # Discussion

303 The OLTT has been used to assess whole-body lipid homeostasis. The ability to quickly
304 normalize hyperlipidemic metabolism following the oral administration of lipids provides
305 integrated information about intestinal lipid digestion and absorption, lipoprotein transportation,
306 and tissue-specific lipid metabolism. However, OLTT methodologies are highly varied and are
307 even different within research groups. Therefore, an appropriate model for the OLTT has not yet
308 been constructed, whereas the methodology of the OGTT has been generally established in both
309 mice [21] and humans by the WHO (2013) [28]. The wide array of available lipid sources and
310 the complexity of lipid metabolism compared to glucose metabolism constitute two reasons for
311 the variation in OLTT methodologies. This present study has proposed a more appropriate model
312 of the OLTT for evaluating lipid-induced hypertriglyceridemia in mice by investigating the
313 differences between mice strains, lipid sources, the fasting period, gender, and diet before OLTT
314 administration.

315 The first experiment of the present study confirmed that the plasma TG levels after the oral
316 administration of lipids were different among the tested mice strains. Yamazaki et al. (2012) first
317 reported that ddY mice were susceptible to lipid-induced hypertriglyceridemia due to an increase
318 in lipoprotein production and a decrease in whole-body plasma lipoprotein lipase (LPL) activity
319 [29]. The higher lipoprotein production and lower LPL activity indicate that chylomicron- and
320 very-low-density-lipoprotein (VLDL)-TG in the plasma cannot be hydrolyzed and transported
321 into the tissues, which often results in hypertriglyceridemia. Saleh et al. (2011) also suggested in
322 their review that LPL may be a causative factor for the acceleration of lipid clearance from the

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323 blood [30]. However, ddY mice are known to possess higher LPL activities and have the
324 potential to induce lipid-induced hypertriglyceridemia; however, they are not often selected for
325 OLTT studies (Table 1). The increases in the plasma TG levels 180 min after oil administration
326 in the C57BL/6N and ICR mice were 128 and 270 mg/dL, respectively, which were remarkably
327 lower than in the ddY mice (412 mg/dL). Considering the smaller increase in plasma TG levels
328 observed in the C57BL/6N and ICR mice, it is likely more difficult to evaluate the suppressive
329 effects of food materials on hypertriglyceridemia in these strains. Correspondingly, in previous
330 studies that used C57BL/6 mice strain, the administration of lipids elevated the plasma TG levels
331 only to about twice the value of the fasting baseline. Yamazaki et al. (2012) reported that the
332 C57BL/6 mice strain did not show postprandial hypertriglyceridemia [29]. Therefore, we
333 contend that the choice of mice strain used for the OLTT is an important factor for the evaluation
334 of lipid-induced hypertriglyceridemia.

335 The lipid source used for the OLTT is considered to be an important factor for lipid-induced
336 hypertriglyceridemia. In rodent studies, olive and corn oils have often been used as the lipid
337 source (Table 1). In human studies, various high-fat meals were utilized as the lipid source. The
338 dominant fatty acids in olive and corn oils are oleic acid (rich in n-9 fatty acids) and linoleic acid
339 (rich in n-6 fatty acids), respectively, but not n-3 fatty acids (Fig 1). In this study, dietary oils
340 rich in n-6 and n-9 fatty acids could easily elevate the plasma TG levels. It has been reported that
341 dietary n-3 fatty acids such as α-linolenic acid, eicosapentaenoic acid (EPA), and
342 docosahexaenoic acid (DHA) improved lipid metabolism in rodents and humans through anti-
343 inflammatory and peroxisome proliferator-activated receptor (PPAR)-α/γ activation mechanisms
344 [31]. The activation of PPAR-α by n-3 fatty acids can increase fatty acid oxidation and decrease
345 TG and VLDL secretion. Furthermore, the activation of PPAR-γ by n-3 fatty acids improves

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346 insulin sensitivity, resulting in the increase of TG clearance [15, 16]. The present results indicate
347 that fish and perilla oils rich in n-3 fatty acids decreased the plasma TG levels and accelerated
348 TG clearance at 180 min after oil administration, although the maximum plasma TG levels were
349 not suppressed. These results indicate that n-3 fatty acids accelerated fatty acid oxidation.
350 Additionally, n-3 fatty acids were identified to be less susceptible to pancreatic lipase activity
351 due to the presence of multiple double bonds and their overall structural complexity, which may
352 delay their digestion and absorption [32–35]. In contrast, the administration of beef tallow,
353 which is rich in saturated fatty acids, unexpectedly suppressed plasma TG elevation. In general,
354 saturated fats such as those found in beef tallow and lard can easily induce abnormalities in
355 glucose and fat metabolism, delaying TG clearance. On the other hand, saturated fats are less
356 susceptible to pancreatic lipase activity, in part due to their higher melting points, resulting in
357 lower fat absorption. Therefore, these fats can take longer time to be digested and absorbed in
358 the small intestine, and the plasma TG levels can be suppressed after the administration of
359 saturated fats. Due to the higher melting points of saturated fats, they are more difficult to orally
360 administer to rodents with a stainless-steel tube at room temperature. On the other hand,
361 saturated fats have often been used for the OLTT in human studies. This must be carefully
362 considered when comparing the results between mice and human studies. This study aimed to
363 propose an appropriate dietary lipid for OLTT that will increase the plasma TG elevation in
364 mice, and we determined that soybean oil is the most suitable lipid source for this purpose.
365 Considering the suppression of the plasma TG elevation and AUC values by n-3 fatty acids, oils
366 rich in n-3 fatty acids are not likely to be appropriate for OLTT.
367 Dietary lipid size can affect the digestion and absorption of lipids in the small intestine,
368 which can easily elevate the plasma TG levels. As shown in Table 1, fat emulsions have often

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369 been utilized as fat sources when considering the intestinal digestion and absorption of lipids [18,
370 23, 36-40]. Generally, dietary lipids are emulsified by endogenous bile acids to smaller lipid
371 droplets in the gut, and then they are digested and absorbed in the small intestine. Administering
372 the mixture of oils and emulsifiers has been observed to accelerate lipid digestion. Some reports
373 have shown that the plasma TG levels reached their maximum 2–3 hours after the
374 administrations of fat emulsions [23, 36–40], which is similar to the timeframe observed in this
375 study and other reports in which no emulsions were used [24, 29, 41]. Higher maximum values
376 of plasma TG levels during the OLTT are needed for easier evaluation of hypertriglyceridemia;
377 hence, different methodologies for lipid preparation should be investigated.

378 The fasting period before the OLTT can greatly affect the resulting plasma TG elevation, but
379 few investigations examining the effects of fasting period before the OLTT on plasma TG
380 elevation have been carried out [17, 42]. Ikeda et al. (2014) demonstrated that a longer fasting
381 period dramatically suppressed mRNA expression associated with lipogenesis but activated
382 mRNA expression associated with lipid β-oxidation in the liver [17]. The fasting period-
383 dependent increase in the AUC values of plasma TG levels suggests that the plasma TG levels
384 did not reach the maximum and that orally administered lipids were effectively absorbed. In the
385 case of 48 h-fasting period, the maximum plasma TG elevations were found to be delayed,
386 indicating that the administered lipids were digested and absorbed in the small intestine but were
387 quickly transported into the liver, before they could enter the blood and be detected in the
388 plasma. Therefore, fasting treatment for 12 hours before the OLTT is more suitable for the
389 evaluation of plasma TG elevation as a fasting period that is too long before an OLTT can
390 greatly affect lipid metabolism; this suggests that a 12 h fasting period is more appropriate than a
391 48 h fasting period.

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392 EGCG is known to have multi-faceted effects [43]. In Japan, EGCG is often utilized as a
393 functional compound in health foods, supplements, and drinks. The inhibition of pancreatic
394 lipase activity and the suppression of dietary lipid absorption are considered to be anti-obesity
395 mechanisms of EGCG [23]. In this study, the administration of EGCG (100 mg/kg) strongly
396 suppressed the plasma TG elevation during the OLTT, and HPTLC data of small intestinal
397 contents showed that TG remained in the small intestine and had not been degraded. EGCG
398 suppressed the elevation of plasma TG to 162, 289, and 186 mg/dL compared to the control
399 (320, 398, and 308 mg/dL) at 60, 120, and 180 min after administration, respectively. Therefore,
400 the investigated protocols (ddY, male, 12 h fasting before OLTT, and soybean oil) have been
401 determined to be appropriate for the evaluation of food-derived compounds in OLTT.
402 Considering the results from Fig 2 (Exp 1), it is not expected that the C57BL/6N and ICR mice
403 represent a strong suppressive effect of EGCG on hypertriglyceridemia as observed in the ddY
404 mice. On the other hand, a single administration of EGCG did not affect TG content and FAS
405 activity in the liver, unlike in the plasma.

406 Gender has a considerable influence on lipid-induced hypertriglyceridemia. Many reports
407 have used male mice or rats for evaluating the effects of food materials on lipid-induced
408 hypertriglyceridemia in OLTT studies (Table 1), although the reasons why male rodents were
409 exclusively used remain unclear. Some review articles have indicated that male mice are more
410 susceptible to postprandial hypertriglyceridemia than female mice by some endogenous factors
411 [30, 44]. Saleh et al. (2011) and Murray et al. (1999) pointed out that acylation-stimulating
412 protein (ASP), which is produced by adipocytes, accelerated postprandial TG clearance in
413 women and that a significant association between progesterone and ASP levels contributed to
414 abdominal fat accumulation in women [30, 44]. In rodents, gender dimorphism was observed in

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415 the postprandial response; female mice displayed larger increases in adipose tissue weights and
416 LPL activities compared to male mice. The rapid clearance of lipids in female mice was
417 suggested to be caused by LPL activity. Our findings indicate that the maximum plasma TG
418 levels during OLTT were higher in male mice and EGCG suppressed the levels in male mice
419 only. Therefore, in the case of OLTT investigations using female mice, the suppressive effects of
420 food materials on plasma TG elevation may be difficult to be evaluated.
421 Finally, the implementation of high-fat diets for over several weeks exacerbates metabolic
422 syndrome parameters observed in the plasma and tissues of mice. These diets could not only
423 exacerbate dysfunctional lipid metabolism, but also exacerbate glucose intolerance and insulin
424 resistance. On the other hand, a short-term regimen of a high-fat diet (1 week) can induce lipid-
425 induced hypertriglyceridemia without affecting fasting plasma TG levels [12, 31]. Hernández
426 Vallejo et al. (2009) suggested that 1-week adaptation to a saturated fat-based high-fat diet could
427 induce postprandial hypertriglyceridemia, but it could not affect the fasting plasma TG levels, by
428 inducing intestinal TG synthesis and decreasing chylomicron secretions [12]. In particular, the
429 serum apo-B48 levels in a fasted state can be a useful marker of postprandial
430 hypertriglyceridemia [45], which may be another method to associate lipid-induced TG elevation
431 in the OLTT.
432

433 # Conclusions

434 This study compared TG responses after the oral administration of various dietary lipids in
435 several strains of fasting mice. These findings helped elucidate more appropriate OLTT models.
436 We determined that male ddY mice fasted for 12 h displayed markedly higher lipid-induced
437 hypertriglyceridemia in response to soybean oils rich in n-6 fatty acids. Lipid-induced

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438 hypertriglyceridemia and postprandial hyperglycemia are determined to be independent risk
439 factors for coronary diseases. Determining standard protocols for lipid-induced
440 hypertriglyceridemia testing is requisite for investigating lipid metabolism in mice.
441

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445

446 References

447 1. World Health Organization. 2018, Global Health Estimates 2016: Deaths by Cause, Age,
448 Sex, by Country and by Region, 2000–2016. Geneva.
449 2. Borén J, Matikainen N, Adiels M, Taskinen MR. Postprandial hypertriglyceridemia as a
450 coronary risk factor. Clin. Chim. Acta. 2014; 431:131–42. doi: 10.1016/j.cca.2014.01.015
451 PMID: 24508990
452 3. Pirillo A, Norata GD, Catapano AL. Postprandial lipemia as a cardiometabolic risk factor.
453 Curr. Med. Res. Opin. 2014; 30(8):1489–503. doi: 10.1185/03007995.2014.909394 PMID:
454 24673475
455 4. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with
456 nonfasting triglycerides and risk of cardiovascular events in women. JAMA. 2007; 298:309–
457 16. doi: 10.1001/jama.298.3.309 PMID: 17635891
458 5. Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG. Nonfasting triglycerides and
459 risk of ischemic stroke in the general population. JAMA. 2008; 300:2142–52. doi:
460 10.1001/jama.2008.621 PMID: 19001625

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461 6. Iso H, Naito Y, Sato S, Kitamura A, Okamura T, Sankai T, et al. Serum triglycerides and
462 risk of coronary heart disease among Japanese men and women. Am. J. Epidemiol. 2001;
463 153:490–9. doi: 10.1093/aje/153.5.490 PMID: 11226981
464 7. Boquist S, Ruotolo G, Tang R, Björkegren J, Bond MG, de Faire U, et al. Alimentary
465 lipemia, postprandial triglyceride-rich lipoproteins, and common carotid intima-media
466 thickness in healthy, middle-aged men. Circulation. 1999; 100:723–8. doi:
467 10.1161/01.cir.100.7.723 PMID: 10449694
468 8. Patsch JR, Miesenböck G, Hopferwieser T, Mühlberger V, Knapp E, Dunn JK, et al.
469 Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial
470 state. Arterioscler. Thromb. 1992; 12:1336–45. doi: 10.1161/01.atv.12.11.1336 PMID:
471 1420093
472 9. Björkegren J, Boquist S, Samnegârd A, Lundman P, Tornvall P, Ericsson CG, et al.
473 Accumulation of apolipoprotein C–I-rich and cholesterol-rich VLDL remnants during
474 exaggerated postprandial triglyceridemia in normolipidemic patients with coronary artery
475 disease. Circulation. 2000; 101:227–30. doi: 10.1161/01.cir.101.3.227 PMID: 10645915
476 10. Tomlinson B, Chan P, Lam CWK. Postprandial hyperlipidemia as a risk factor in patients
477 with type 2 diabetes. Expert Rev. Endocrinol. Metab. 2020; 15(3):147–57. doi:
478 10.1080/17446651.2020.1750949 PMID: 32292091
479 11. Lairon D, Defoort C. Effects of nutrients on postprandial lipemia. Curr. Vasc. Pharmacol.
480 2011; 9:309–12. doi: 10.2174/157016111795495576 PMID: 21314626
481 12. Hernández Vallejo SJ, Alqub M, Luquet S, Cruciani-Guglielmacci C, Delerive P, Lobaccaro
482 JM, et al. Short-term adaptation of postprandial lipoprotein secretion and intestinal gene

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483 expression to a high-fat diet. Am. J. Physiol. Gastrointest. Liver Physiol. 2009;296(4):
484 G782–92. doi: 10.1152/ajpgi.90324.2008 PMID: 19196952
485 13. Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological
486 influences on postprandial lipid metabolism. Br. J. Nutr. 2007; 98:458–73. doi:
487 10.1017/S000711450774268X PMID: 17705891
488 14. Perez-Martinez P, Garcia-Rios A, Delgado-Lista J, Perez-Jimenez F, Lopez-Miranda J.
489 Nutrigenetics of the postprandial lipoprotein metabolism: evidences from human
490 intervention studies. Curr. Vasc. Pharmacol. 2011. 9(3):287–91. doi:
491 10.2174/157016111795495495 PMID: 21314629
492 15. Schmitz G, Ecker J. The opposing effects of n-3 and n-6 fatty acids. Prog. Lipid Res. 2008;
493 47(2): 147–55. doi: 10.1016/j.plipres.2007.12.004 PMID: 18198131
494 16. Calder PC. Mechanisms of action of (n-3) fatty acids. J. Nutr. 2012; 142(3):592S–9S. doi:
495 10.3945/jn.111.155259 PMID: 22279140
496 17. Ikeda I, Metoki K, Yamahira T, Kato M, Inoue N, Nagao K, et al. Impact of fasting time on
497 hepatic lipid metabolism in nutritional animal studies. Biosci. Biotechnol. Biochem. 2014;
498 78(9):1584–91. doi: 10.1080/09168451.2014.923297 PMID: 25209508
499 18. Morentin Gutierrez P, Yates J, Nilsson C, Birtles S. Evolving data analysis of an Oral Lipid
500 Tolerance Test toward the standard for the Oral Glucose Tolerance Test: Cross species
501 modeling effects of AZD7687 on plasma triacylglycerol. Pharmacol. Res. Perspect. 2019;
502 7(2):e00465. doi: 10.1002/prp2.465 PMID: 30899516
503 19. King AJ, Segreti JA, Larson KJ, Souers AJ, Kym PR, Reilly RM, et al. In Vivo Efficacy of
504 Acyl CoA: Diacylglycerol Acyltransferase (DGAT) 1 Inhibition in Rodent Models of

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505 Postprandial Hyperlipidemia. Eur J. Pharmacol. 2010; 637(1-3):155–61. doi:
506 10.1016/j.ejphar.2010.03.056 PMID: 20385122
507 20. Hong DJ, Jung SH, Kim J, Jung D, Ahn YG, Suh KH, et al. Synthesis and biological
508 evaluation of novel thienopyrimidine derivatives as diacylglycerol acyltransferase 1
509 (DGAT-1) inhibitors. J. Enzyme Inhib. Med. Chem. 2020; 35(1):227–234. doi:
510 10.1080/14756366.2019.1693555 PMID: 31752563
511 21. Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance
512 test in mice. Am. J. Physiol. Endocrinol. Metab. 2008; 295(6):E1323–32. doi:
513 10.1152/ajpendo.90617.2008 PMID: 18812462
514 22. Ochiai M, Inada M, Horiguchi S. Nutritional and safety evaluation of locust (Caelifera)
515 powder as a novel food material. J. Food Sci. 2020; 85(2):279–88. doi: 10.1111/1750-
516 3841.15024 PMID: 31976553
517 23. Ikeda I, Tsuda K, Suzuki Y, Kobayashi M, Unno T, Tomoyori H, et al. Tea catechins with a
518 galloyl moiety suppress postprandial hypertriacylglycerolemia by delaying lymphatic
519 transport of dietary fat in rats. J. Nutr. 2005; 135(2):155–9. doi: 10.1093/jn/135.2.155
520 PMID: 15671206
521 24. Kurihara H, Shibata H, Fukui Y, Kiso Y, Xu JK, Yao XS, et al. Evaluation of the
522 hypolipemic property of Camellia sinensis Var. ptilophylla on postprandial
523 hypertriglyceridemia. J. Agric. Food Chem. 2006; 12; 54(14):4977–81. doi:
524 10.1021/jf0603681 PMID: 16819905
525 25. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of
526 total lipides from animal tissues. J. Biol. Chem. 1957; 226(1):497–509. PMID: 13428781

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527 26. Yoshizumi K, Murota K, Watanabe S, Tomi H, Tsuji T, Terao J. Chiisanoside is not
528 absorbed but inhibits oil absorption in the small intestine of rodents. Biosci Biotechnol
529 Biochem. 2008; 72(4):1126–9. doi: 10.1271/bbb.70761 PMID: 18391464
530 27. Nepokroeff CM, Lakshmanan MR, Porter JW. Fatty-acid synthase from rat liver. Methods
531 Enzymol. 1975; 35:37–44. doi: 10.1016/0076-6879(75)35136-7 PMID: 1121291
532 28. World Health Organization. 2013, Diagnostic criteria and classification of hyperglycaemia
533 first detected in pregnancy (WHO/NMH/MND/13.2)
534 29. Yamazaki T, Kishimoto K, Ezaki O. The ddY mouse: a model of postprandial
535 hypertriglyceridemia in response to dietary fat. J. Lipid Res. 2012; 53(10):2024–37. doi:
536 10.1194/jlr.M023713 PMID: 22735545
537 30. Saleh J, Al-Wardy N, Farhan H, Al-Khanbashi M, Cianflone K. Acylation stimulating
538 protein: a female lipogenic factor? Obes. Rev. 2011; 12(6):440–8. doi: 10.1111/j.1467-
539 789X.2010.00832.x PMID: 21348923
540 31. Kimura R, Takahashi N, Lin S, Goto T, Murota K, Nakata R, et al. DHA attenuates
541 postprandial hyperlipidemia via activating PPARα in intestinal epithelial cells. J. Lipid Res.
542 2013; 54(12):3258–68. doi: 10.1194/jlr.M034942 PMID: 24133194
543 32. Akanbi TO, Sinclair AJ, Barrow CJ. Pancreatic lipase selectively hydrolyses DPA over EPA
544 and DHA due to location of double bonds in the fatty acid rather than regioselectivity. Food
545 Chem. 2014; 160:61–6. doi: 10.1016/j.foodchem.2014.03.092 PMID: 24799209
546 33. Zhu X, Ye A, Verrier T, Singh H. Free fatty acid profiles of emulsified lipids during in vitro
547 digestion with pancreatic lipase. Food Chem. 2013; 139(1-4):398–404. doi:
548 10.1016/j.foodchem.2012.12.060 PMID: 23561123

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549 34. Ye Z, Li R, Cao C, Xu YJ, Cao P, Li Q, et al. Fatty acid profiles of typical dietary lipids
550 after gastrointestinal digestion and absorption: A combination study between in-vitro and in-
551 vivo. Food Chem. 2019; 280:34–44. doi: 10.1016/j.foodchem.2018.12.032 PMID: 30642504
552 35. Ye Z, Cao C, Li R, Cao P, Li Q, Liu Y. Lipid composition modulates the intestine digestion
553 rate and serum lipid status of different edible oils: a combination of in vitro and in vivo
554 studies. Food Funct. 2019; 10(3):1490–503. doi: 10.1039/c8fo01290c PMID: 30783644
555 36. Kobayashi M, Ichitani M, Suzuki Y, Unno T, Sugawara T, Yamahira T, et al. Black-tea
556 polyphenols suppress postprandial hypertriacylglycerolemia by suppressing lymphatic
557 transport of dietary fat in rats. J. Agric. Food Chem. 2009; 57(15):7131–6. doi:
558 10.1021/jf900855v PMID: 19722586
559 37. Suzuki Y, Unno T, Kobayashi M, Nozawa A, Sagesaka Y, Kakuda T. Dose-dependent
560 suppression of tea catechins with a galloyl moiety on postprandial hypertriglyceridemia in
561 rats. Biosci Biotechnol Biochem. 2005; 69(7):1288–91. doi: 10.1271/bbb.69.1288 PMID:
562 16041132
563 38. Kido Y, Hiramoto S, Murao M, Horio Y, Miyazaki T, Kodama T, et al. Epsilon-polylysine
564 inhibits pancreatic lipase activity and suppresses postprandial hypertriacylglyceridemia in
565 rats. J. Nutr. 2003; 133(6):1887–91. doi: 10.1093/jn/133.6.1887 PMID: 12771334
566 39. Zhou Y, Inoue N, Ozawa R, Maekawa T, Izumo T, Kitagawa Y, et al. Effects of heat-killed
567 Lactobacillus pentosus S-PT84 on postprandial hypertriacylglycerolemia in rats. Biosci.
568 Biotechnol. Biochem. 2013; 77(3):591–4. doi: 10.1271/bbb.120830 PMID: 23470742
569 40. Tamaru S, Ohmachi K, Miyata Y, Tanaka T, Kubayasi T, Nagata Y, et al.
570 Hypotriglyceridemic potential of fermented mixed tea made with third-crop green tea leaves

bioRxiv preprint doi: https://doi.org/10.1101/2020.06.25.170605; this version posted June 25, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

571 and camellia (Camellia japonica) leaves in Sprague-Dawley rats. J. Agric. Food Chem.
572 2013; 61(24):5817–23. doi: 10.1021/jf400938h PMID: 23705670
573 41. Hiel S, Neyrinck AM, Rodriguez J, Pachikian BD, Bouzin C, Thissen JP, et al. Inulin
574 Improves Postprandial Hypertriglyceridemia by Modulating Gene Expression in the Small
575 Intestine. Nutrients. 2018; 10(5):532. doi: 10.3390/nu10050532 PMID: 29693598
576 42. Palou M, Priego T, Sánchez J, Villegas E, Rodríguez AM, Palou A, et al. Sequential changes
577 in the expression of genes involved in lipid metabolism in adipose tissue and liver in
578 response to fasting. Pflugers Arch. 2008; 456(5):825–36. doi: 10.1007/s00424-008-0461-1
579 PMID: 18493788
580 43. Ikeda I. Multifunctional effects of green tea catechins on prevention of the metabolic
581 syndrome. Asia Pac. J. Clin. Nutr. 2008; 17(Suppl 1):273–4. PMID: 18296354
582 44. Murray I, Sniderman AD, Cianflone K. Mice lacking acylation stimulating protein (ASP)
583 have delayed postprandial triglyceride clearance. J. Lipid Res. 1999; 40(9):1671–6. PMID:
584 10484614
585 45. Masuda D, Sakai N, Sugimoto T, Kitazume-Taneike R, Yamashita T, Kawase R, et al.
586 Fasting serum apolipoprotein B-48 can be a marker of postprandial hyperlipidemia. J.
587 Atheroscler. Thromb. 2011; 18(12):1062–70. doi: 10.5551/jat.10470 PMID: 21946533
588 46. Kimura R, Takahashi N, Murota K, Yamada Y, Niiya S, Kanzaki N, et al. Activation of
589 peroxisome proliferator-activated receptor-α (PPARα) suppresses postprandial lipidemia
590 through fatty acid oxidation in enterocytes. Biochem. Biophys. Res. Commun. 2011;
591 410(1):1–6. doi: 10.1016/j.bbrc.2011.05.057 PMID: 21640707

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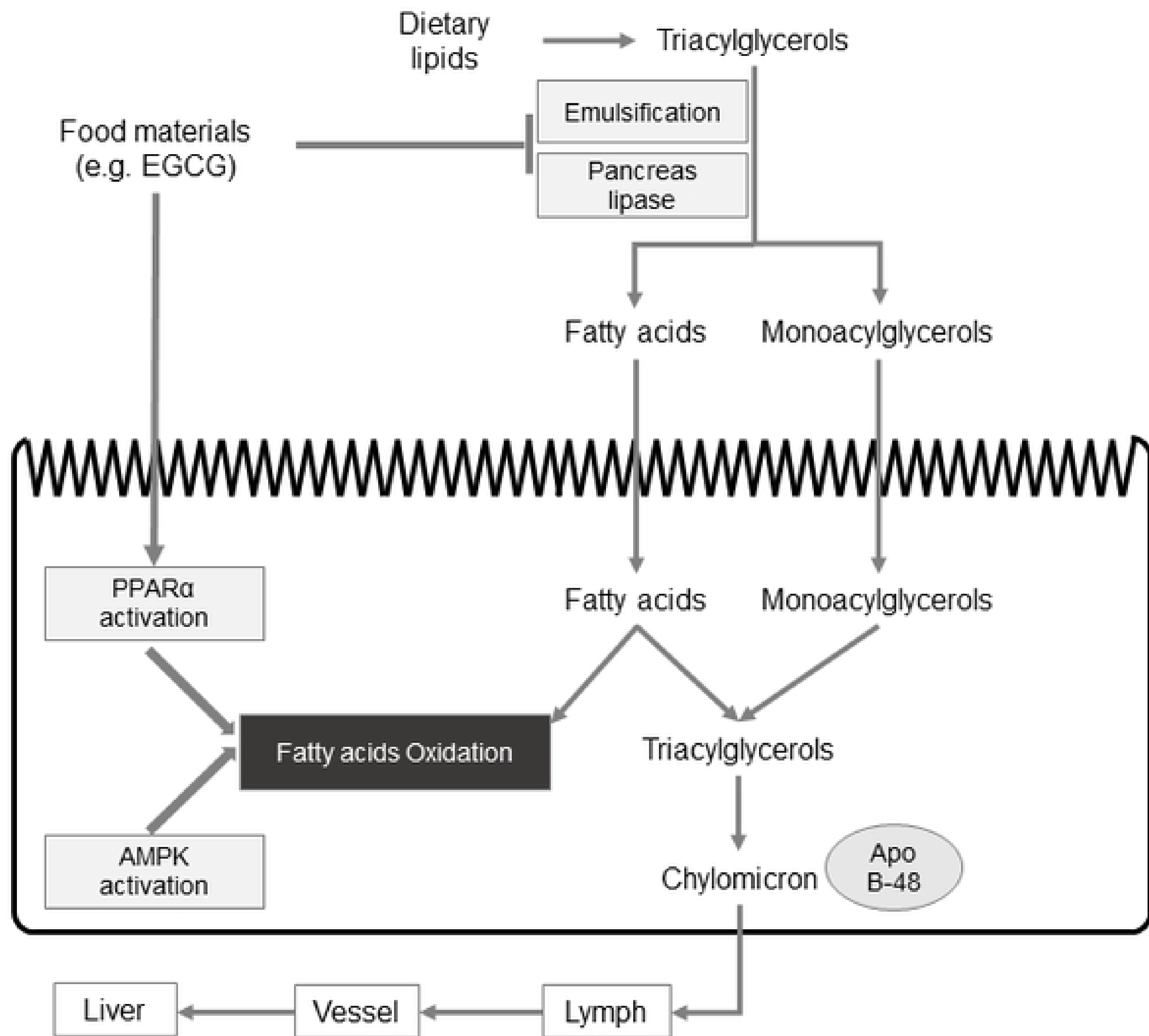
592 47. Toyoda-Ono Y, Yoshimura M, Nakai M, Fukui Y, Asami S, Shibata H, et al. Suppression of
593 postprandial hypertriglyceridemia in rats and mice by oolong tea polymerized polyphenols.
594 Biosci. Biotechnol. Biochem. 2007; 71(4):971–6. doi: 10.1271/bbb.60635 PMID: 17420597
595 48. Sairyo M, Kobayashi T, Masuda D, Kanno K, Zhu Y, Okada T, et al. A Novel Selective
596 PPARα Modulator (SPPARMα), K-877 (Pemafibrate), Attenuates Postprandial
597 Hypertriglyceridemia in Mice. J. Atheroscler. Thromb. 2018; 25(2):142–52. doi:
598 10.5551/jat.39693 PMID: 28781340
599 49. Maeda T, Miki S, Morihara N, Kagawa Y. Aged garlic extract ameliorates fatty liver and
600 insulin resistance and improves the gut microbiota profile in a mouse model of insulin
601 resistance. Exp. Ther. Med. 2019; 18(1):857–66. doi: 10.3892/etm.2019.7636 PMID:
602 31281460
603 50. Matsuda H, Kumazaki K, Otokozawa R, Tanaka M, Udagawa E, Shirai T. Resistant starch
604 suppresses postprandial hypertriglyceridemia in rats. Food Res. Int. 2016; 89(Pt 1):838–42.
605 doi: 10.1016/j.foodres.2016.10.022 PMID: 28460986



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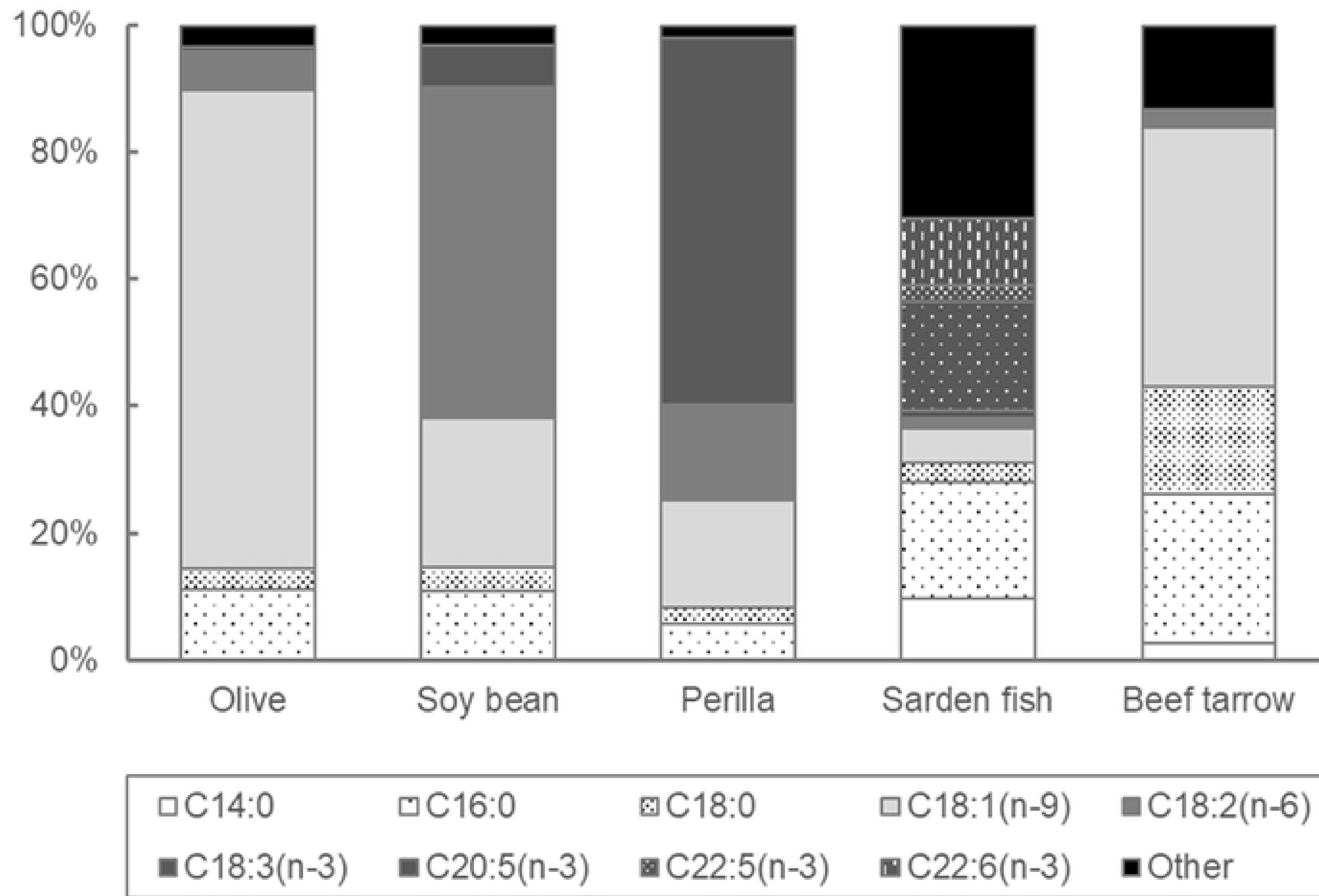

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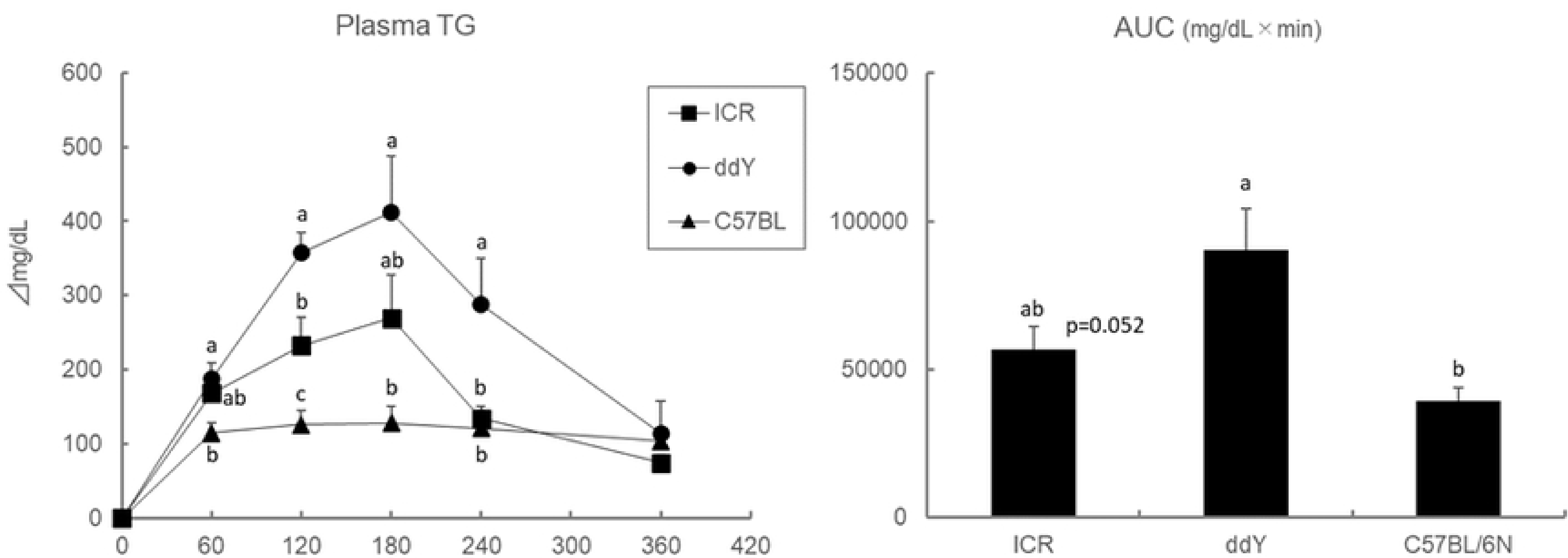

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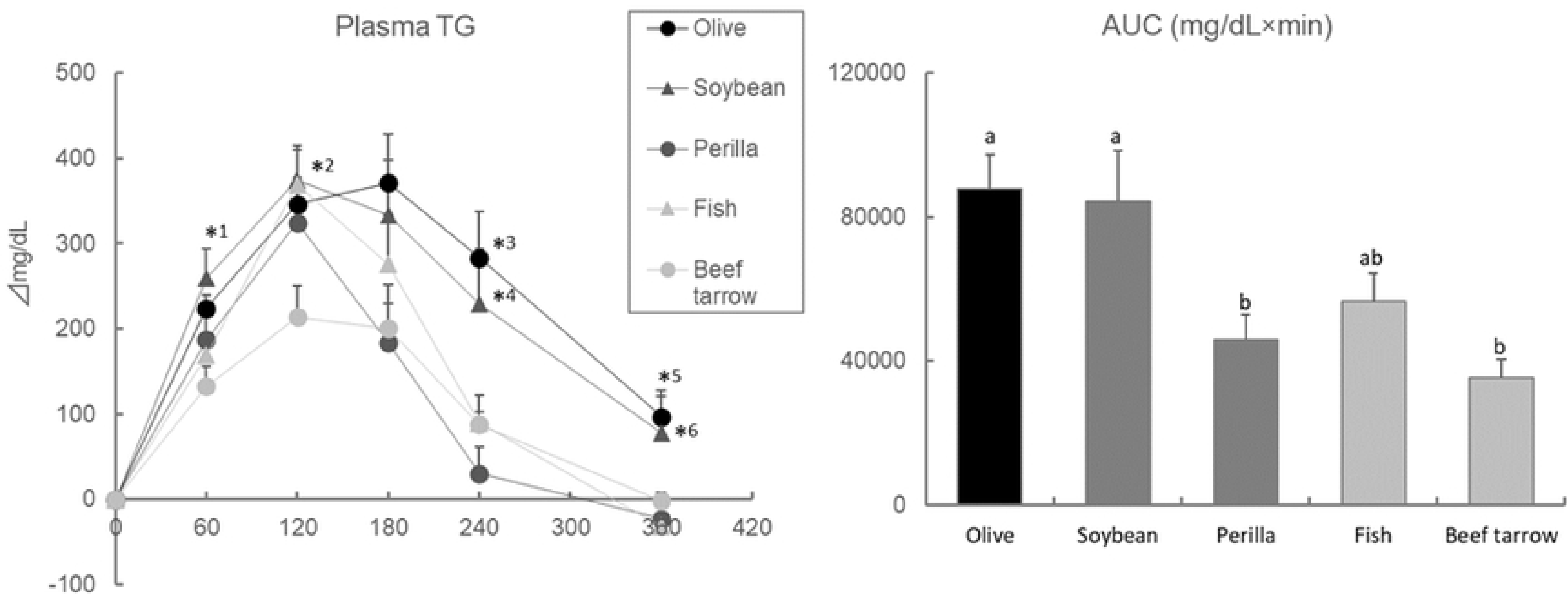

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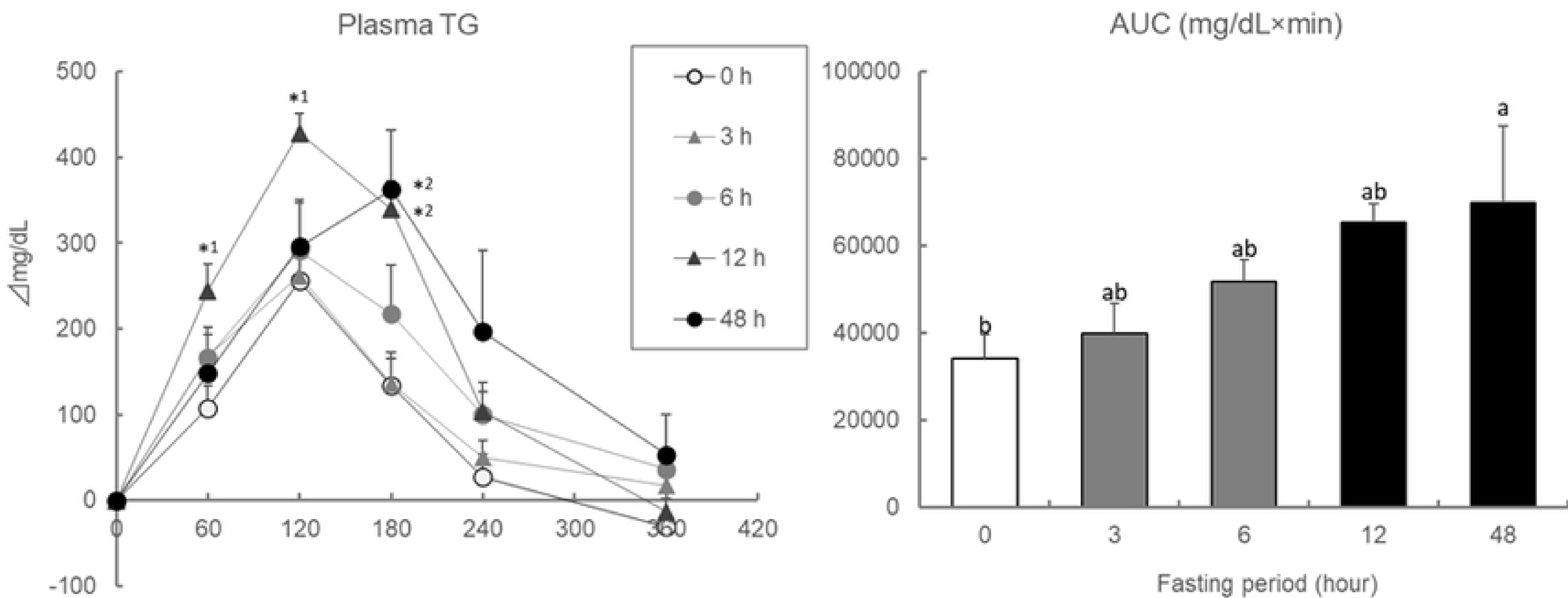

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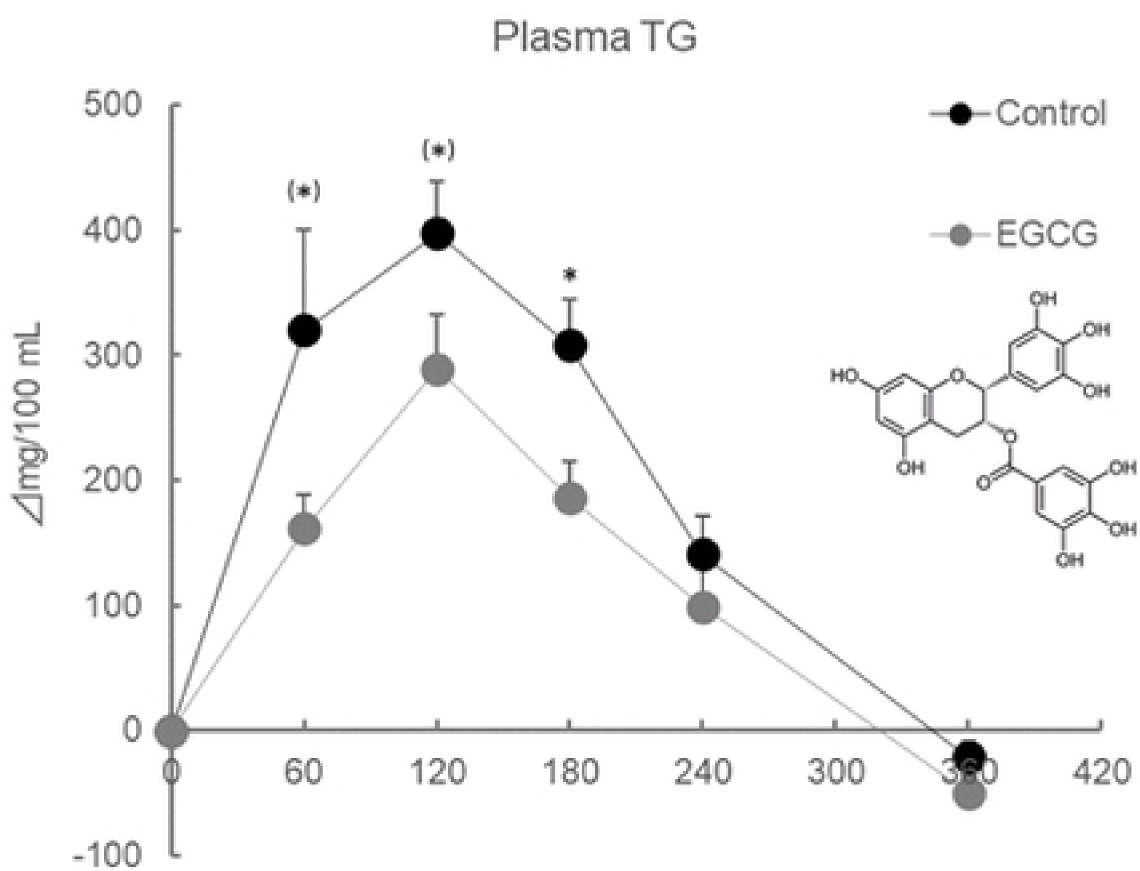

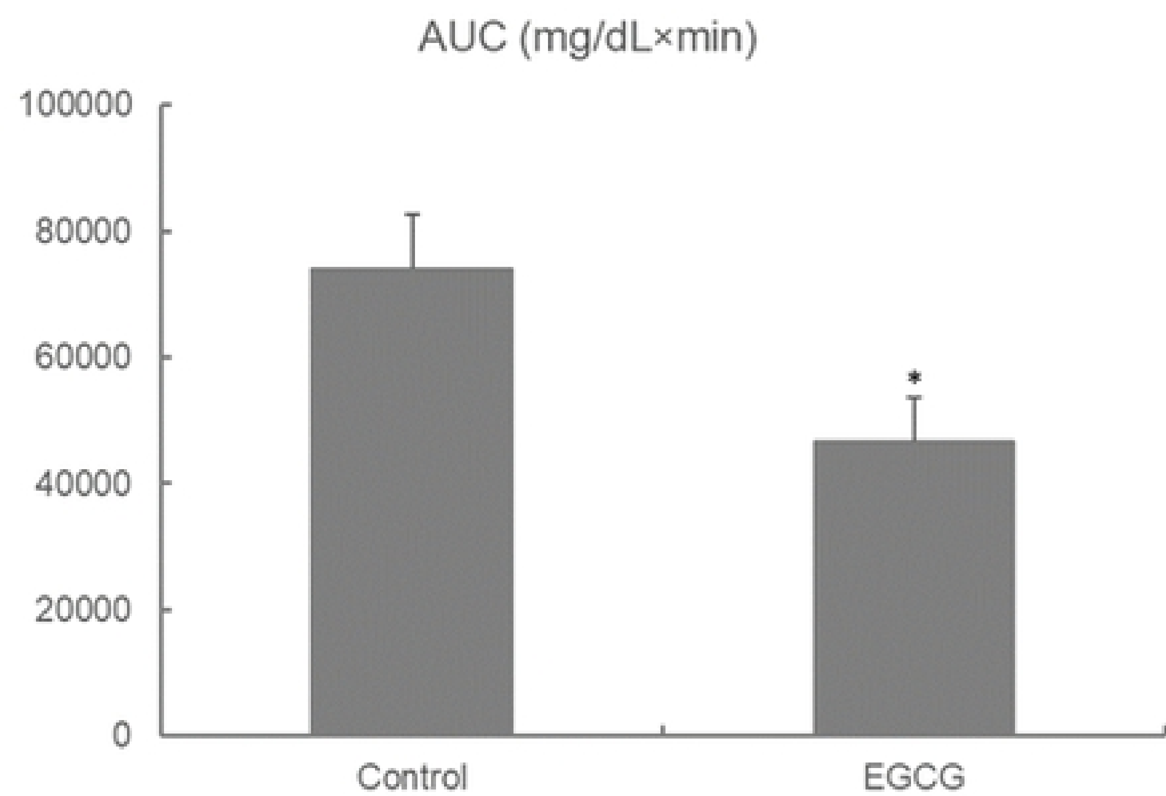

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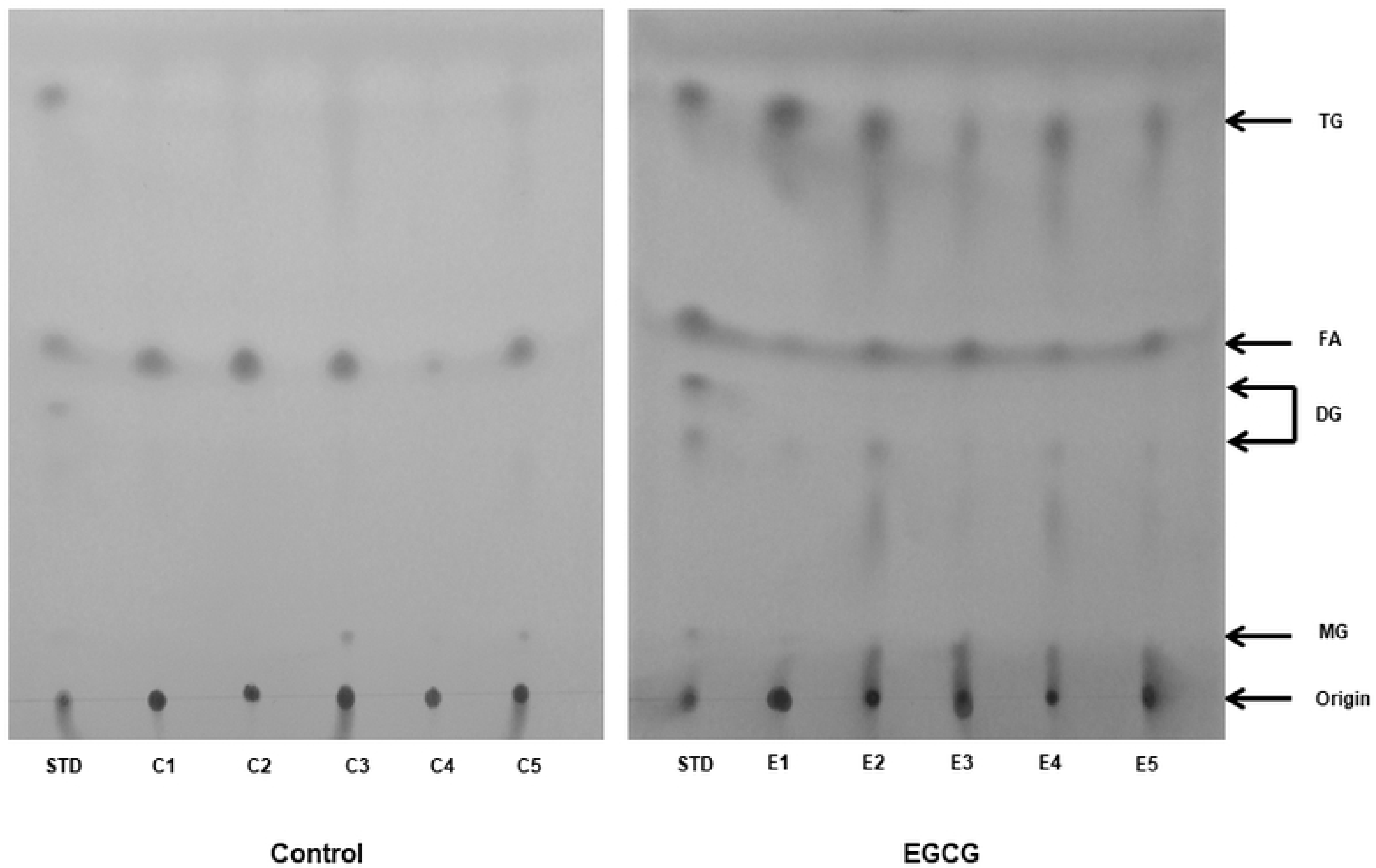

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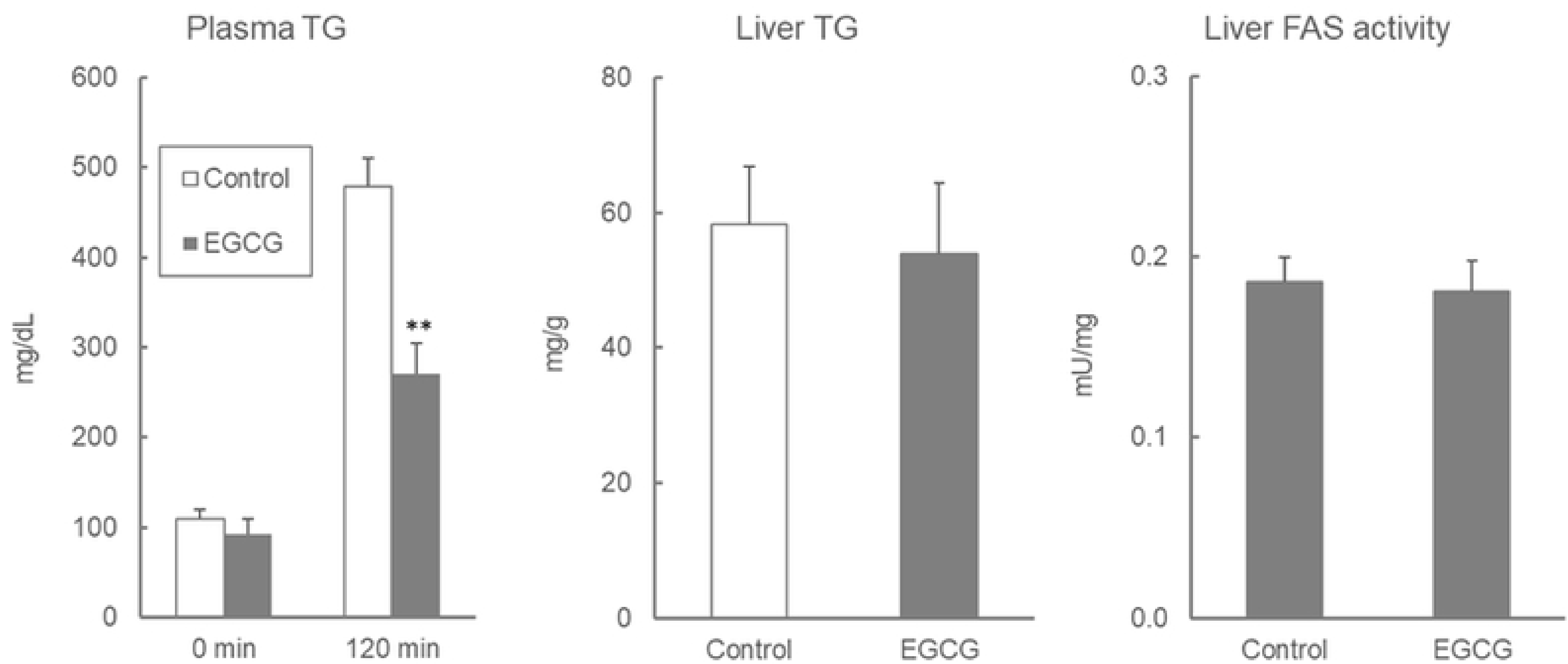

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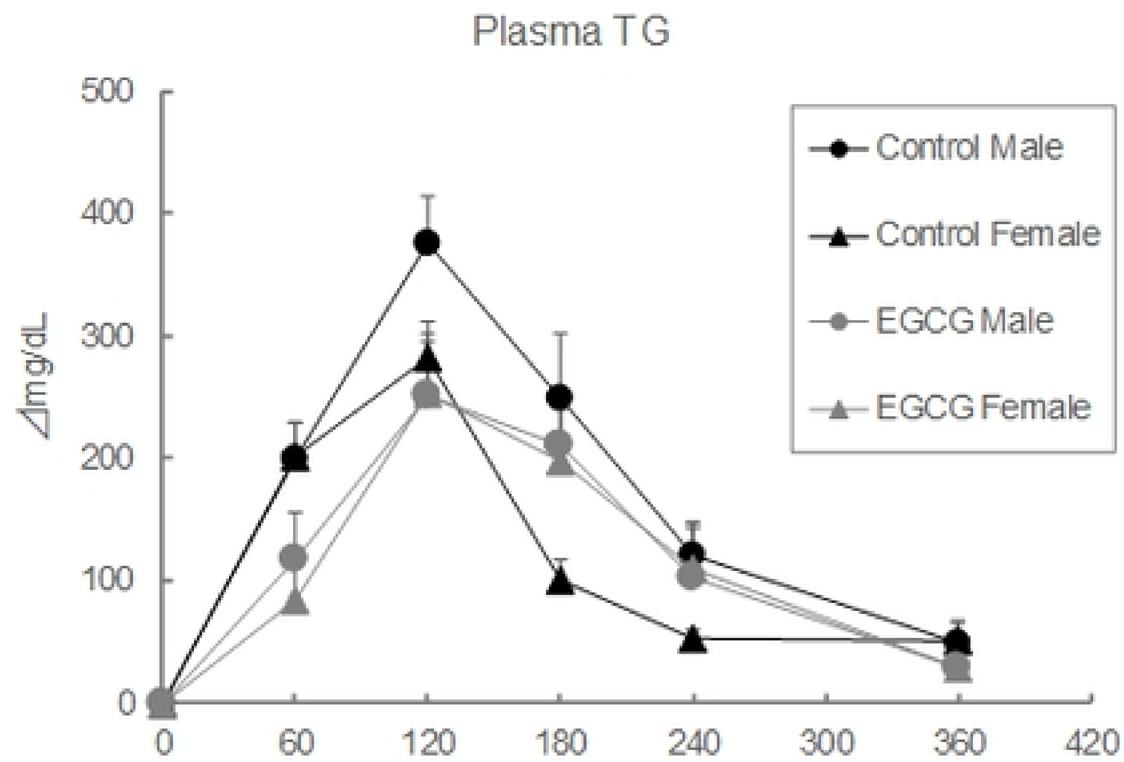
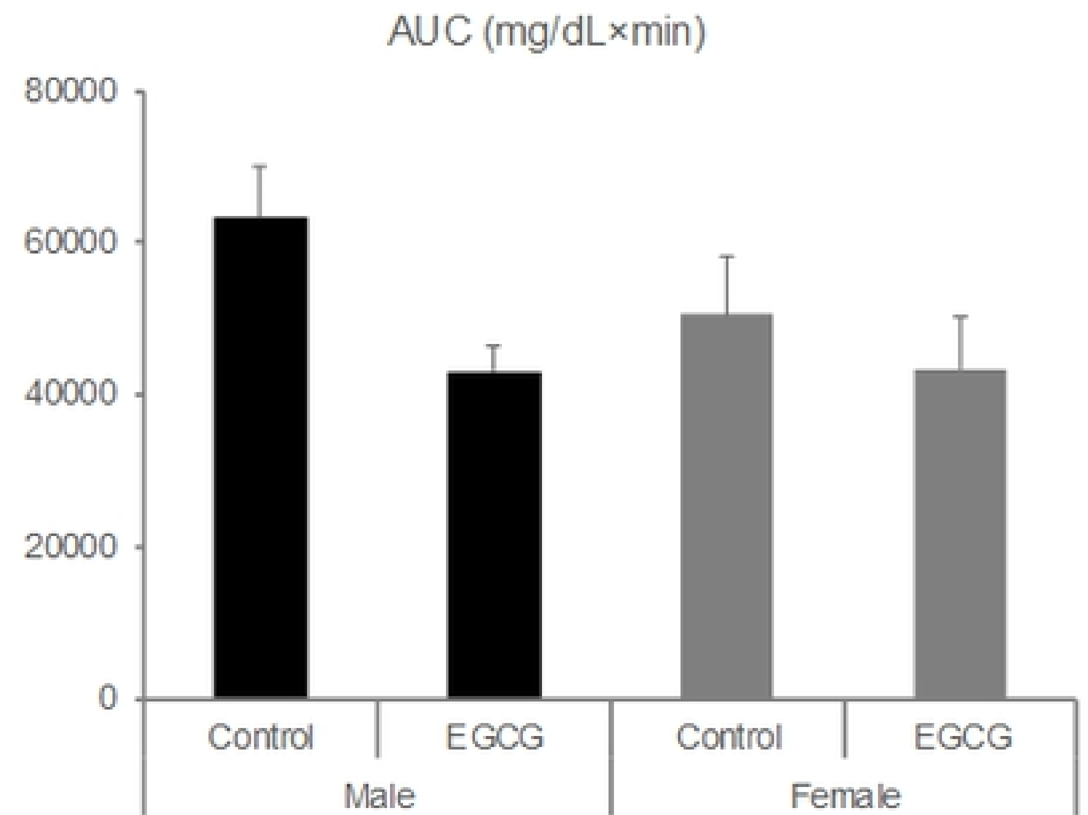
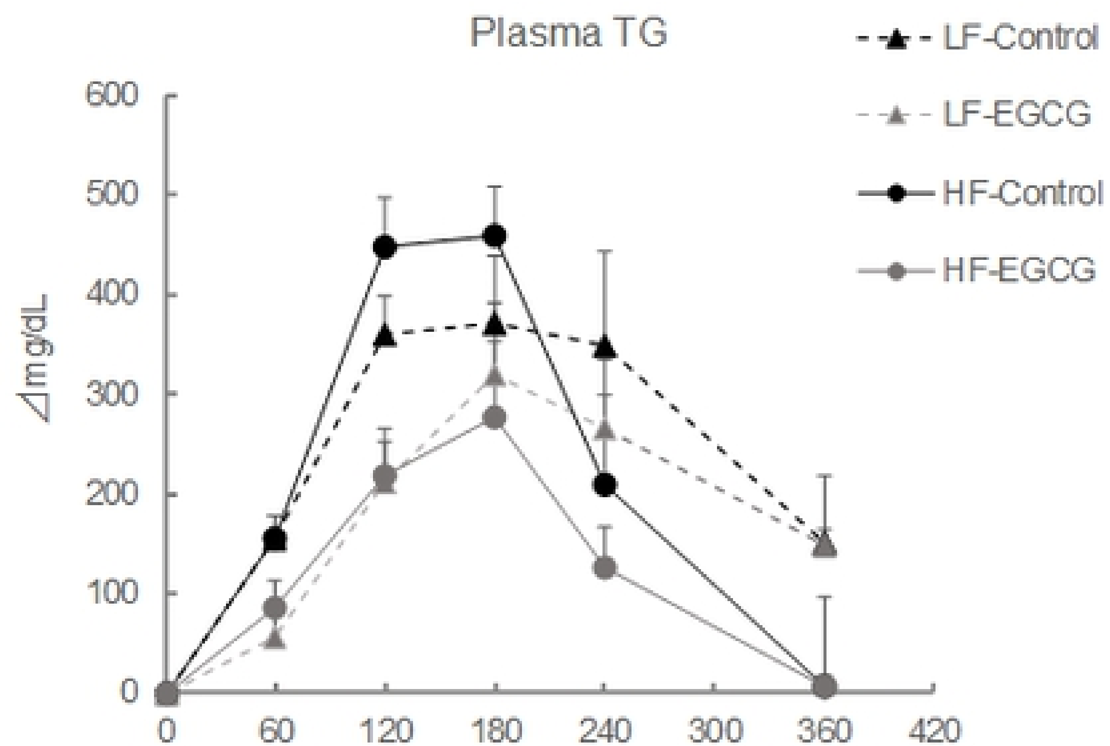


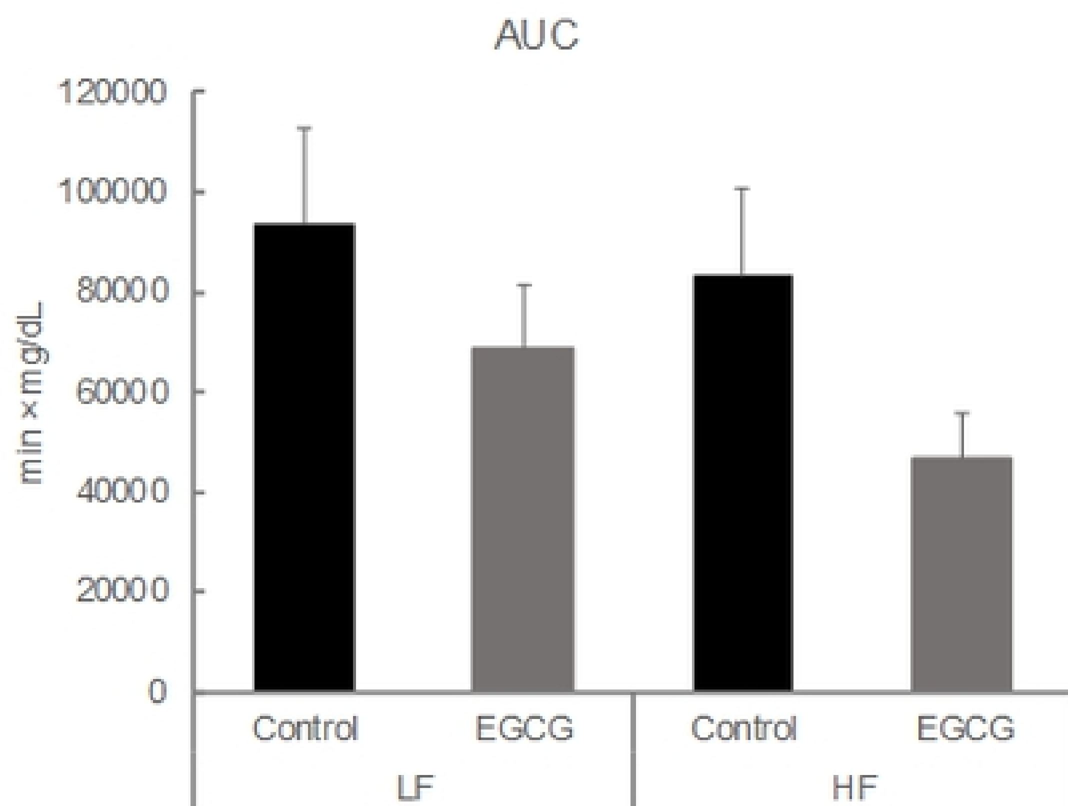


ANOVA					
	60	120	180	240	360
Gender	NS	NS	NS ($p < 0.1$)	NS	NS
EGCG	$p < 0.01$	N.S. ($p < 0.1$)	NS	NS	NS
Gender × EGCG	NS	NS	N.S. ($p < 0.1$)	NS	NS

ANOVA	
Gender	$p < 0.05$
EGCG	NS
Gender × EGCG	NS

Figure 9





ANOVA					
	60	120	180	240	360
HF	N.S.	N.S.	N.S.	N.S. ($p < 0.1$)	$p < 0.05$
EGCG	$p < 0.01$	$p < 0.001$	N.S. ($p < 0.1$)	N.S.	N.S.
HF×EGCG	N.S.	N.S.	N.S.	N.S.	N.S.

ANOVA	
HF	N.S.
EGCG	N.S. ($p < 0.1$)
HF×EGCG	N.S.

Figure 10