



<b>Title</b>	Squalene modulates fatty acid metabolism: Enhanced EPA/DHA in obese/diabetic mice (KK-A(y)) model
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1 **Squalene modulates fatty acid metabolism: Enhanced EPA/DHA, in obese/diabetic mice**  
2 **(*KK-A<sup>y</sup>*) model**

3

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21 **Running title:** Squalene effect on EPA/DHA levels in obese/diabetic mice

22 **Abbreviations:**

23 ALA -  $\alpha$ -Linolenic Acid; Con – Control; DPA – Docosapentanoic Acid; FAME – Fatty Acid  
24 Methyl Ester; SO – Soybean Oil; SQ – Squalene; WAT – White Adipose Tissue

25

26 **Abstract**

27 Biosynthesis of long-chain n-3 fatty acids from precursors is limited. *In-vivo* effect of  
28 squalene (SQ) on the metabolic fate of n-3 fatty acid precursors in obese/diabetic *KK-A<sup>y</sup>*  
29 rodent model was evaluated in our work. Soybean oil, being rich in ALA (18:3 n-3; a known  
30 precursor of EPA/DHA), was chosen as the n-3 fatty acid precursor rich source. A high-fat  
31 diet (20%) containing 7% soybean oil (SO) was fed to obesity/diabetes-prone male *KK-Ay*  
32 mice (control). In the case of diets fed to test groups, soybean oil was replaced with 5% SO &  
33 2% SQ. Hepatic DHA (6 fold) levels increased in SQ fed group over control (p<0.05). Gene  
34 and protein expressions of  $\Delta^5$  and  $\Delta^6$  desaturases, key enzymes involved in the fatty acid  
35 metabolism, further supported the results. Also, SQ exhibited a hypotriglyceridemic and  
36 hypoglycemic effect. The results clearly indicated the effect of SQ in modulating the n-3 fatty  
37 acid metabolism, including EPA/DHA synthesis in the presence of n-3 fatty acid precursor.  
38 This is the first report of enhancement of *in-vivo* DHA/EPA by SQ and in turn, modulating the  
39 physiological fatty acid profile.

40

41 **Practical Applications (150 words)**

42 Squalene (SQ) is an important marine biofunctional material that is found in some terrestrial  
43 sources as well. Squalene, being a cholesterol precursor, forms an interesting subject of  
44 research for its effect *in-vivo*. SQ significantly enhanced proportions of EPA and/or DHA  
45 when their n-3 fatty acid precursors were available in the diet. The study further establishes  
46 the usefulness of SQ in functional food formulations. The work provides an important basis  
47 for further evaluation of the role of SQ in normal and disease conditions.

48

49 **Keywords:** Docosahexanoic acid; eicosapentanoic acid; *KK-A<sup>y</sup>* mice, n-3 PUFA, squalene

50

## 51 **1 Introduction**

52 Long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) have drawn considerable attention  
53 due to their diverse and dynamic biofunctional properties. Among n-3 PUFA,  
54 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to be essential for  
55 brain and retinal development during infancy, a factor for longer/completion of gestation in  
56 pregnant women in addition to maintenance of homeostasis of various functions and organs  
57 in adults [1]. Also, EPA and DHA are essential components of the membrane, necessary for  
58 retinal and neuronal function, and can reduce the risk of inflammatory, cardiovascular  
59 diseases, cancer, hypertension and diabetes [1-3]. The primary sources of pre-formed long  
60 chain n-3 fatty acids such as EPA and DHA include foods of marine origin such as  
61 unicellular phytoplankton, seaweeds and fish [1]. The precursor of n-3 fatty acids,  $\alpha$ -linolenic  
62 acid (ALA) is abundant in plant sources such as linseed/flaxseed oil [2]. Though humans and  
63 other animals have a biosynthetic pathway for the synthesis of EPA, docosapentaenoic acid  
64 (DPA) and DHA, the rate of conversion to EPA has been found to be very limited (up to 8%)  
65 [4]. Further, the bioconversion is still more meagre in the case of DHA (0.01%) while  
66 pregnant women may exhibit greater (9%) bioconversion [5-6]. Hence, while biosynthesis of  
67 EPA from precursors may be feasible, DHA production is grossly inadequate [7]. Also, a sex  
68 dependent difference with greater conversion to DHA in women as compared to men has also  
69 been reported [8]. Overall, preformed DHA has been the only effective means to increase  
70 DHA to desirable levels in blood and target organs [7-11].

71 Squalene is a triterpenoid that is a precursor in the cholesterol/sterol biosynthetic pathway.  
72 Skin is the major organ for squalene storage (13%), and adipose tissue is also known to store  
73 considerable amounts of squalene. Since squalene is a sterol/cholesterol precursor compound,  
74 it is an important intermediate component in the formation of eukaryotic sterols and bacterial  
75 hopanoids and is present in almost all cells [12]. Some reports attribute anticancerous

76 properties to squalene as it enhances cellular antioxidant status due to its antioxidant activity  
77 [13-15]. Our recent study in macrophages demonstrated increased protection against lipid  
78 peroxidation by the xanthophyll carotenoid, astaxanthin, in the presence of squalene [16].  
79 Besides, many researchers have attributed the anti-cancerous properties of squalene to the  
80 down-regulation of phenylation action of the RAS oncogene [17,18]. Though varied in results,  
81 several reports are available on the effect of squalene on lipid components as a result of  
82 feeding including hypocholesterolemic effect [19-22] and hypercholesterolemic effect [23-  
83 25]. These have led the scientific community to suggest exercising caution with regards to  
84 squalene. On the other hand, recently, deCastro et al [26] and Smith et al [27] have reported  
85 that squalene had no effect on plasma lipid parameters; while, enhancement of glucose-  
86 induced insulin secretion and insulin content indicating its possible hypoglycemic effect is  
87 also reported[28].

88 From the above published reports, squalene's role in altering the lipid profile, positively or  
89 negatively, is probable. However, none of the studies have attempted to decipher the role of  
90 squalene in fatty acid metabolism, biosynthesis in particular, especially in the presence of n-3  
91 fatty acids precursor. We hypothesized that squalene could affect fatty acid metabolism and  
92 EPA/DHA in particular. A model with altered metabolic status (*KK-A<sup>y</sup>* mice) was fed a high  
93 fat diet and used for the experiments. *KK-A<sup>y</sup>* mice were chosen as it would reflect better any  
94 effect of squalene feeding on lipid related parameters as this mice model responds to altered  
95 metabolic status with rapid development of obesity and diabetes. Based on previous studies  
96 evaluating other effects of squalene that reported dosages varying from 1 to 4% [22, 26, 27],  
97 a 2% squalene dosage was employed in our study. Against this background, our study was  
98 designed to analyse the effect of orally administered squalene (2% of diet) on the fatty acid  
99 profile in the presence of an n-3 fatty acids precursor (ALA) responsible for EPA/DHA

100 formation. In addition to this, other lipid parameters and effect of orally fed squalene on  
101 glucose levels was also monitored.

## 102 **2Materials and Methods**

### 103 **2.1 Materials**

104 Squalene (SQ) was from Wako Pure Chemicals, Ltd., Osaka, Japan. Dietary lipids, soybean  
105 oil and lard, were obtained from Wako, Summit Oil Mill Co. Ltd., Chiba, Japan, and Junsei  
106 Chemical Co. Inc., Tokyo, Japan, respectively. All the other chemicals and solvents used in  
107 the study were of analytical grade.

### 108 **2.2 Animals and diets**

109 Obese/diabetic model KK-A<sup>y</sup> mice (male, four weeks age) employed in the study were  
110 obtained from Japan CREA Co., Osaka, Japan. The diet preparation was as per the  
111 recommendations of American Institute of Nutrition (AIN-93G) [29]. All the procedures and  
112 protocols for the use and care of animals were approved by the Ethical Committee for  
113 Experimental Animal Care of the Hokkaido University, Japan (Approval no. 14-0072).

114 After acclimation for 1 week, the mice were randomly divided into control and experimental  
115 groups (n=7). Mice fed with 20% fat diet [13% lard and 7% soybean oil (SO)] formed the  
116 control (Con) (Table 1). The test diet (SO+SQ) contained 13% lard,5% SO and 2% SQ. The  
117 animals were maintained at  $23 \pm 2^{\circ}\text{C}$  and  $60 \pm 5\%$  humidity under a 12 hour light/dark cycle.  
118 All the animals had free access to food and water.

119 The food and water intakes along with body weights were measured and recorded on a daily  
120 basis. At the end of the experimental period of 4 weeks, both control and test animals were  
121 sacrificed to collect blood and other organs. The animals were fasted overnight before culling.  
122 The blood collected in vacuettes was used for separation of serum. Organs were excised,  
123 weighed, divided and stored in liquid nitrogen, RNA later solution (Life Technologies, USA)  
124 and stored in the deep freezer ( $-40^{\circ}\text{C}$ ) until further analyses.

125 **2.3 Fatty acid analysis of dietary lipids**

126 Total lipid was extracted with chloroform/methanol (2:1,v/v) from each diet. Fatty acyl  
127 groups in the lipid of diets and dietary oils were transmethylated to obtain methyl esters. The  
128 fatty acid methyl esters (FAME) were prepared according to the method of Prevot and  
129 Mordret[30] and used for determining the fatty acid composition by GC. Briefly, 1 mL of n-  
130 hexane and 0.2 mL of 2 N NaOH in methanol were added to an aliquot of total lipid (ca.20  
131 mg), vortexed and incubated at 50 °C for 30 min. Post incubation, 0.2 mL of 2 N HCl in  
132 methanol was added to the solution and vortexed. The mixture was separated by  
133 centrifugation at 3000 rpm for 5 min. The upper hexane layer containing FAME was  
134 recovered and subjected to GC analysis on a Shimadzu GC-14B (Shimadzu Seisakusho,  
135 Kyoto, Japan) equipped with a flame-ionization detector(FID) and a capillary  
136 column(Omegawax-320; 30 m x0.32 mm i.d.; Supelco, Bellefonte, PA, USA). The detector,  
137 injector, and column temperatures were 260, 250, and 200 °C, respectively. The carrier gas  
138 was helium at a flow rate of 50 kPa. The peaks were identified by comparing with retention  
139 of FAME of different fatty acid standards (Nu-Check-Prep, Inc, Elysian MN) and were  
140 quantified by an online integrator (Shimadzu Chromatopack C-R8A). The fatty acids content  
141 was expressed as weight % of total fatty acids by comparing the retention times with standard  
142 fatty acid mix and heptadecanoic acid (C17:0) was used as an internal standard (Supelco,  
143 Bellefonte, PA, USA)

144 **2.4 Profiling lipids of tissues and blood glucose**

145 Total lipids from serum and tissues were extracted by the method of Folch et al [31]. An  
146 aliquot of the total lipids was subjected to transmethylation with 2M methanolic sodium  
147 hydroxide followed by 2M methanolic hydrochloric acid as described above [30]. The FAME  
148 thus obtained were dissolved in hexane and analysed on a GC (Shimadzu GC-14B,  
149 Shimadzu) under the same conditions as described in section 2.3. The relative amount of each

150 fatty acid was expressed as weight % of total fatty acids. Fatty acid composition of the liver  
151 of *KK-A<sup>y</sup>* mice was determined by the method as mentioned earlier.

152 Triglycerides (TG) and cholesterol content in tissue and serum were measured by LabAssay  
153 Kits (Wako Pure Chemical Industries, Osaka, Japan) by spectrophotometric method using a  
154 plate reader. Blood glucose levels (overnight fasting) were measured in the live animals by  
155 using tail blood on a glucose monitor, the Glutest Neo Sensor (Sanwa Kagaku Kenkyusho Co.  
156 Ltd., Aichi, Japan). This sensor is an amperometric sensor with flavin adenine dinucleotide  
157 (FAD)-dependent glucose dehydrogenase and  $\text{Fe}(\text{CN})_6^{3-}$ .

## 158 **2.4 Quantitative real-time PCR**

159 Total RNA was extracted from the liver of mice using the RNeasy Lipid Tissue Mini Kit  
160 (Qiagen, Tokyo, Japan) according to the manufacturer's protocol. The cDNA was then  
161 synthesised from total RNA using the High-Capacity cDNA Reverse Transcription Kit  
162 (Applied Biosystems Japan Ltd., Tokyo, Japan). Quantitative real-time PCR analysis of  
163 individual cDNA was performed with ABI Prism 7500 (Applied Biosystems Japan Ltd.,  
164 Tokyo, Japan) using TaqMan Gene Expression Assays (Applied Biosystems Japan Ltd.,  
165 Tokyo, Japan; Acox1: Mm01246834\_m1, Acox2: Mm00446408\_m1, Elovl2:  
166 Mm00517086\_m1 Elovl5: Mm00506717\_m1, Elovl6: Mm00851223\_s1, Fads1:  
167 Mm00507605\_m1, Fads2: Mm00517221\_m1, Fasn: Mm00662319\_m1, GAPDH:  
168 Mm99999915\_g1. PCR cycling conditions were 50 °C for 2 min, 95 °C for 10 min, followed  
169 by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

## 170 **2.5 Western Blotting**

171 Livers of mice (30 mg) were homogenised in 5-10 volumes of a solution containing 10 mM  
172 Tris-HCl, 1 mM EDTA (pH 7.4) and 1% protease inhibitor for 30 s with a Polytron. The  
173 homogenate was subjected to centrifugation (15000 rpm, 20 min, 4°C) and the fat-free  
174 supernatant was collected and used for Western Blot analysis of  $\Delta^5$  desaturase (FADS1) and

175  $\Delta^6$  desaturase (FADS2). The total protein concentration of liver was estimated with the  
176 DCprotein assay kit (Bio-Rad). Separation of proteins in the solution (30 mg protein/lane)  
177 was achieved by 10% SDS-polyacrylamide gel electrophoresis. The separated proteins were  
178 blotted onto polyvinylidene difluoride membranes and incubated with the antibody against  
179 FADS 1 or FADS 2(1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 hour.  
180 Next step involved incubation with a secondary antibody mouse IgG-conjugated horseradish  
181 peroxidase (1:2000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 hr at room  
182 temperature. Visualisation of bands was achieved by treating the membranes with the  
183 reagents in the chemiluminescence detection kit (ECL system, Amersham PharmaciaBiotech,  
184 Piscataway, NJ, USA) as per the protocol provided by the manufacturer.  $\beta$ -Actin served as a  
185 loading control with  $\tau\eta\epsilon$   $\beta$ -actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

## 186 **2.6 Statistical analysis**

187 Mean separation of the data in the groups was achieved using Student's t-test with a  
188 confidence interval of 95% (significant when  $p < 0.05$ ). **3 Results**

### 189 **3.1 Fatty acid profile of diets and dietary oils**

190 Fatty acid composition of dietary oils and the lipids from the diet are shown in Table 2. The  
191 20% fat diet with and without squalene fed to *KK-A<sup>y</sup>* mice was found to contain common  
192 fatty acids such as palmitic, stearic, oleic, linoleic and ALA, which were contributed by the  
193 lard and SO present in the diet.

### 194 **3.2 Weight gain in the mice and weight of organs**

195 The gain in body weight of the mice, monitored on a daily basis, showed no significant  
196 difference ( $p > 0.05$ ) between the groups as a result of feeding the experimental diet (data not  
197 shown). Similarly, the weight of major organs (data not shown) also was not significantly  
198 different ( $p > 0.05$ ), except for a slight increase in the liver weight (18%) of the  
199 SO+SQ ( $p > 0.05$ ) -fed group ( $p > 0.05$ ).

### 200 **3.3 Fatty acid composition of liver**

201 Fatty acid profiles of the liver lipids of KK-A<sup>y</sup> mice fed SO, or SO+SQ diets are presented in  
202 Table 3. The results reveal increased proportions ( $p<0.05$ ) of the long-chain n-3 fatty acid,  
203 DHA, in the squalene fed group when compared to control (SO), indicating the possible  
204 modulation of fatty acid metabolism. The results demonstrated a significant ( $p<0.05$ ) increase  
205 in the long chain n-3 fatty acids, EPA and/or DHA, in the experimental group that was fed  
206 squalene.

### 207 **3.4 Triglycerides(TG), cholesterol, and glucose levels**

208 In addition to the fatty acid profile, it was of interest to study the effect of squalene on other  
209 lipid parameters including the cholesterol and TG, together with blood glucose. The results  
210 obtained with KK-A<sup>y</sup> mice revealed significant decrease ( $p<0.05$ ) in TG (Figure 1a) in the  
211 liver and epididymal WAT of squalene fed experimental groups as compared to the control.  
212 Whereas, TG levels in serum and cholesterol levels in serum, liver and epididymal WAT  
213 were not significantly ( $p>0.05$ ) different from control (data not shown).. On the other hand, a  
214 marked decrease ( $p<0.05$ ) in the blood glucose levels of mice in the group fed with the diet  
215 containing SQ with that of control was observed, as can be seen in Figure 1 (c).

### 216 **3.5 mRNA expression of elongase, desaturase and peroxisomal enzymes involved in** 217 **EPA/DHA synthesis**

218 Elongases and desaturases are essential for the biosynthesis of long chain PUFA. Many of  
219 these are common to the n-3 and n-6 fatty acid pathways. The effect of squalene feeding on  
220 the mRNA expression of important elongases and desaturases as well as other enzymes such  
221 as fatty acid synthase and peroxisomal acyl-CoA oxidases associated with DHA synthesis  
222 from DPA is shown in Figure 2 (a). Increase ( $p<0.05$ ) was observed in the  $\Delta^5$ -desaturase  
223 enzymes in liver of mice fed squalene (Figure 2a).

### 224 **3.6 Protein expression of $\Delta^5$ and $\Delta^6$ desaturase enzymes involved in EPA/DHA synthesis**

225 The protein expressions for the  $\Delta^5$  and  $\Delta^6$  desaturases was determined using Western blot  
226 from the protein extract from liver of control and experimental mice. The results indicate a  
227 significant increase in the protein expression of  $\Delta^5$  desaturase. Besides, the protein expression  
228 of the  $\Delta^6$  desaturase (Figure 2b) showed an increase ( $p<0.05$ ).

#### 229 **4 Discussion**

230 The results of our study indicated that squalene influenced the lipid metabolism as seen by  
231 the TG levels and fatty acid profiles in test diet fed to KK- $A^y$  mice. No significant differences  
232 ( $p>0.05$ ) were observed in organ as a result of squalene feeding. Though several previous  
233 studies have shown the conversion of n-3 fatty acid precursor, ALA to EPA and DPA,  
234 increase in the DHA levels have rarely been reported [4-6]. In a previous study by our  
235 research team, fucoxanthin, a marine carotenoid, was observed to increase the DHA levels in  
236 the liver of KK- $A^y$  mice [32]. In the present study also, an increase in the proportion of DHA  
237 in the fatty acid profile of the liver with a 4.0 fold increase ( $p<0.05$ ) and elevation ( $p<0.05$ ) in  
238 liver DHA levels (6 fold) was observed in the squalene fed KK- $A^y$  mice. Several studies have  
239 reported the conversion of ALA to EPA and DPA in different experimental models. However,  
240 the reports have demonstrated very limited conversion to DHA [4-6]. Squalene is a naturally  
241 occurring lipid substance that is a precursor to cholesterol and is present in many foods with  
242 shark liver oil being the primary source. It is also present in other food sources such as olive  
243 oil, amaranth seed oil, etc. The results of this study offer another evidence for the utility of  
244 squalene as a dietary component to enhance the long chain n-3 fatty acids from their  
245 precursors present in food, *in vivo*.

246 A minor but interesting observation was the decrease in arachidonic acid proportions on  
247 feeding squalene. A possible reason for this occurrence could be the increased levels of EPA  
248 and DHA that have been previously associated with decreased arachidonic acid levels [9].  
249 The same  $\Delta^5$ -desaturase enzyme - that is responsible for the formation of arachidonic acid

250 (AA, C<sub>20:4,n-6</sub>) from C<sub>20:3,n-6</sub> (intermediate formed during the synthesis of C<sub>20:4</sub> from C<sub>18:2,n-6</sub>)  
251 - is involved in the formation of EPA (C<sub>20:5,n-3</sub>) from C<sub>20:4,n-3</sub> (intermediate formed during  
252 synthesis of EPA from ALA) [9]. The role of uncontrolled synthesis of arachidonic acid  
253 metabolites in the development of CVD and cancer is well documented[18] and, squalene by  
254 reducing the AA content effectively prevents those ill effects.

255 An attempt was made to study the effect of squalene on mRNA gene expression levels of  
256 desaturases and elongases and related molecules involved in the synthesis of the long chain n-  
257 3 fatty acids. Moreover, the protein expression of the desaturases ( $\Delta^5$  and  $\Delta^6$ ) were also  
258 ascertained. The mRNA gene expression of EPA synthesis related  $\Delta^5$ -desaturase enzyme was  
259 increased (p<0.05) in experimental group fed squalene. However, slight increase in  
260 expression of elongases - Elovl2 and Elovl5 - in experimental groups was not significant  
261 (p>0.05). While this suggests the involvement of squalene in the fatty acid metabolism, no  
262 definite link could be established between the increase in the fatty acids and the mRNA  
263 expression of other related genes such as the elongases, fatty acid synthase or  $\Delta^6$ -desaturase.  
264 Similar explanation is seen in the instance of Barceló-Coblijn and Murphy [33] who have  
265 stated that measures of gene expression of fatty acid metabolism may not directly relate to  
266 enzyme activity and accumulated products. The lack of consistency in the mRNA expression  
267 of the involved genes observed in our study can be explained by their theory. However, the  
268 protein expression studies revealed an increased expression of  $\Delta^5$  and  $\Delta^6$  desaturases, lending  
269 support to the gene expression studies and confirming that squalene is capable of modulating  
270 the fatty acid metabolism.

271 In addition to its regulation of fatty acid metabolism resulting in increased long chain n-3  
272 fatty acids, squalene also was found to regulate the TG in the tissues of KK-A<sup>y</sup> mice. While  
273 previous studies have reported the hypocholesterolemic effect of squalene [19-22], squalene  
274 was not found to decrease cholesterol levels in the present study. Contradictory results

275 resulting in hypercholesterolemia have also been reported as a result of feeding trials with  
276 squalene [23-25]. Squalene has also been reported to protect the long chain fatty acids from  
277 oxidation, in particular, linolenic, arachidonic and docosahexaenoic acids [33], affording an  
278 added benefit of squalene.

279 Another significant observation was the ability of squalene to exhibit hypoglycemic effect.  
280 Squalene was found to decrease blood glucose levels ( $p < 0.05$ ) in high fat/sugar fed *KK-A<sup>y</sup>*  
281 mice, as compared to control in the present study. The ability of squalene to enhance glucose  
282 stimulated insulin secretion as well as insulin content could be the reasons [28].

## 283 **5 Conclusion**

284 The results collated from this study clearly indicate that squalene significantly enhances liver  
285 DHA levels when n-3 fatty acid precursors are available in the diet. For the first time, the  
286 results show the fatty acid modulation by squalene. Further, squalene also exhibits  
287 hypotriglyceridemic effect apart from decreasing the blood glucose levels. Further studies on  
288 oral feeding of squalene in other *in-vivo* models including normal phenotypes (e.g.,  
289 C57BL/6J) are warranted so that the mechanism of squalene action can be understood better  
290 and comparisons may be drawn to its role in normal and disease conditions.

## 291 **Conflict of interest**

292 Authors declare that there are no conflicts of interest.

## 293 **Acknowledgement**

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299 Invitation Fellowship, respectively.

300

301

302

303 Table 1. Composition (g/kg) of the control and experimental (SO+SQ) diets.

304

Ingredient	Control	SO+SQ
$\beta$ -Corn starch	224.6	224.6
$\alpha$ -Corn starch	84.9	84.9
Milk casein	260	260
Sucrose	130	130
Cellulose	50	50
Soybean oil	70	50
Lard	130	130
Squalene	-	20
L-cystine	3	3
AIN93G mineral mix	35	35
AIN93G vitamin mix	10	10
Choline bitartrate	2.5	2.5
Tert-butyl hydroquinone	0.014	0.014

305 SO=Soybean oil, SQ=Squalene

306

307

308

309 Table 2. Fatty acid composition (g/100g total fatty acids) of control (con) and experimental  
 310 (SO+SQ) diets and fatty acid profile of oils used in the diets.

311

FA	Control	SO+SQ	Lard	SO
16:0	4.1	3.9	25.39	10.98
16:1	ND	ND	2.64	0.11
17:0	ND	ND	ND	0.1
18:0	2.1	2.0	13.67	4.02
18:1	7.7	7.2	46.03	24.66
18:2	4.8	3.8	9.19	51.66
18:3	0.4	0.3	0.48	4.92
20:0	ND	ND	ND	0.33
20:1	ND	ND	0.97	0.34
20:5	ND	ND	ND	ND
22:0	ND	ND	ND	0.34
22:6	ND	ND	ND	ND
24:0	ND	ND	ND	0.12

312

SO=Soybean oil, SQ=Squalene, ND=Not Detected

313

314

315 Table 3. Fatty acid composition (%) of liver in high fat fed control and experimental (squalene  
 316 supplemented) KK-A<sup>y</sup> mice.

FA	Control	SO+SQ
C <sub>16:0</sub>	19.63±0.8	19.02±0.8
C <sub>18:0</sub>	5.49±1.3	3.98±0.88 <sup>x</sup>
C <sub>18:1</sub>	36.76±3.4	36.16±1.5
C <sub>18:2 n-6</sub>	22.35±1.2	17.62±1.9 <sup>x</sup>
C <sub>18:3 n-3</sub>	0.82±0.07	0.66±0.07 <sup>x</sup>
C <sub>20:4 n-6</sub>	4.15±1.3	2.67±0.72 <sup>x</sup>
C <sub>20:5 n-3</sub>	0.05±0.05	0.02±0.02 <sup>x</sup>
C <sub>22:5 n-3</sub>	0.27±0.04	0.20±0.04 <sup>x</sup>
C <sub>22:6 n-3</sub>	2.56±0.5	10.30±1.4 <sup>x</sup>

317 SO=Soybean oil, SQ=Squalene

318 <sup>x</sup>Statistically significant (p<0.05) compared to control.

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323 **Figure titles**

324 Figure 1. Effect of squalene on:

325 (a) Triglycerides (TG) in per g liver (L) and epididymal white adipose tissue (WAT),

326 (b) Fasting glucose levels per dl blood.

327 SO=soybean oil, SQ=squalene

328 x = Statistically significant ( $p < 0.05$ ) compared to SO (Con)

329 Figure 2. (a) mRNA expression of genes associated with biosynthesis of long chain n-3 PUFA

330 in control (con) and experimental (SO+SQ) KK-Ay mice.

331 (b) Protein expression of  $\Delta^5$  and  $\Delta^6$  desaturases: L1 =  $\Delta^5$  in con, L2 =  $\Delta^5$  in SO+SQ,

332 L3 =  $\Delta^6$  in con, L4 =  $\Delta^6$  in SO+SQ. Representative bands for each group are

333 represented in L1 to L4.

334 SO=soybean oil, SQ=squalene, D5-D= $\Delta^5$ -Desaturase, D6-D= $\Delta^6$ -Desaturase.

335 x = Statistically significant ( $p < 0.05$ ) compared to SO (Con)

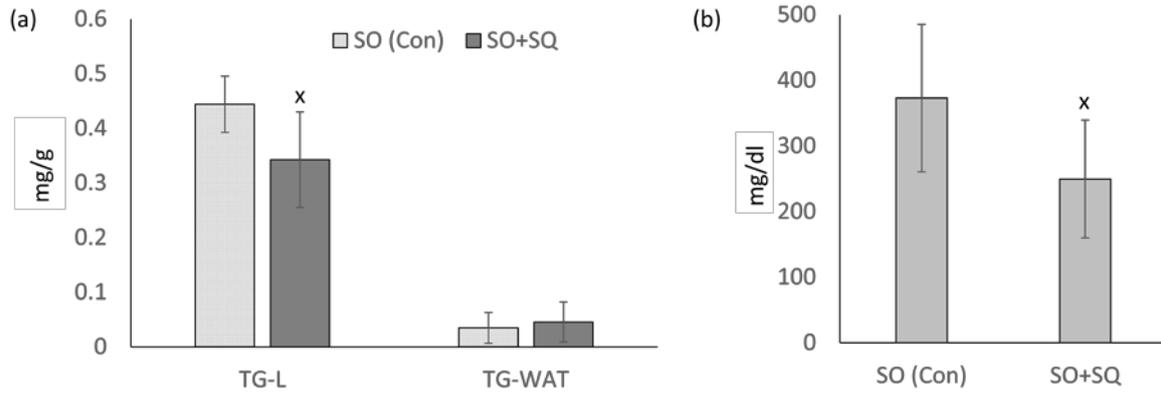
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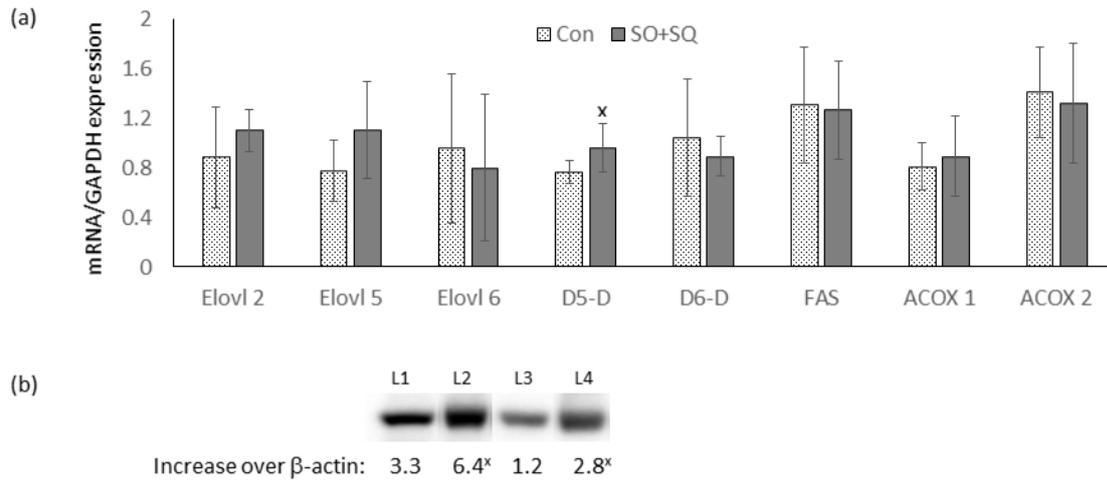
344 (a) Triglycerides (TG) in per g liver (L) and epididymal white adipose tissue (WAT),

345 (b) Fasting glucose levels per dl blood.

346 SO=soybean oil, SQ=squalene

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354 Figure 2.(a) mRNA expression of genes associated with biosynthesis of long chain n-3 PUFA  
 355 in control (con) and experimental (SO+SQ) KK-Ay mice.

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359 SO=soybean oil, SQ=squalene, D5-D= $\Delta^5$ -Desaturase, D6-D= $\Delta^6$ -Desaturase.

360 x = Statistically significant ( $p < 0.05$ ) compared to SO (Con)

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