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Methylsulfonylmethane and sesame seed oil improve hyperlipidemia and polyunsaturated fatty

acid metabolism in the *db/db* mouse

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Abstract

Background: An estimated 463 million people worldwide suffer from diabetes, and that number is projected to rise significantly to 700 million by 2045. One of the hallmarks of type 2 diabetes and metabolic syndrome is alterations in the lipid profile and polyunsaturated fatty acid metabolism.

Objective: The objective of this study was to identify lipid alterations in leptin receptor deficient *db/db* mice, a type 2 diabetes model, to establish a baseline biological signature for the evaluation of natural products with purported lipid-altering activity.

Methods: Six-week old male *db/db* mice (n = 10/group) were randomized to the following groups: 1) diabetic control with no treatment, 2) methylsulfonylmethane (MSM) treatment (3.81 $\pm$ 0.33 g/kg), 3) sesame seed oil (SSO) treatment (23.54 $\pm$ 2.91 mg/kg), and 4) MSM and SSO combination treatment, receiving the same dosages of MSM and SSO as the single treatment groups. Eight-week old male C57BL6/J mice (n=10) were used as a non-diabetic control group.

Results: Serum triglycerides and total cholesterol were significantly increased in the *db/*db model compared to nondiabetic control, mimicking diabetes in people. High-density lipoprotein (HDL) was significantly increased in all *db/db* treatment groups, with the most significant effect in the MSM and SSO combination group, with a corresponding decrease in non-HDL cholesterol . Serum total polyunsaturated fatty acid (PUFA) levels were significantly reduced in diabetic mice compared with control mice. In contrast, treatment with SSO alone reversed this effect such that fed mice exhibited serum PUFA levels comparable to control mice.

Conclusions: Treatment of *db/db* mice with MSM and SSO improved commonly measured clinical parameters in serum lipid panels. The combination of MSM and SSO treatment's effects

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42   on HDL and non-HDL cholesterol and fatty acid metabolism could lead to improved clinical

43   outcomes in diabetics, such as reduced incidence of atherosclerosis and hepatic steatosis.

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Introduction

The International Diabetes Federation estimates there are at least 463 million people (1 in 11 adults aged 20-79 years) worldwide suffering from diabetes, and that number is projected to rise significantly to 700 million by 2045 [1]. One of the hallmarks of type 2 diabetes mellitus and related metabolic syndrome and obesity is dyslipidemia and impaired lipid metabolism [1, 2]. While most type 2 diabetic patients require pharmaceutical intervention such as insulin therapy and peroxisome proliferator-activated receptor (PPARγ) agonists, changes in the diet may also improve dyslipidemia and the diabetic condition [3]. An important contributor to the rise in the number of diabetic patients is the increasing rates of obesity due to the Modern Western Diet. In the past three decades, total fat and saturated fat intake as a percentage of total calories has continuously increased in Western diets, while the intake of omega-6 fatty acids increased and omega-3 fatty acids decreased. This shift has resulted in a large increase in the omega-6: omega-3 ratio from 1:1 during evolution to 20:1 today or even higher [4]. This high omega-6: omega-3 ratio is directly linked to chronic inflammatory diseases including obesity, metabolic syndrome, and type 2 diabetes mellitus [5].

Current anti-diabetic therapeutic strategies, e.g., insulin, metformin, and PPARγ agonists, are employed once an individual has already developed type 2 diabetes. A more recent approach to controlling the diabetes epidemic is to elucidate the mechanisms of pre-diabetes as a means to understand factors that contribute to conversion of pre-diabetes to diabetes. Pre-diabetes, or impaired glucose tolerance and insulin resistance prior to onset of type 2 diabetes, has been historically diagnosed based on glycemic criteria including hemoglobin A1c of 5.7-6.4%, fasting blood glucose of 100-125 mg/dL, and blood glucose of 140-199 mg/dL two hours post oral glucose

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66   tolerance test [1, 6]. Early detection of the pre-diabetic condition and insulin resistance (IR) can

67   lead to intervention and prevention of the development of type 2 diabetes [1]. The recent

68   development of tests such as the Quantose^{TM} IR test, which measures biomarkers of insulin

69   sensitivity other than blood glucose and A1c, can detect pre-diabetes even earlier in the disease

70   process than these standard glycemic markers [7]. One biomarker included in the Quantose^{TM} IR

71   is linoleoylglycerophosphocholine (LGPC), also known as lysophosphatidylcholine-linoleic acid

72   (LPC-LA, 18:2), which is a representative omega-6 polyunsaturated fatty acid (PUFA) that

73   decreases in people with pre-diabetes [6]. Dietary supplements and foods that can improve this

74   dyslipidemia found in the diabetic and pre-diabetic patient could lead to improved clinical

75   outcomes; thus there is a growing need for research into lipid altering compounds.

76   The dietary ingredient sesamin, a phytonutrient of the lignans class found abundantly in

77   sesame seed oil, is known to decrease formation of the pro-inflammatory omega-6 metabolite

78   arachidonic acid in the liver by inhibiting the delta-5 desaturase activity. This effect results in

79   enhanced liver formation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

80   relative to arachidonic acid (AA) [8]. Sesamin's impact on the PUFA balance is limited by the fact

81   that the liver inefficiently delivers EPA and DHA to the systemic circulation and thus the tissues

82   in the setting of diabetes. This occurs because a key contributor to PUFA release from the liver is

83   the presence of methyl donors such as vitamin B12 and folic acid, which are significantly reduced

84   in diabetes [9]. Methylsulfonylmethane, with the chemical formula $(CH_3)_2SO_2$, also known as

85   MSM, DMSO2, methyl sulfone, or dimethyl sulfone, is a naturally-derived sulfur compound found

86   in many plants and food sources and a putative methyl donor [7, 8]. MSM provides remarkable

87   defense against oxidative stress and inflammatory injuries [9, 10]. The objective of this research

88   was to investigate whether supplementation with sesame seed oil (SSO), MSM, or the combination

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89   of SSO and MSM will improve the dyslipidemia seen in the diabetic condition, and thus reverse

90   the pro-inflammatory environment found in an animal model of type 2 diabetes mellitus, the leptin

91   receptor deficient *db/db* mouse.

Materials and methods

Animals and housing

94   Six-week old male BKS.Cg-*Dock7^m +/+ Lepr^{db}*/J (hereafter referred to as *db/db*) mice

95   were purchased from The Jackson Laboratory as a model for type 2 diabetes mellitus. This strain

96   is leptin receptor deficient; therefore, leptin is unable to bind and produce satiety, resulting in

97   development of obesity and type 2 diabetes mellitus. Eight-week old male C57BL6/J mice

98   (n=10) were used as a non-diabetic control group. Nondiabetic mice (n=10) received no

99   treatment, and diabetic mice were divided into four experimental groups (n=10 per group): 1)

100 diabetic control with no treatment, 2) MSM treatment, 3) SSOtreatment, and 4) MSM and

101 SSOcombination treatment. Upon arrival and prior to treatments, mice were placed in

102 individually ventilated caging at a housing density of 5 mice per cage on corncob bedding

103 (Shepherd Specialty Papers, Watertown, TN) with a 12:12 light: dark cycle. Chlorinated water

104 was delivered in polysulfone bottles (Alternative Design, Siloam Springs, AR) ad libitum. Mice

105 were fed Tekland 7912 rodent diet ad libitum (Envigo, Madison, WI). Animal work was

106 performed in an Association for Assessment and Accreditation of Laboratory Animal Care

107 International (AAALAC) accredited animal facility and approved by the University of Tennessee

108 Health Science Center Institutional Animal Care and Use Committee (Protocol 17-095.0).

109

Diabetes confirmation and treatments

111   One week after arrival, non-fasted blood glucose was measured to confirm hyperglycemia

112   in the *db/db* mice. Blood glucose was measured by handheld glucometer (FreeStyle Lite) on a drop

113   of blood obtained from the tail vein with the mouse in a plastic mouse restraining device. Mice

114   with blood glucose measurements greater than 250 mg/dL were considered diabetic. Records were

115   maintained of daily individual mouse weight, daily water and food intake per cage of five mice,

116   and weekly individual mouse blood glucose levels. The criteria for early euthanasia was 20%

117   weight loss from the start of experiment or hunched posture, however, none of the mice in this

118   study reached these euthanasia criteria. After confirmation of diabetes, *db/db* mice then began

119   treatment with either MSM alone, SSO alone, or a combination of MSM and SSO for four weeks

120   prior to euthanasia via isoflurane overdose. MSM provided by Bergstrom Nutrition was dissolved

121   in Millipore filtered water in a polysulfone bottle (Alternative Design, Siloam Springs, AR) at one

122   percent v/v. Tekland 7912 feed was soaked in sesame seed oil (Sigma Aldrich) for five minutes,

123   blotted dry with an absorbent paper towel, then stored in a sealed plastic container and fed ad

124   libitum. Nondiabetic C57BL6/J mice and diabetic control *db/db* mice continued to receive

125   unaltered 7912 diet and Millipore filtered water in polysulfone bottles for four weeks prior to

126   euthanasia via isoflurane overdose.

127   All drug solutions were sterile filtered using a 0.22-micron filter prior to administration.

128   Blood was collected via cardiocentesis under isoflurane anesthesia at euthanasia, and then

129   centrifuged at 2500 rpm for fifteen minutes for serum separation. Serum was aliquoted for Rodent

130   Lipid Profiles and fatty acid analysis via LC-MS/MS. Samples of 250 uL of serum per mouse were

131   mailed on ice overnight to IDEXX BioAnalytics for Rodent Lipid Profile measurement, including

132   cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels. Non-HDL

133   cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. At euthanasia,

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134   tissues (eyes, brain, and liver) were harvested for fatty acid analysis using LC-MS/MS. Sections

135   of liver were preserved in formalin, cryosectioned, and stained with Oil Red O for lipid content.

136   Histology slides were read and scored numerically from 1 to 5 for degree of hepatocyte steatosis,

137   or fatty change, by a blinded veterinary pathologist. The scores of 1 through 5 were none, mild,

138   moderate, marked, and pathologic, respectively.

LC-MS/MS parameters

140   LC-MS/MS analysis was performed following previous papers with minor modifications

141   [11, 12]. The LC-MS/MS system comprised a Sciex (Framingham, MA) 5500 triple quadrupole

142   mass spectrometer, equipped with a Turboionspray™ ionization interface in negative ion mode.

143   The heated capillary temperature was 600°C, the sheath gas pressure was 50 psi, the auxiliary gas

144   setting was 50 psi, and the heated vaporizer temperature was 350°C. The collision gas was argon

145   at a pressure of 1.5 mTorr. The analytical data were processed using the software program Analyst

146   (Version 1.3). Analyses were performed in the multiple reaction monitoring (MRM) mode, in

147   negative ion mode. The collision energy (CE), collision cell exit potential (CXP), entrance

148   potential (EP), and declustering potential (DP) were optimized for each compound to obtain

149   optimum sensitivity.

150   A reverse-phase column (Analytical UPELCO C18; 2.1mm ID; 15mm length; 3 um

151   particle size; 175A pore size) with a gradient elution of solvent A (5 mM ammonium formate in

152   water, pH 4.0) and solvent B (5 mM ammonium formate in 95% (v/v) acetonitrile, pH 4.0) was

153   used for chromatographic separation. Flowrate was at 0.5 ml/min. Solvent A was prepared by

154   mixing 95 ml of Milli-Q (Millipore) water and 5 ml of 100 mM ammonium formate, then pH was

155   adjusted to 4.0 with formic acid. Similarly, solvent B was prepared by mixing 95 ml acetonitrile

156   and 5 ml of 100 mM ammonium formate, adjusting pH 4.0 with formic acid. The gradient started

157   at 30% of mobile phase B, maintained 30% B for 1 minute and then followed by a linear gradient

158   to 100% B from 1 to 8 minutes, and held at 100% B for 1 minute. Subsequently, the mobile phase

159   was immediately returned to the initial condition 30% B and maintained for 1 minute until the end

160   of the run. Column effluents were introduced into the mass spectrometer between 1 and 9 minutes

161   after injections. The run time for a single sample was 11 minutes with most of the lipids eluting

162   from 3 minutes to 5 minutes.

Lipid extraction

164   Liver, brain, and eye tissues were weighed and placed into 1.5 ml siliconized sample tubes.

165   500 µl volumes of acidic methanol (pH 4.0) with 200 ng/ml deuterium-labeled arachidonic acid

166   (AA-d_6) were added for the internal standard. The obtained mixtures were homogenized for 1

167   minute by Bio-Gen PRO200 Homogenizer (with a 5 mm probe, 3,000 rpm, on ice). Mixtures were

168   continuously homogenized for 10 minutes on ice in an ultrasonic bath. After an initial

169   centrifugation at 1,000 g for 10 minutes at 4°C, the supernatants were further centrifuged at 13,500

170   g for 10 minutes at 4°C. The resulting supernatants were filtered with a 0.2 µm pore size and 4

171   mm inner diameter filters. Approximately 150 µl of filtrations were transferred to a 96-well plate

172   for LC-MS/MS.

173   Serum was incubated at 37°C for 1 hour, allowed to stand at 4°C for 12 hours, and

174   centrifuged at 1,500 g for 10 minutes at 25°C. The serum samples (30 µl each) were placed in 1.5

175   ml siliconized sample tubes, deproteinized by mixing with 150 µl acidic methanol (pH 4.0), and

176   homogenized for 10 minutes on ice in an ultrasonic bath. After an initial centrifugation at 1,000 g

177   for 10 minutes, the supernatants were further centrifuged at 13,500 g for 10 minutes at 4°C, as was

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178   done with the liver, brain, and eye samples. The resulting supernatants were filtered and 150 µl of

179   filtrations were subjected to LC-MS/MS.

Statistics

181   Mouse weights, blood glucose measurements, and Rodent Lipid Panels, were analyzed via

182   two-way ANOVA with Tukey posthoc analysis for statistical significance. Results of the fatty acid

183   analysis via LC-MS/MS were analyzed via Kruskal-Wallis H test for statistical significance. All

184   data were analyzed in SPSS and considered significant if the p value was less than 0.05.

Results

Weight, water and food intake, and blood glucose

187   Mice gained weight appropriately according to their age throughout the study, and no mice

188   reached early euthanasia criteria. There were no significant differences in diabetic mouse weights

189   among the treatment groups at the end of treatment (Fig 1). Diabetic mice weighed significantly

190   more than nondiabetic control mice ($p<0.05$), which was to be expected due to the *db/db* obese

191   condition. The average daily intake of MSM in water was 4.49 ± 0.53 g/kg of body weight for the

192   MSM treatment group and 3.81 ± 0.33 g/kg of body weight for the MSM + SSO treatment group.

193   The average daily intake of the ingredient sesamin in sesame seed oil-soaked rodent feed was

194   23.54 ± 2.91 mg/kg of body weight for the SSO treatment group and 22.70 ± 2.57 mg/kg of body

195   weight for the MSM + SSO treatment group. Blood glucose readings on day 0 and day 28 of

196   treatment did not significantly differ within any treatment group, although the blood glucose of

197   *db/db* mice on day 28 of treatment with MSM (326 ± 21 mg/dL) was mildly decreased compared

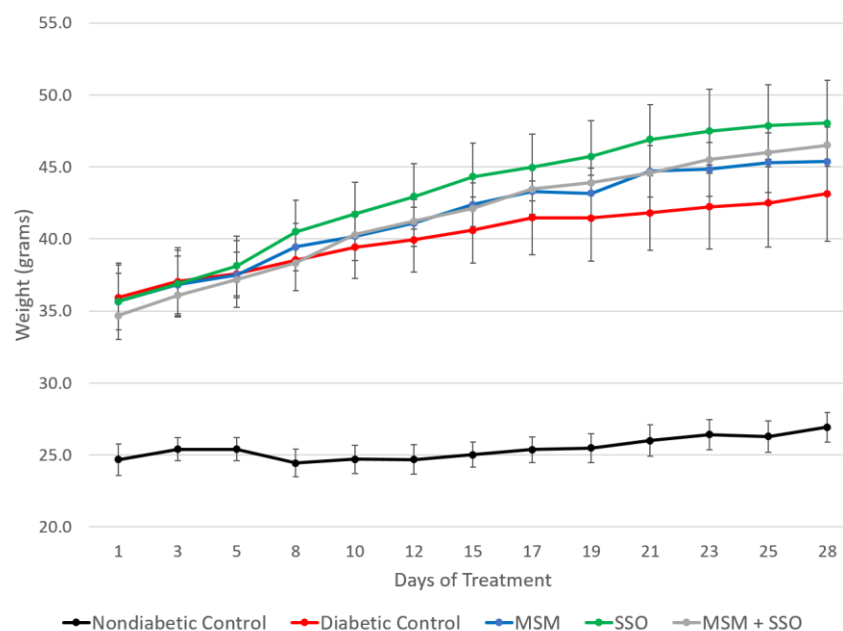198   with day 0 of treatment (375 ± 30 mg/dL).

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Fig 1. Type 2 Diabetic Mice Weights. Body weights of nondiabetic C57BL/6J mice and *db/db* mice over the 4-week treatment period.

Rodent lipid panels

Results of the Rodent Lipid Panels received from IDEXX demonstrated significant differences between the nondiabetic control group and the diabetic groups. Serum cholesterol was significantly increased in the type 2 diabetic control and all type 2 diabetic treatment groups compared with the nondiabetic control group (Fig 2). Serum triglycerides were significantly increased in the diabetic control, SSO treated, and MSM plus SSO treated diabetic groups compared with the nondiabetic control group. In the MSM treated diabetic mice, serum triglycerides were not significantly different from the nondiabetic control group (Fig 3).
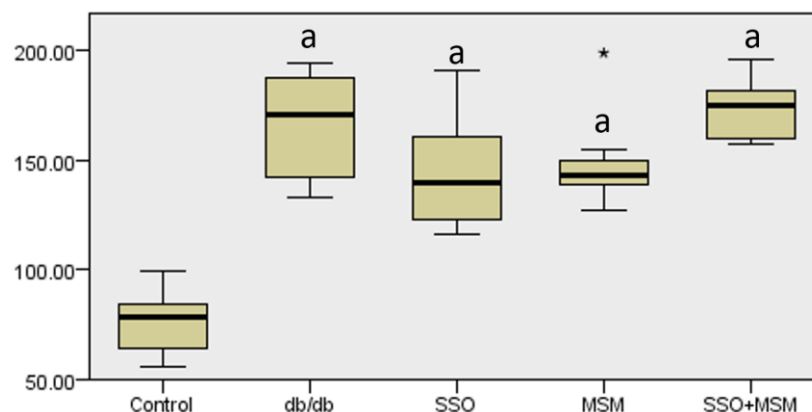
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Fig 2. Total Cholesterol. Total cholesterol levels of C57BL/6J mice (non-diabetic control) and *db/db* mice after the 4-week treatment period, where a denotes p<0.05 compared with nondiabetic control and * denotes one outlier measurement.
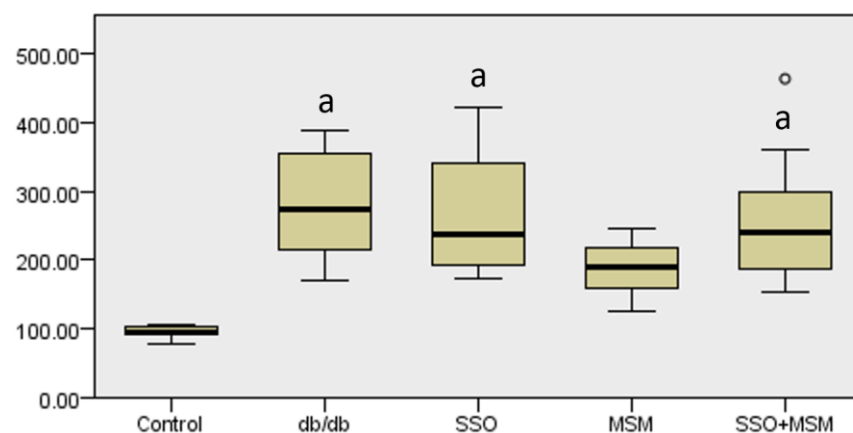
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Fig 3. Triglycerides. Triglyceride levels of C57BL/6J mice (non-diabetic control) and *db/db* mice after the 4-week treatment period, where a denotes p<0.05 compared with nondiabetic control and ° denotes one outlier measurement.

219   Serum HDL was significantly increased in all diabetic groups compared with the

220   nondiabetic control group. Diabetic mice treated with MSM, SSO, or a combination of the two

221   had significantly increased HDL levels compared with diabetic control mice. Additionally, the

222   HDL levels of diabetic mice receiving treatment with MSM/SSO combination were significantly

223   increased compared with treatment with either MSM alone or SSO alone (Fig 4). Serum non-HDL

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224   cholesterol levels were significantly increased in all diabetic groups compared with the nondiabetic

225   control. There was a decreasing trend in non-HDL cholesterol levels in all diabetic mice receiving

226   treatment with either MSM, SSO, or combination of the two, although the differences were not

227   statistically significant (Fig 5).

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Fig 4. HDL Cholesterol. HDL cholesterol levels of C57BL/6J mice (non-diabetic control) and *db/db* mice after 4-week treatment period, where a denotes $p<0.05$ compared with nondiabetic control and b denotes $p<0.05$ compared with diabetic control.
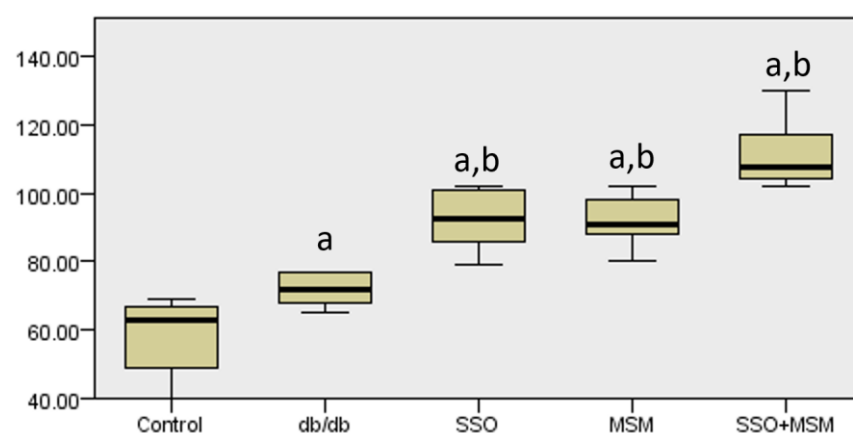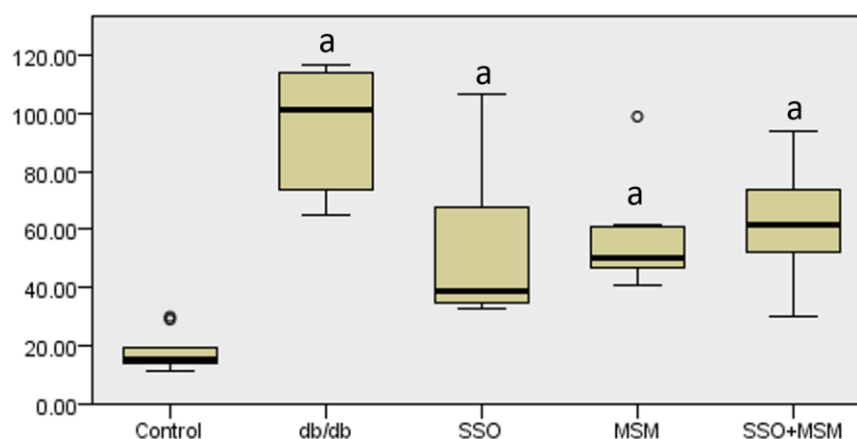

Fig 5. Non-HDL Cholesterol. Non-HDL cholesterol levels of C57BL/6J mice (non-diabetic control) and *db/db* mice after 4-week treatment period, where a denotes $p<0.05$ compared with nondiabetic control and ° denotes outlier measurements.

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Liver histology

In the diabetic mice, liver histology revealed variable microvesicular steatosis with intrahepatic variability and increased red-staining lipid found in the centrilobular regions around the central veins of the liver. There was intragroup variability and no significant differences among treatment groups (Fig 6A-D). All liver histology slides were scored either a 1 for appearing normal or a 2 for mild hepatic steatosis, but there was no trend among the groups, and therefore, no statistics were performed on the liver histology scores.
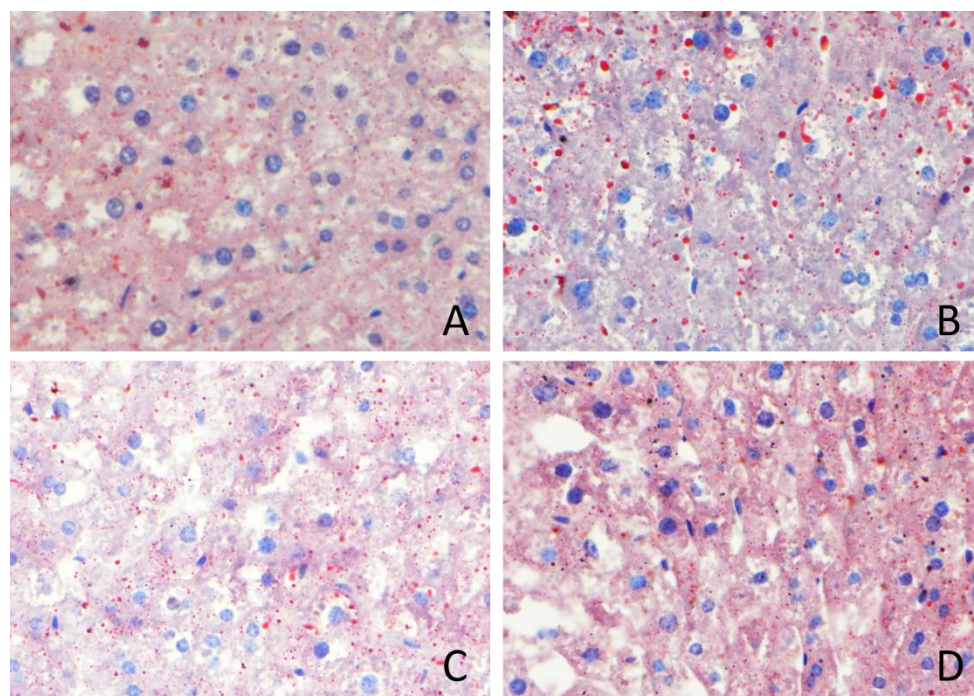

Fig 6. Liver Histology. Representative liver histology stained with Oil Red O from diabetic control group (A), diabetic mice treated with MSM (B), diabetic mice treated with SSO (C), and diabetic mice treated with both MSM and SSO (D).

Free Fatty Acids

Serum

249   In the serum, linoleic acid (LA) levels were significantly increased in *db/db* mice treated

250   with SSO or MSM compared with nondiabetic and diabetic control mice (Table 1). Dihomo-

251   gamma-linolenic acid (DGLA) and arachidonic acid (AA) levels were significantly decreased in

252   diabetic control mice compared with nondiabetic control mice, and this effect was reversed in

253   mice treated with either SSO or MSM alone, where these levels were significantly increased

254   compared with *db/db* control mice and not significantly different from nondiabetic control mice.

255   In diabetic control and diabetic mice treated with MSM/SSO combination therapy, alpha

256   linolenic acid (ALA) levels were significantly increased compared with nondiabetic controls.

257   In diabetic mice treated with SSO alone, ALA levels were returned to levels that were not

258   significantly different from nondiabetic control and were significantly decreased compared with

259   diabetic control mice. EPA levels were significantly increased in diabetic mice treated with

260   MSM alone compared with both nondiabetic control and diabetic control mice. DHA levels were

261   significantly decreased in diabetic control mice and diabetic mice treated with MSM/SSO

262   combination therapy compared with nondiabetic control, but this effect was reversed in diabetic

263   mice treated with SSO alone or MSM alone, whose DHA levels were significantly increased

264   compared with diabetic control mice and not significantly different from nondiabetic control

265   mice. Levels of neuroprotectin D1 (NPD1), a mediator derived from DHA that protects eye and

266   brain from oxidative stress, was not significantly different among groups. Prostaglandin E2

267   (PGE2), a proinflammatory mediator derived from AA, was significantly increased in diabetic

268   control mice and mice treated with SSO compared with nondiabetic control mice, but PGE2 was

269   not significantly different in MSM treated or MSM/SSO treated mice compared with nondiabetic

270   control mice.

271   **Table 1. Serum Free Fatty Acids**

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Free fatty acids	Non-diabetic Control (Mean ± SD)	Diabetic Control (Mean ± SD)	SSO Treatment (Mean ± SD)	MSM Treatment (Mean ± SD)	MSM + SSO Treatment (Mean ± SD)
AA	102 ± 8.37	52 ± 17 [a]	117 ± 7.46 [b]	113 ± 14.6 [b]	79 ± 19
DGLA	1.1 ± 0.3	0.13 ± 0.06 [a]	1.5 ± 0.1 [b]	1.7 ± 0.5 [b]	0.17 ± 0.06[a]
LA	4.2 ± 0.6	3.8 ± 1.1	6.3 ± 1.0 [ab]	5.9 ± 1.2 [ab]	5.4 ± 1.1
DHA	157 ± 12.7	100 ± 29.7[a]	148 ± 13.2[b]	156 ± 18.4 [b]	107 ± 23.4[a]
EPA	30 ± 7.5	34.4 ± 14.0	41 ± 7.2	97 ± 12.8 [ab]	17 ± 7.8
ALA	0.6 ± 0.2	1.7 ± 0.5 [a]	0.89 ± 0.21 [b]	1.0 ± 0.3	1.8 ± 0.4 [a]
NPD1	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.01
PGE2	0.10 ± 0.01	0.16 ± 0.07 [a]	0.13 ± 0.01 [a]	0.12 ± 0.03	0.12 ± 0.03
total omega-3	187 ± 19.6	136 ± 42.7	189 ± 18.1	254 ± 30.5 [b]	126 ± 30.7 [a]
total omega-6	107 ± 8.56	56 ± 18 [a]	15 ± 7.94 [b]	122 ± 15.9 [b]	84 ± 19
Total PUFAs	295 ± 25.3	193 ± 60.0	314 ± 25.2 [a]	376 ± 45.1 [a]	210 ± 48
omega-6/omega-3	0.6 ± 0.1	0.41 ± 0.05 [a]	0.66 ± 0.04 [b]	0.48 ± 0.03	0.7 ± 0.1 [b]
AA/DHA	0.7 ± 0.1	0.52 ± 0.06	0.8 ± 0.1 [ab]	0.73 ± 0.04 [b]	0.7 ± 0.1 [b]
AA/EPA	3.7 ± 1.1	1.6 ± 0.3 [a]	2.9 ± 0.4 [a]	1.2 ± 0.1 [a]	5.2 ± 1.5 [b]

272   Serum free fatty acid levels of C57BL/6J mice (non-diabetic control) and *db/db* mice after 4-week

273   treatment period, measured as a ratio with the internal standard, AA-d_6.

274   [a]p<0.05 compared with nondiabetic control

275   [b]p<0.05 compared with diabetic control

276   [ab]p<0.05 compared with nondiabetic control and with diabetic control

277   **Liver**

278   The livers of diabetic mice treated with SSO alone and MSM/SSO combination therapy

279   had significantly increased levels of LA compared with nondiabetic controls (Table 2). ALA

280   levels were significantly increased in diabetic control mice and diabetic mice treated with either

281   SSO alone or MSM alone compared with nondiabetic control mice. This effect was reversed in

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282   diabetic mice treated with MSM and SSO combined, and the ALA levels were not significantly

283   different from nondiabetic control mice. EPA levels were significantly increased in diabetic

284   control mice and diabetic mice treated with MSM alone compared with nondiabetic control mice

285   but were significantly decreased in those treated with SSO alone compared with diabetic control.

286   Mice receiving MSM/SSO combination therapy had levels of EPA comparable to those seen in

287   nondiabetic control mice, and the two groups were not significantly different. There were no

288   significant differences regarding AA,DHA, NPD1, or PGE2 levels among groups.

289   **Table 2. Liver Free Fatty Acids**

Free fatty acids	Non-diabetic Control (Mean ± SD)	Diabetic Control (Mean ± SD)	SSO Treatment (Mean ± SD)	MSM Treatment (Mean ± SD)	MSM + SSO Treatment (Mean ± SD)
AA	38 ± 11	36 ± 6.2	46 ± 15	41 ± 16	45 ± 10
DGLA	0.07 ± 0.03	0.07 ± 0.02	0.08 ± 0.03	0.09 ± 0.04	0.09 ± 0.02
LA	0.9 ± 0.3	1.2 ± 0.3	1.5 ± 0.5 [a]	1.2 ± 0.3	1.5 ± 0.3 [a]
ALA	0.09 ± 0.03	0.18 ± 0.06 [a]	0.17 ± 0.05 [a]	0.21 ± 0.09 [a]	0.11 ± 0.03
DHA	50 ± 14	40 ± 5.1	45 ± 14	48 ± 14	46 ± 7.4
EPA	6.8 ± 2.3	32 ± 5.0 [a]	13 ± 1.6 [b]	42 ± 12 [a]	16 ± 4.3
NPD1	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
PGE2	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02
total omega-3	57 ± 15	72 ± 8.3	58 ± 15	91 ± 25 [a]	62 ± 11
total omega-6	39 ± 11	38 ± 6.4	47 ± 16	42 ± 16	46 ± 10
Total PUFAs	96 ± 26	110 ± 14.2	106 ± 30.0	133 ± 39.4	108 ± 21.6
omega-6/omega-3	0.7 ± 0.1	0.5 ± 0.1	0.80 ± 0.12 [b]	0.46 ± 0.10 [a]	0.75 ± 0.05 [b]
AA/DHA	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.2 [a]	0.84 ± 0.18	1.0 ± 0.1 [a]
AA/EPA	5.9 ± 1.4	1.2 ± 0.2 [a]	3.4 ± 0.9 [b]	0.96 ± 0.25 [a]	2.9 ± 0.4

290   Liver free fatty acid levels of C57BL/6J mice (non-diabetic control) and *db/db* mice after 4-week

291   treatment period, measured as a ratio with the internal standard, AA-d_6.

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292   [a]p<0.05 compared with nondiabetic control

293   [b]p<0.05 compared with diabetic control

Brain

295   In the brain, LA levels were significantly decreased in diabetic mice treated with MSM

296   alone compared with diabetic control mice (Table 3). Diabetic control mice and diabetic mice

297   treated with MSM/SSO combination therapy had significantly increased DGLA levels compared

298   with nondiabetic controls. This effect was reversed in diabetic mice treated with MSM alone,

299   whose DGLA levels were significantly decreased compared with diabetic control mice and were

300   not significantly different from nondiabetic control mice. AA levels in diabetic mice treated with

301   MSM alone were significantly increased compared with both nondiabetic and diabetic control

302   mice. Diabetic control mice and diabetic mice treated with MSM alone had significantly

303   increased levels of EPA. This effect was reversed in diabetic mice treated with SSO or

304   MSM/SSO combination therapy, with EPA levels that were not significantly different from

305   nondiabetic control mice and were significantly decreased compared with diabetic control mice.

306   DHA levels were significantly increased in diabetic mice treated with MSM alone compared

307   with nondiabetic control mice. There was no significant difference in ALA, NPD1, or PGE2

308   levels among the groups.

309   **Table 3. Brain Free Fatty Acids**

Free fatty acids	Non-diabetic Control (Mean ± SD)	Diabetic Control (Mean ± SD)	SSO Treatment (Mean ± SD)	MSM Treatment (Mean ± SD)	MSM + SSO Treatment (Mean ± SD)
AA	1,025 ± 195.9	1,163 ± 314.1	1,254 ± 451.9	1,503 ± 205.1 [ab]	1,358 ± 323.9
LA	9.7 ± 2.2	12 ± 2.0	14 ± 3.8	8.7 ± 1.8 [b]	13 ± 2.5
DGLA	7.2 ± 2.9	15 ± 4.0 [a]	11 ± 3.1	10 ± 1.4 [b]	12 ± 2.4 [a]

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DHA	$1,039 \pm 223.5$	$1,181 \pm 354.5$	$1,150 \pm 340.7$	$1,490 \pm 226.1$ [a]	$1,365 \pm 312.9$
EPA	98 ± 21	150 ± 29.3 [a]	82 ± 18 [b]	199 ± 33.6 [a]	105 ± 28.6 [b]
ALA	1.0 ± 0.8	1.5 ± 0.5	1.2 ± 0.4	1.1 ± 0.4 [b]	1.3 ± 0.5
NPD1	0.5 ± 0.8	0.4 ± 0.3	0.23 ± 0.15	0.2 ± 0.1	0.3 ± 0.2
PGE2	1.7 ± 0.7	1.9 ± 0.3	1.7 ± 0.3	2.0 ± 0.3	2.1 ± 0.4
total omega-3	$1,138 \pm 222.4$	$1,333 \pm 369.7$	$1,233 \pm 355.4$	$1,690 \pm 243.3$ [a]	$1,471 \pm 330.9$
total omega-6	$1,042 \pm 197.0$	$1,189 \pm 312.1$	$1,278 \pm 452.8$	$1,522 \pm 204.5$ [a]	$1,382 \pm 324.0$
total PUFAs	$2,180 \pm 402.2$	$2,521 \pm 675.7$	$2,511 \pm 775.1$	$3,212 \pm 446.2$ [a]	$2,853 \pm 645.7$
omega-6/omega-3	0.9 ± 0.1	0.90 ± 0.07	1.0 ± 0.2 [b]	0.90 ± 0.02	0.9 ± 0.1
AA/DHA	1.0 ± 0.1	1.00 ± 0.09	1.1 ± 0.2	1.01 ± 0.03	1.0 ± 0.1
AA/EPA	11 ± 4.1	7.8 ± 2.0	15 ± 4.0 [b]	7.7 ± 1.0	14 ± 3.8 [b]

310   Brain free fatty acid levels of C57BL/6J mice (non-diabetic control) and *db/db* mice after 4-week

311   treatment period, measured as a ratio with the internal standard, AA-d$_6$.

312   [a] $p<0.05$ compared with nondiabetic control

313   [b] $p<0.05$ compared with diabetic control

314   [ab] $p<0.05$ compared with nondiabetic control and with diabetic control

315   **Eye**

316   In the eye, the levels of NPD1, LA, and ALA were significantly decreased in all diabetic

317   mice regardless of treatment compared with nondiabetic control mice (Table 4). DGLA and AA

318   were significantly increased in diabetic mice receiving MSM, SSO, or MSM/SSO combination

319   therapy compared with nondiabetic control mice. Diabetic mice receiving SSO or MSM/SSO

320   combination treatment had significantly decreased EPA levels compared with diabetic control

321   mice and were not significantly different from nondiabetic control mice. DHA was significantly

322   increased in diabetic mice treated with MSM alone or in combination with SSO compared with

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323 nondiabetic control mice. There were no significant differences in PGE2 levels among the

324 groups.

325 **Table 4. Eye Free Fatty Acids**

Free fatty acids	Non-diabetic Control (Mean ± SD)	Diabetic Control (Mean ± SD)	SSO Treatment (Mean ± SD)	MSM Treatment (Mean ± SD)	MSM + SSO Treatment (Mean ± SD)
AA	71 ± 4.5	76 ± 7.9	84 ± 5.4 [a]	85 ± 6.4 [a]	87 ± 10 [ab]
LA	6.1 ± 1.2	1.6 ± 0.7 [a]	1.9 ± 0.4 [a]	1.5 ± 0.3 [a]	1.9 ± 0.6 [a]
DGLA	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1 [a]	0.8 ± 0.1 [a]	0.9 ± 0.1 [a]
DHA	149 ± 14.2	175 ± 12.9 [a]	168 ± 8.5	178 ± 9.97 [a]	185 ± 12.8 [a]
EPA	10 ± 1.5	12 ± 2.0	8.4 ± 1.0 [b]	15 ± 1.7 [a]	9.9 ± 1.6 [b]
ALA	0.5 ± 0.1	0.2 ± 0.1 [a]	0.17 ± 0.04 [a]	0.18 ± 0.04 [a]	0.2 ± 0.1 [a]
NPD1	0.05 ± 0.01	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]
PGE2	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.01
total omega-3	160 ± 15.0	187 ± 13.5 [a]	176 ± 8.56	192 ± 10.1 [a]	195 ± 13.6 [a]
total omega-6	78 ± 4.3	78 ± 8.4	87 ± 5.4	87 ± 6.6	89 ± 11 [ab]
total PUFAs	237 ± 17.5	265 ± 20.9 [a]	263 ± 11.6	280 ± 14.6 [a]	284 ± 22.1 [a]
omega-6/omega-3	0.49 ± 0.04	0.42 ± 0.03 [a]	0.49 ± 0.03 [b]	0.45 ± 0.03	0.46 ± 0.04 [b]
AA/DHA	0.48 ± 0.04	0.43 ± 0.03	0.50 ± 0.03 [b]	0.48 ± 0.04	0.47 ± 0.05
AA/EPA	7.2 ± 0.8	6.3 ± 0.8	10 ± 1.0 [ab]	5.9 ± 0.5	8.9 ± 0.8 [b]

326 Eye free fatty acid levels of C57BL/6J mice (non-diabetic control) and *db/db* mice after 4-week

327 treatment period, measured as a ratio with the internal standard, AA-d_6.

328 [a]p<0.05 compared with nondiabetic control

329 [b]p<0.05 compared with diabetic control

330 [ab]p<0.05 compared with nondiabetic control and with diabetic control

Discussion

331

332 Dyslipidemia and impaired lysophospholipid metabolism are hallmarks of type 2 diabetes

333 mellitus and related metabolic syndrome and obesity [1]. Diet and exercise, along with

334 administration of cholesterol-lowering pharmaceuticals such as statins, are recognized as standard

335 interventions to control hyperlipidemia. In recent years, however, there has been increasing interest

336 and compelling evidence to support the adjuvant use of natural products to help control

337 hyperlipidemia [13]. Our goal was to identify lipid alterations in *db/db* mice, a murine model of

338 type 2 diabetes, for the purpose of establishing a baseline biological signature for the subsequent

339 evaluation of natural products with purported lipid-altering activity. In this article, we utilized

340 mass spectrometry-based lipidomics to investigate diabetes-associated changes in fatty acid

341 metabolism and the extent to which the dietary ingredients sesame seed oil and/or

342 methylsulfonylmethane impacted biomarkers of metabolic syndrome.

343 Sesamin, the primary constituent lignan found in SSO, exerts potent lipid-lowering effects

344 by targeting key enzymes fatty acid synthesis (e.g., delta-5 desaturase), fatty acid oxidation (e.g.,

345 PPARα), and cholesterol metabolism (e.g., 3-hydroxy-3-methyl-glutaryl-CoA reductase) [14]. For

346 example, low-density lipoprotein (LDL) receptor deficient mice fed a diet of SSO were found to

347 have reduced plasma total cholesterol, TG, very low-density lipoprotein cholesterol (VLDL), and

348 LDL cholesterol, while high-density lipoprotein (HDL) was significantly increased compared with

349 atherosclerotic diet-fed animals [15]. Similarly, we found that diabetic mice fed SSO alone

350 showed decreased non-HDL cholesterol (LDL + VLDL) accompanied by an increase in HDL (Fig

351 4 and 5), an effect that has been well recognized in human patients. Sesame seed oil

352 supplementation has been shown to increase HDL cholesterol with a corresponding decrease in

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353   total cholesterol, triglycerides, and LDL cholesterol in hypercholesterolemic patients [16] and in

354   rheumatoid arthritis patients, as well as reduce body weight, body mass index, and systolic blood

355   pressure [17]. Similarly, Sankar et al. found that supplementation with 35 grams of sesame seed

356   oil daily for 60 days significantly reduces triglycerides, total cholesterol, and LDL cholesterol

357   while significantly increasing HDL cholesterol in type 2 diabetics [18] and hypertensive patients

358   [19]. In postmenopausal women supplemented with 50 grams of sesame powder daily for five

359   weeks, the same decrease in total cholesterol and LDL:HDL cholesterol was seen, with a

360   corresponding increase in antioxidant status [20]. Our results affirm these findings in the type 2

361   diabetic *db/db* mouse.

362   Regarding fatty acid metabolism (Fig 7), serum total polyunsaturated fatty acid (PUFA)

363   levels were significantly reduced in diabetic mice compared to control mice (Table 1). This finding

364   is consistent with literature data demonstrating that in diabetics there is a reduction in the activity

365   of delta-5 desaturase, the rate-limiting step in PUFA metabolism [21]. In contrast, feeding of only

366   SSO reversed this effect such that fed mice exhibited serum PUFA levels comparable to control

367   mice. SSO contains high amounts of omega-6 PUFAs such as linoleic acid [22], which partially

368   explains the effect of SSO on omega-6 PUFA levels. However, since SSO is not an appreciable

369   source of omega-3 PUFAs, dietary PUFAs in SSO cannot entirely account for the increase in total

370   PUFAs, *viz*., omega-6 plus omega-3 PUFAs. A clue to an alternative mechanism to explain SSO's

371   effect on total PUFA levels may be found in its effect on the delta-5 desaturase index (AA:DGLA),

372   which represents a measure of PUFA metabolism where a higher ratio reflects greater delta-5

373   desaturase activity. The delta-5 desaturase activity in diabetic mice (~500) was higher than either

374   control (~100) or SSO only fed mice (~100). Paradoxically, it appears that total serum PUFAs are

375   reduced in diabetic mice compared to SSO-only fed mice despite delta-5 desaturase being five

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376   times greater in diabetic mice. Regardless of the mechanism, reduced PUFA availability can

377   significantly impact lysophospholipid metabolism.
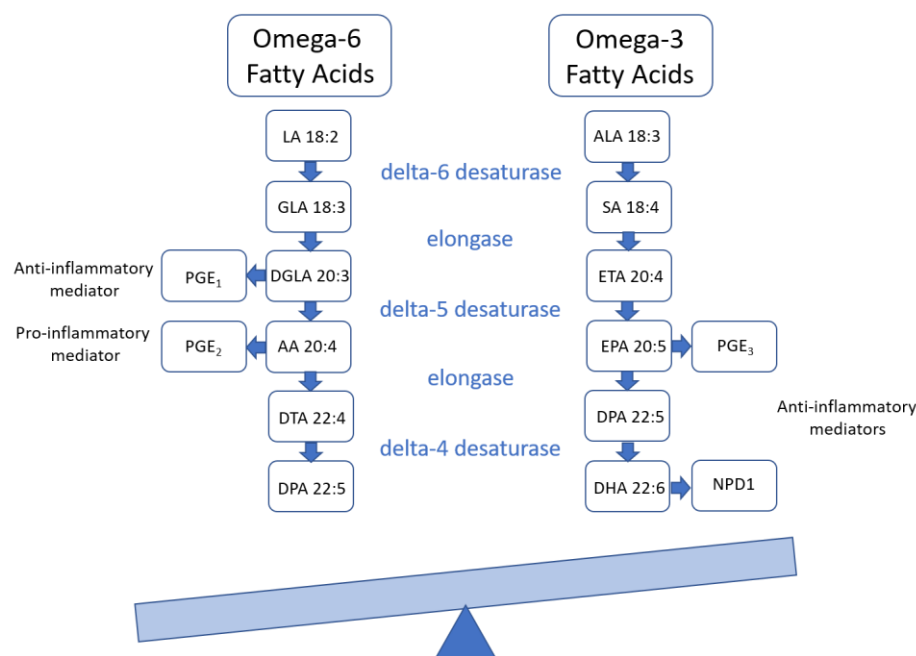

378

379   **Fig 7. Fatty Acid Metabolism.** This schematic demonstrates the metabolism of omega-6 and

380   omega-3 fatty acids into pro-inflammatory and anti-inflammatory mediators, a balance which is

381   disrupted in diabetes and metabolic syndrome.

382   Oral administration of methylsulfonylmethane to *db/db* mice results in a dose-dependent

383   decrease in liver cholesterol, triglycerides, lipogenesis-dependent gene expression (e.g., fatty acid

384   synthase), and fatty oxidation[8]. Since MSM contains two methyl groups, the authors speculated

385   that MSM may serve as a methyl donor to ameliorate hepatic steatosis. Building on this hypothesis,

386   we sought to demonstrate that MSM, acting as a methyl donor, could mitigate diabetes-related

387   hyperlipidemia and aberrant PUFA metabolism. Similar to SSO, administration of MSM alone

388   led to decreased non-HDL cholesterol (LDL + VLDL) and increased HDL (Fig 4 and 5).

389   The human genes for delta-5 (*FADS1*) and delta-6 (*FADS2*) desaturase enzymes are

390   situated head-to-head (5'-5') in a cluster configuration on chromosome 11 (11q12.2) [23]. This

23

391 arrangement affords a common promoter for *FADS1* and *FADS2* that contains a potential

392 "enhancer signature" comprising methylation loci located within a 5' regulatory region [23].

393 Methylation probe studies demonstrated an inverse relationship between methylation proportion

394 and both delta-5 (AA/DGLA) and delta-6 (GLA/LA) desaturase activity [23]. These data could

395 potentially explain the association between methyl donor deficiency and reduced delta-5

396 desaturase activity in diabetics [21]. As mentioned above, our data demonstrate that delta-5

397 desaturase activity is greatly enhanced in *db/db* mice compared to control or MSM alone treated

398 mice (Table 1). These data are consistent with the notion that reduced methyl donor availability in

399 diabetic mice is associated with increased delta-5 desaturase activity and that administration of the

400 putative methyl donor MSM significantly decreases delta-5 desaturase activity. Future studies

401 examining the serum and liver methyl donor status (e.g., choline, betaine, etc.) in *db/db* mice are

402 needed to test this hypothesis.

403   In conclusion, treatment of *db/db* mice with MSM and SSO improved commonly

404 measured clinical parameters in serum lipid panels. Serum triglycerides and total cholesterol

405 were significantly increased in the *db/db* model compared to nondiabetic control, mimicking the

406 diabetic condition in people. Most notably, HDL-cholesterol was significantly increased in all

407 *db/db* treatment groups, with the most significant treatment effect in the MSM + SSO

408 combination group. Because the HDL-cholesterol level is increasing with treatment, the ratio of

409 LDL:HDL is in turn decreasing. This change in the LDL:HDL ratio due to combination MSM

410 and SSO treatment could lead to improved clinical outcomes in people such as a reduced

411 incidence of atherosclerosis.

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Acknowledgements

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