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Decreased saturated fatty acids, total cholesterol and LDL-C in *sdd17* mice

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1. ABSTRACT

SDD17, a delta-15 desaturase from the fungus *Saprolegnia* can convert arachidonic acid to eicosapentanoic acid in yeast, plant embryos, and mammalian cells. Here, we generated transgenic mice that carried two copies of codon-optimized *sdd17* cDNA within a non-coding domain of chromosome 6. RT-PCR analysis revealed that the foreign gene was expressed in the transgenic tissues. Gas chromatography showed that the levels of total unsaturated fatty acids in muscle, liver, and spleen tissues were significantly ($p<0.05$) increased in transgenic mice compared to non-transgenic mice at 3 or 8 weeks of age. In addition, the serum concentrations of total cholesterol and low-density lipoprotein cholesterol in transgenic females, but not in males, were significantly lower than those in sex-matched non-transgenic mice. These results suggest that endogenous *sdd17* expression is beneficial for mammalian health and that its effects on fatty acid profiles may differ between sexes.

2. INTRODUCTION

Long chain fatty acids (FAs) include saturated- (SFAs), monounsaturated- (MUFAs), and polyunsaturated- (PUFAs) acids. In mammals, SFAs can be converted into MUFAs through the action of delta-9 desaturase, whereas MUFAs cannot be converted into PUFAs because mammalian cells are unable to introduce double bonds at the omega-3 (n3) or omega-6 (n6) positions of the fatty acids because they lack the delta-15 and delta-12 desaturase enzymes. Therefore, PUFAs must be provided from the diet. In general, each class of fatty acids has a different set of biological effects. For instance, SFAs such as C16:0 and C14:0 are usually linked to cardiovascular disease, stroke and type 2 diabetes mellitus (1), yet stearic acid (C18:0) has been shown to have a neutral or beneficial effect on health (2). MUFAs are beneficial to humans and are associated with lower risks of chronic disease (3, 4). PUFAs, including n3 and n6 fatty acids that are not inter-convertible in the mammalian body, play critical roles in

infant growth, neural development (5), and immune function (6). The most common n6 fatty acid is linoleic acid (C18:2n6, LA), which can be inverted into active arachidonic acid (C20:4n6, ARA). The basic n3 fatty acid is alpha-linolenic acid (C18:3n3, ALA), which can be inverted into physiologically active forms, including eicosapentaenoic acid (C20:5n3, EPA), docosapentaenoic acid (C22:5n3, DPA), and docosahexaenoic acid (C22:6n3, DHA), in mammals. In humans, n3 deficiency results in several neuron-specific defects, whereas n6 deficiency causes numerous abnormalities such as cancer, reduced growth, reproductive failure, skin lesions, fatty liver, and polydipsia (7, 8).

Studies in animal models or humans fed a specific dietary PUFA regimen often exhibit bias or inaccurate results. Because there are many confounding factors in a diet, there are many limitations for tissue fatty acid composition analyses as an approach to study the effects of a diet on fatty acid metabolism and the differences in PUFA metabolism among different tissues (9, 10). In contrast, an appropriate animal model that can synthesize endogenous PUFAs without the need of a dietary fatty acid supply will be very helpful for the evaluation of the health effects of specific nutrients. In 2004, a *fat-1* transgenic mouse (11) and a delta-12 fatty acid desaturase-2 transgenic pig (12) were successfully produced in different labs. These models are especially useful because transgenic and wild type littermates fed identical diets produce different fatty acid profiles. These models thus allow well-controlled studies for understanding human disease and health, without the interference of the potential confounding factors from different diets.

In the fungus *Saprolegnia*, EPA is the sole n3 PUFA, and the novel gene *sdd17*, which encodes a delta-15 desaturase, can recognize a 20-carbon substrate (13). This desaturase mainly converts exogenous C20:4n6 to C20:5n3 when expressed in yeast and soybean embryos, and it can also produce trace amounts of C20:4n3 from C20:3n6 (13). In our previous study, we demonstrated that the codon-optimized *sdd17* gene could be functionally expressed in murine NIH 3T3 cells and observed an increase in EPA and decrease in ARA in transformed cell lines (14). In the current study, we further generated *sdd17* transgenic mice that produce their own desaturase to allow the study of de novo PUFA metabolism and its effect on mammalian health. The healthy changes in the fatty acid compositions of transgenic tissues and levels of serum lipoprotein in transgenic mice indicated that endogenous *sdd17* expression is beneficial for mammalian health.

3. MATERIALS & METHODS

3. 1. Chemicals

All chemicals were purchased from Sigma, unless otherwise stated.

3. 2. Animals

Kunming mice at 8 weeks of age were obtained from the Beijing Experimental Animal Center (Beijing, China) and used for transgenesis. All animals were maintained in a light-controlled room (14L:10D, lights on at 0600 h) at a temperature of 22°C and raised on a normal diet. All animal procedures in the present study were approved by the Committee for Experimental Animals of China Agricultural University.

3. 3. Gene construction

The previously described codon-optimized sequence of the *sdd17* gene (14) was used in the present study. In brief, the DNA fragment for microinjection contained the optimized *sdd17* gene driven by the cytomegalovirus enhancer and the chicken beta-actin promoter, which allowed high and broad expression of the foreign gene in mice tissues. The linear 3.4-kb transgenic cassettes were digested with *Sal*I + *Hin*dIII, purified using a QIAquick Gel Extraction Kit (Qiagen), and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) to a final concentration of 4.5 μg/ml.

3. 4. Generation of transgenic mice

Fertilized eggs were collected from superovulated females at 8 week of age and released from the cumulus layers by treatment with HEPES-buffered CZB medium containing 300 IU/ml hyaluronidase. Embryos with an obvious polar body and two pronuclei were used for DNA microinjection. Ten to twelve embryos were transferred into each oviduct of 0.5 day-post-coitum pseudopregnant females. Pregnant recipients were then housed individually and allowed to carry the pregnancies to term.

3. 5. Transgene detection by PCR

Genomic DNA was extracted from the tail tissues of pups at 3 week of age utilizing a standard phenol-chloroform method and dissolved in TE buffer (pH 8.0) for nucleic acid analysis. The presence of the transgene was assayed by PCR amplification using one set of primers (forward: 5'-tgctgcctacgcttatct-3', reverse: 5'-tctcatcagtcgctcttgg-3') and amplified a 468-bp fragment that spanned the *sdd17* gene. PCR amplification was performed as follows: 95°C for 5 min; 30 cycles of 94°C for 40 sec, 60°C for 40 sec, and 72°C for 60 sec, and a final extension at 72°C for 8 min.

3. 6. Thermal asymmetric interlaced PCR

To determine the integration site of the *sdd17* gene in mice, three transgene-specific primers (tgp1: 5'-ccttgagcatctgacttct-3'; tgp2: 5'-gttggctataaagaggtcatcag-3'; tgp3: 5'-ccctgctgtccattcctt-3') and an arbitrary degenerate primer (AD: 5'-wgtgnagwancanaga-3') were used for thermal asymmetric interlaced-PCR (Tail-PCR) amplification as previously described (15, 16). The thermal cycling conditions were as described in our previous studies (15). After the third Tail-PCR reaction, the products were separated on a 1.5% agarose gel, and a single band from each sample was gel purified and sequenced directly. The resulting sequences were analyzed using online BLAST from the *Mus musculus* (http://www.ensembl.org) database.

3. 7. Real-time PCR

Real-time PCR was used to determine the transgene copy number using the SYBR® Premix Ex

Table 1. Total lipid contents of the standard mouse diets

Fatty acids	Mean ± SD
C16:0	21.30 ± 0.15
C16:1n7	0.42 ± 0.02
C18:0	2.67 ± 0.04
C18:1n9	16.62 ± 0.08
C18:1n7	1.29 ± 0.02
C18:2n6	48.65 ± 0.21
C18:3n6	0.27 ± 0.01
C20:1n9	4.08 ± 0.01
C18:3n3	0.32 ± 0.00
C20:3n6	0.26 ± 0.01
C20:4n6	0.10 ± 0.02
C20:5n3	0.08 ± 0.01
C22:6n3	1.35 ± 0.15
Total saturated	24.88 ± 0.13
Total monounsaturated	22.41 ± 0.05
Total n6	49.28 ± 0.19
Total n3	1.75 ± 0.16
Others	1.64 ± 0.05

Note: Each value represents the mean ± SD from six independent measurements. Total saturated fatty acids was calculated as C16:0 + C18:0 + C24:0. Total monounsaturated FAs was calculated as C16:1n7 + C18:1n9, C18:1n7 + C20:1n9. Total n6 was calculated as C18:2n6 + C18:3n6 + C20:3n6 + C20:4n6. Total n3 was calculated as C18:3n3 + C20:5n3 + C22:6n3.

TaqTM II (Perfect Real Time) kit (Takara) according to the manufacturer's protocol. Genomic DNA was used for real-time PCR amplification with the primer pairs specific to the *sdd17* (forward: 5'-ccttgagcatctgacttct-3', reverse: 5'-gccaaactctaaaccaaatac-3') or *hprt* (forward: 5'-cacgcctattttccacct-3', reverse: 5'-agccaaagcccattattc-3') genes. Each reaction contained 10 ng of genomic DNA, 0.4 μM of each primer, and 1× SYBR® *Premix Ex Taq*™ II Mix in a final reaction volume of 20 μl, and the assay was performed using an ABI Prism 7500 Sequence Detection System (Applied Biosystems) at 95°C, 30 sec, 1 cycle; 95°C, 5 sec, 60°C, 34 sec, 40 cycles. Each reaction was performed as four replicates and measured at least four times. In accordance with Brunner *et al* (17), the copy number was determined using the equation $2^{-\Delta Ct}$, where Ct is the PCR cycle number at which the accumulated fluorescence signal in each reaction crosses a threshold above background and $-\Delta Ct = Ct_{hprt} - Ct_{transgene}$.

3. 8. RT-PCR

Total RNA was extracted from fresh muscle, liver, spleen, or heart tissues from transgenic mice or their non-transgenic littermates using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions and treated with DNase (TaKaRa) prior to RT-PCR. The purified RNA was used for first-strand cDNA synthesis, and reverse transcription was performed using M-MLV reverse transcriptase with oligo-dT primers according to the manufacturer's instructions (Promega, Madison, USA). Reactions in the absence of reverse transcriptase were also performed for each RNA sample tested to check for genomic contamination. The resulting cDNA was used for PCR amplification with the *sdd17* specific primers (forward: 5'-tgctgcctacgcttatct-3', reverse: 5'-tctcatcagtcgctcttgg-3') to produce a 468-bp fragment. As a control, a 352-bp fragment of the *hprt* gene (forward: 5'-gtcaagggcatatccaacaacaaac-3', reverse: 5'-cctgctggattacattaaagcactg-3') was amplified under the same conditions. PCR amplification was performed as follows: 95°C for 5 min; 30 cycles of 94°C for 40 sec, 60°C for 40 sec, and 72°C for 60 sec, and a final extension at 72°C for 8 min.

3. 9. Gas chromatography

A heterozygous *sdd17* male was mated with a Kunming female to obtain non-transgenic (wild type) and heterozygous *sdd17* littermates. Three-week-old female offspring (fed by non-transgenic milk) or 8-week-old littermates from each breeding pair fed a normal diet were used for fatty acid analysis by gas chromatography as previously described (14, 18). The total lipids of the normal diet are listed in Table 1. In brief, fatty acid methyl esters were quantified using a fully automated 7890 Network GC System (Agilent Technologies) with a flame ionization detector. The esters were separated on a fused-silica capillary column (HP-88, J & W 112-88A7, Agilent). The injector and detector were set at 250°C and 280°C, respectively. FAME were eluted using the following temperature program: 1 min at 120°C, increased to 175°C at 10°C/min and held at 175°C for 10 min, increased to 210°C at 5°C/min and held at 210°C for 5 min, increased to 240°C at 5°C/min and held at 240°C for 10 min. Nitrogen was used as the carrier gas, and the split ratio was 10:1. Peaks were identified by comparison with fatty acid standards, and the area percentage for all resolved peaks was analyzed using GC ChemStation Software (Agilent).

3. 10. Blood sampling and analyses

Blood was taken from 20 *sdd17* mice (10 male and 10 female) and 20 non-transgenic littermates after an overnight fast at the age of 3 or 8 weeks, and the serum was analyzed using an automatic analyzer (Toshiba TBA-40FR, Co., Ltd. Tokyo, Japan) immediately or within two days. In brief, the high- (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in serum were measured by the direct method using an HDL-C and LDL-C Assay kit (Chivd, Beijing, China). Total cholesterol (CHO) and triglyceride (TG) measurements were performed with the Cholesterol Oxidase Endpoint Method using CHO Assay Kit (Chivd) and the enzymatic method using the TG Assay Kit (Chivd), respectively.

3. 11. Statistical analysis

All experiments were repeated at least four times, and statistical analyses were performed using SPSS (version 13.0 for Windows). All values are presented as the means ± SD. A ratio was considered significant if the mean t-test P value was less than or equal to 0.05 for each sample.

4. RESULTS

4. 1. Gene analysis

PCR analysis of DNA samples derived from the 20 offspring demonstrated the presence of transgenes in mouse-12 and mouse-31 (Figure 1A). Progeny analysis revealed germ-line transmission of the *sdd17* transgene for both founders. Tail-PCR analysis revealed that *sdd17*

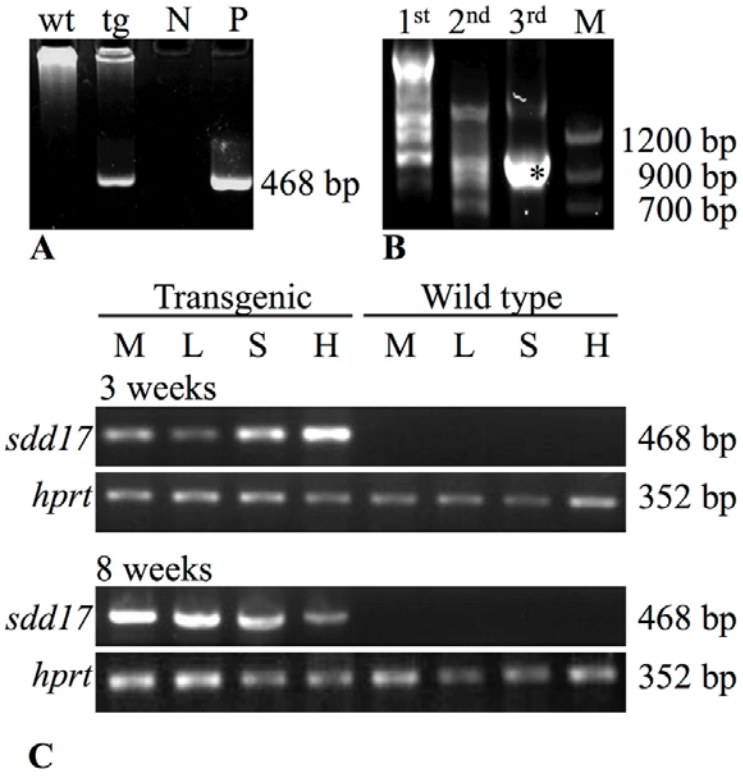

Figure 1. Transgene analysis. (A) Conventional PCR analysis of wild-type (wt), mouse-12 (tg), no DNA control (N), and *sdd17* plasmid (P) samples show that the mouse-12 line is *sdd17* transgenic. The PCR products are 468 bp spanning *sdd17* gene. (B) Gel analysis of the primary, secondary and tertiary Tail-PCR products amplified from the *sdd17* mouse. Total DNA from mouse-12 was used for Tail-PCR, and the sequencing results of specific products (*) from the third amplification reveal that the *sdd17* transgene was inserted into a non-coding region in chromosome 6. Lane M indicates the DNA marker. (C) RT-PCR analysis of *sdd17* mice. Total RNAs without DNA contamination were extracted from muscle (M), liver (L), spleen (S), and heart (H) tissues from the offspring of a transgenic founder (mouse-12), including both transgenic (tg) and non-transgenic (wt) littermates, at 3 or 8 weeks of age. As a control, the *hprt* gene (352-bp fragment) was used for concurrent RT-PCR amplification. The amplification of the specific fragment of *sdd17* (468-bp) confirms its transcription in the above transgenic tissues.

transgene was inserted into a single site within the non-coding domain of chromosome 6 in mouse-12 (Figure 1B) and in chromosome 17 in mouse-31, respectively. Real-time PCR further revealed that there were two copies (1.98 ± 0.17) of the *sdd17* transgene in the mouse-12 line. RT-PCR patterns revealed that the *sdd17* gene was expressed in the muscles, liver, spleen, and heart in 3- and 8-week-old animals from the mouse-12 line (Figure 1C). No transgenic signal was detected in mouse-31 samples.

4. 2. Fatty acids analysis

Heterozygous *sdd17* mice and their non-transgenic littermates were used for fatty acids analysis. Mice at 3 and 8 weeks of age were used for gas chromatography analysis. Mice at 3 weeks of age were fed fresh milk from their non-transgenic mothers, and 8-week-old mice were raised from normal diet. Consistent with the RT-PCR analysis, the gas chromatography results showed that SDD17 expression resulted in substantial fatty acid changes in samples from the mouse-12 line but not those from the mouse-31 line. In transgenic samples of 3-week-old animals from the mouse-12 line (Table 2), the levels of total unsaturated fatty acids consisting of n7, n6, and n3 in muscle (54.39 ± 0.80 vs. 52.48 ± 1.11), liver (60.26 ± 0.47 vs. 56.67 ± 0.39), and spleen (47.28 ± 0.62 vs. 44.16 ± 0.30) tissue were significantly ($p<0.05$) increased compared to non-transgenic age-matched tissues; only the heart tissue did not have significantly increased fatty acid levels (55.87 ± 1.84 vs. 57.43 ± 0.70, $p>0.05$). Similarly, in 8-week-old mouse-12 samples (Table 3), the levels of total unsaturated fatty acids in the muscle (55.96 ± 0.81 vs. 53.07 ± 0.30), liver (57.75 ± 0.31 vs. 55.29 ± 0.16), and spleen (59.87 ± 4.01 vs. 45.65 ± 1.87) were significantly ($p<0.05$) increased compared to the levels in non-transgenic age-matched tissues. In contrast, the levels of total saturated fatty acids in muscle (32.70 ± 0.73 vs. 34.19 ± 0.31), liver (35.87 ± 0.37 vs. 37.94 ± 0.76), and spleen (29.87 ± 1.50 vs. 40.66 ± 2.09) were significantly ($p<0.05$) decreased compared to the levels in non-transgenic age-matched tissues. In addition, among examined tissues, the concentrations of EPA or total omega-3 fatty acids were not significantly increased. Consequently, no change in the n6/n3 or (EPA+DPA+DHA)/(LA+AA) ($p>0.05$) ratios were detected in all of the transgenic organs tested (Tables 2 and 3).

Table 2. Total lipids in muscle, liver, spleen and heart from wild type (WT) and *sdd17* transgenic (TG) mice at 3 weeks of age

Fatty acids	Muscle		Liver		Spleen		Heart	
	WT (n=5)	TG (n=3)	WT (n=5)	TG (n=5)	WT (n=3)	TG (n=4)	WT (n=4)	TG (n=5)
C16:0	17.74 ± 0.88	19.81 ± 0.50*	18.89 ± 0.50	19.38 ± 0.61	20.72 ± 0.28	23.36 ± 1.20*	11.75 ± 0.38	13.17 ± 1.20
C18:0	13.93 ± 1.29	12.62 ± 0.07	13.94 ± 0.74	14.52 ± 1.71	13.53 ± 0.01	12.56 ± 1.34	18.06 ± 0.29	17.51 ± 1.72
C20:0	0.18 ± 0.03	0.15 ± 0.02	0.08 ± 0.01	0.05 ± 0.04	0.27 ± 0.01	0.24 ± 0.03	0.43 ± 0.06	0.41 ± 0.05
C22:0	0.41 ± 0.04	0.31 ± 0.07	0.24 ± 0.06	0.22 ± 0.11	0.68 ± 0.08	0.71 ± 0.06	0.44 ± 0.02	0.57 ± 0.13
C24:0	0.57 ± 0.32	0.35 ± 0.03	0.21 ± 0.10	0.22 ± 0.08	1.48 ± 0.29	0.98 ± 0.23	0.25 ± 0.10	0.20 ± 0.03
C16:1n7	0.39 ± 0.10	0.49 ± 0.06	0.23 ± 0.05	0.26 ± 0.11	0.36 ± 0.03	0.35 ± 0.20	0.15 ± 0.03	0.38 ± 0.24
C18:1n7	1.88 ± 0.13	1.41 ± 0.0*	0.87 ± 0.08	0.72 ± 0.07*	1.56 ± 0.04	1.31 ± 0.07*	1.79 ± 0.08	1.21 ± 0.13*
C18:1n9	12.91 ± 2.66	11.80 ± 1.09	9.63 ± 1.30	7.67 ± 1.32*	10.41 ± 0.99	11.38 ± 1.49	9.71 ± 3.71	10.05 ± 2.14
C24:1n9	0.57 ± 0.10	0.31 ± 0.03*	0.32 ± 0.08	0.31 ± 0.13	1.38 ± 0.10	1.27 ± 0.17	0.24 ± 0.01	0.20 ± 0.02*
C18:2n6	15.32 ± 1.68	15.67 ± 0.39	18.34 ± 2.24	18.95 ± 1.20	7.42 ± 0.96	8.50 ± 1.05	17.18 ± 0.82	15.74 ± 4.69
C20:2n6	1.19 ± 0.10	0.99 ± 0.13	0.49 ± 0.07	0.45 ± 0.13	0.96 ± 0.04	0.96 ± 0.11	1.53 ± 0.06	1.12 ± 0.19*
C20:3n6	1.01 ± 0.09	0.89 ± 0.09	0.56 ± 0.08	0.88 ± 0.13*	0.83 ± 0.07	0.91 ± 0.04	0.71 ± 0.03	0.59 ± 0.06*
C20:4n6	13.66 ± 1.00	14.67 ± 0.75	14.95 ± 2.91	18.29 ± 2.17	16.10 ± 0.90	16.24 ± 1.31	11.75 ± 0.68	11.89 ± 2.13
C18:3n3	0.12 ± 0.03	0.19 ± 0.02*	0.13 ± 0.05	0.20 ± 0.06	ND	ND	ND	0.17 ± 0.08
C20:5n3	0.14 ± 0.02	0.20 ± 0.04	0.10 ± 0.01	0.19 ± 0.04*	ND	0.15 ± 0.09*	ND	ND
C22:5n3	1.26 ± 0.15	1.07 ± 0.13	0.53 ± 0.14	0.52 ± 0.12	1.28 ± 0.17	1.47 ± 0.18	1.41 ± 0.06	1.20 ± 0.22
C22:6n3	4.53 ± 0.38	5.51 ± 0.24*	9.53 ± 1.42	8.98 ± 3.04	3.33 ± 0.48	3.76 ± 0.64	10.13 ± 0.50	10.49 ± 1.10
Saturated	33.01 ± 0.66	33.24 ± 0.35	33.36 ± 1.25	34.40 ± 1.47	36.67 ± 0.12	37.95 ± 0.80	30.93 ± 0.50	31.57 ± 0.06
Monounsaturated	15.09 ± 0.78	13.72 ± 0.02*	11.03 ± 0.48	8.47 ± 0.06*	14.04 ± 0.89	15.17 ± 0.58	14.27 ± 0.36	13.19 ± 1.55
Total n6	31.72 ± 0.24	32.29 ± 0.43	34.99 ± 3.07	38.85 ± 1.38*	25.51 ± 0.05	26.78 ± 0.27*	31.59 ± 0.44	31.07 ± 0.67
Total n3	6.50 ± 0.07	6.79 ± 0.22	10.80 ± 0.92	12.02 ± 0.52	4.61 ± 0.64	5.14 ± 0.50	11.41 ± 0.21	12.32 ± 0.95
Total unsaturated	52.48 ± 1.11	54.39 ± 0.80*	56.67 ± 0.39	60.26 ± 0.47*	44.16 ± 0.30	47.28 ± 0.62*	57.43 ± 0.70	55.87 ± 1.84
(EPA+DPA+DHA)/(LA+ARA)	0.20 ± 0.01	0.22 ± 0.01	0.33 ± 0.05	0.32 ± 0.00	0.20 ± 0.03	0.21 ± 0.02	0.40 ± 0.02	0.42 ± 0.04
n6/n3	5.20 ± 0.57	4.75 ± 0.07	2.98 ± 0.30	3.21 ± 0.02	5.58 ± 0.79	5.24 ± 0.54	2.82 ± 0.03	2.48 ± 0.24

Note: Each value represents the mean ± SD of at least 3 independent measurements. Total saturated fat was calculated as C16:0 + C18:0 + C20:0 + C22:0 + C24:0. Total monounsaturated fat was calculated as C16:1n7 + C18:1n9 + C18:1n7 + C20:1n9 + C24:1n9. Total n6 was calculated as C18:2n6 + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6. Total n3 was calculated as C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3. Total unsaturated was calculated as total monounsaturated + total n6 + total n3. WT: wild type; TG: *sdd17* transgenic mice. ND: not detected. The student's t-test was used to assess the differences in fatty acids profiles between wild type and *sdd17* transgenic mice. *P < 0.05.

Table 3. Total lipids in muscle, liver, spleen and heart tissues from 8-week-old wild type (WT) and *sdd17* transgenic (TG) mice

Fatty acids	Muscle		Liver		Spleen		Heart	
	WT (n=4)	TG (n=4)	WT (n=4)	TG (n=4)	WT (n=3)	TG (n=3)	WT (n=4)	TG (n=4)
C16:0	20.91 ± 0.67	20.20 ± 0.41	21.58 ± 2.40	21.51 ± 0.96	20.19 ± 1.81	18.69 ± 2.24	13.82 ± 0.35	13.63 ± 0.73
C18:0	11.60 ± 0.62	10.68 ± 0.89	13.86 ± 1.00	13.84 ± 1.54	14.98 ± 1.66	12.05 ± 2.49	14.41 ± 1.18	14.29 ± 1.45
C20:0	0.17 ± 0.07	0.16 ± 0.05	0.10 ± 0.05	0.08 ± 0.03	0.42 ± 0.16	0.35 ± 0.13	0.34 ± 0.08	0.35 ± 0.09
C22:0	0.26 ± 0.08	0.28 ± 0.10	0.36 ± 0.19	0.26 ± 0.15	0.79 ± 0.16	0.64 ± 0.14	0.62 ± 0.12	0.60 ± 0.08
C24:0	1.03 ± 0.66	0.89 ± 0.55	0.41 ± 0.11	0.34 ± 0.14	2.22 ± 1.46	0.96 ± 0.31	0.59 ± 0.55	1.92 ± 2.29
C16:1n7	0.29 ± 0.05	0.35 ± 0.12	0.70 ± 0.33	0.46 ± 0.16	0.39 ± 0.12	0.44 ± 0.16	0.22 ± 0.09	0.16 ± 0.07
C18:1n7	1.71 ± 0.28	1.57 ± 0.22	1.45 ± 0.40	1.08 ± 0.42	1.31 ± 0.37	1.13 ± 0.40	1.08 ± 0.15	0.93 ± 0.19
C18:1n9	9.09 ± 0.71	11.55 ± 1.16*	8.84 ± 0.47	8.37 ± 1.35	10.76 ± 4.16	17.26 ± 6.99	11.54 ± 2.67	10.34 ± 3.77
C24:1n9	0.21 ± 0.07	0.21 ± 0.10	0.29 ± 0.04	0.28 ± 0.08	1.19 ± 0.18	0.91 ± 0.33	0.14 ± 0.03	0.16 ± 0.02
C18:2n6	14.16 ± 1.19	15.50 ± 0.93	14.26 ± 0.76	15.70 ± 0.85*	11.46 ± 3.72	16.97 ± 5.78	20.01 ± 1.41	19.15 ± 2.12
C20:2n6	0.67 ± 0.05	0.58 ± 0.08	0.52 ± 0.10	0.49 ± 0.06	1.03 ± 0.18	0.79 ± 0.25	0.51 ± 0.05	0.47 ± 0.10
C20:3n6	0.97 ± 0.11	0.80 ± 0.04*	1.26 ± 0.32	1.22 ± 0.12	0.82 ± 0.19	0.62 ± 0.21	0.48 ± 0.05	0.48 ± 0.07
C20:4n6	14.67 ± 0.97	12.38 ± 1.03*	18.67 ± 1.60	18.69 ± 1.41	15.29 ± 2.46	11.61 ± 3.78	9.81 ± 1.97	8.95 ± 0.66
C18:3n3	0.35 ± 0.04	0.37 ± 0.04	0.25 ± 0.05	0.23 ± 0.04	0.45 ± 0.08	0.50 ± 0.05	0.44 ± 0.07	0.37 ± 0.06
C20:5n3	ND	ND	0.34 ± 0.19	0.28 ± 0.08	0.29 ± 0.58	0.54 ± 1.03	0.49 ± 0.93	ND
C22:5n3	0.84 ± 0.16	0.91 ± 0.07	0.39 ± 0.28	0.43 ± 0.06	0.66 ± 0.16	0.58 ± 0.25	0.67 ± 0.07	0.74 ± 0.17
C22:6n3	9.60 ± 1.41	7.81 ± 2.58	9.02 ± 0.69	8.44 ± 1.62	3.17 ± 0.55	2.66 ± 0.98	12.69 ± 1.29	12.93 ± 4.19
Saturated	34.19 ± 0.31	32.70 ± 0.73*	37.94 ± 0.76	35.87 ± 0.37*	40.66 ± 2.09	29.87 ± 1.50*	29.71 ± 0.64	29.96 ± 0.39
Monounsaturated	11.40 ± 0.53	13.84 ± 1.29*	10.75 ± 0.32	12.00 ± 0.12*	11.79 ± 1.45	24.27 ± 2.55*	14.32 ± 0.81	10.53 ± 3.23
Total n6	29.24 ± 0.31	30.19 ± 0.76	34.15 ± 0.96	35.55 ± 0.87	29.95 ± 1.26	31.31 ± 0.67	31.02 ± 1.33	30.05 ± 0.04
Total n3	11.55 ± 0.66	10.96 ± 1.48	10.63 ± 0.10	10.08 ± 0.86	4.58 ± 0.84	3.89 ± 0.79	13.90 ± 0.80	13.75 ± 1.97
Total unsaturated	53.07 ± 0.30	55.96 ± 0.81*	55.29 ± 0.16	57.75 ± 0.31*	45.65 ± 1.84	59.87 ± 4.01*	58.40 ± 1.95	55.70 ± 3.08
(EPA+DPA+DHA)/(LA+ARA)	0.40 ± 0.04	0.38 ± 0.07	0.31 ± 0.03	0.28 ± 0.02	0.16 ± 0.04	0.13 ± 0.04	0.44 ± 0.04	0.47 ± 0.09
n6/n3	2.50 ± 0.16	2.95 ± 0.49	3.15 ± 0.02	3.74 ± 0.31	5.85 ± 0.59	8.25 ± 1.40	2.20 ± 0.15	2.38 ± 0.02

Note: Each value represents the mean ± SD of at least 3 independent measurements. Total saturated fat was calculated as C16:0 + C18:0 + C20:0 + C22:0 + C24:0. Total monounsaturated fat was calculated as C16:1n7 + C18:1n9 + C18:1n7 + C20:1n9 + C24:1n9. Total n6 was calculated as C18:2n6 + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6. Total n3 was calculated as C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3. Total unsaturated was calculated as total monounsaturated + total n6 + total n3. WT: wild type; TG: sdd17 transgenic mice. ND: not detected. The student's t-test was used to assess the differences in fatty acid profiles between wild type and sdd17 transgenic mice. *P < 0.05.

4.3. Serum lipoprotein analysis

The total cholesterol, triglyceride, HDL-C and LDL-C levels in the serum were also measured in 3- and 8-week-old *sdd17* mice-12 (Figure 2). Although none of the serum parameters in the *sdd17* male was altered in comparison with the non-transgenic males (p>0.05), the

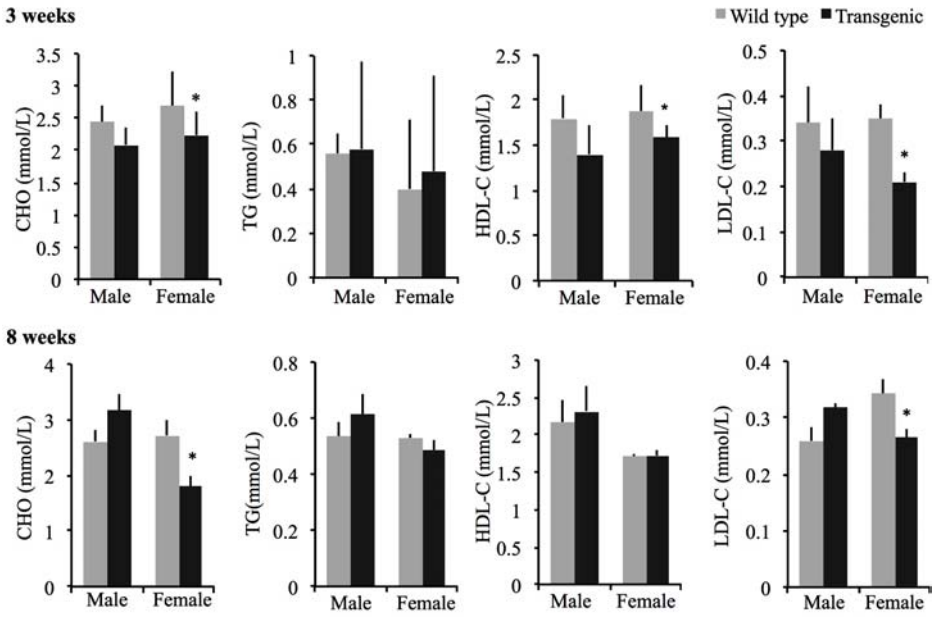

Figure 2. Comparison of serum levels of total cholesterol (CHO), triglycerides (TG), high (HDL-C) and low (LDL-C) density lipoprotein cholesterol in *sdd17* mice. The serum levels of CHO and LDL-C were significantly decreased in *sdd17* females but not in males compared to its non-transgenic (wild type) littermates at both 3 and 8 weeks of age. Each value represents the mean ± SD of measurements from five mice in each group. Statistically significant differences ($p<0.05$) are marked by asterisks within a catalogue.

serum levels of total cholesterol and LDL-C in transgenic females at both timepoints were significantly ($p<0.05$) reduced compared to those of the non-transgenic females. In contrast, the triglyceride and HDL-C levels in *sdd17* female at ages of 3 or 8 weeks were not significantly ($p>0.05$) altered compared with the non-transgenic samples (Figure 2), except the HDL-C content at 3 weeks.

5. DISCUSSION

Mammals are unable to synthesize PUFAs from LA (C18:2n6) or ALA (C18:3n3) substrates because their cells lack the delta-12 and delta-15 desaturases that are necessary to synthesize PUFAs (19). Furthermore, n3 and n6 PUFAs are not inter-convertible in mammalian cells. Previous studies proved that the delta-12 and -15 desaturases from plants (12, 20) and *C. elegans* (11, 21) could be expressed functionally in mammals and resulted in subsequent changes in fatty acid profiles. For instance, the spinach *fad2*-3 gene driven by an adipocyte-specific P2 promoter that converts endogenous oleic acid (C18:1n9) to LA was constitutively expressed in transgenic pigs, resulting in 20% increase of LA in white adipose tissue after feeding with a high-oleic-acid diet (12). The *fat-1* gene from *C. elegans*, which converts endogenous LA to ALA was also used to produce transgenic animals and strongly converted n6 to n3 PUFAs in these animals. The ratio of n6 to n3 in various tissues was to lowered from 20-50 to almost 1 in transgenic mice (11) and from 10-21 to 1-11 in transgenic pigs (21). Previous studies *in vitro* have demonstrated that *sdd17*, a delta-15 desaturase from the fungus *Saprolegnia*, can increase C20:4n3 and C20:5n3 content in yeast (13). We also found that the levels of ARA decreased, while EPA increased significantly in transformed 3T3 cell lines (14). In the current study, we have not found evidence of decreased ARA or increased EPA in 3- or 8-week-old tissues, although RT-PCR indicated that the gene was expressed in various tissues. Furthermore, no significant change in the composition of a single fatty acid was been detected in any of the examined transgenic tissues. Thus, it is difficult to clarify the delta-15 activity of *sdd17* based on the available data. However, it was interesting to note that the total unsaturated fatty acid levels of 3- or 8-week-old transgenic tissues were significantly increased. At the same time, the total saturated fatty acids of 8-week-old tissues were decreased.

Some studies had been carried out to investigate the effects of replacing saturated fatty acids with unsaturated fatty acids on serum lipid and lipoprotein levels and found that the MUFAs in foods can lower harmful LDL-cholesterol in humans (22, 23). Further, diets high in LA or ALA, compared to the average diet, decreased serum total cholesterol, LDL-C, and TG (24). Similarly, replacing snacks that are high in SFA or trans-fatty acids with snacks that are high in PUFA reduced LDL-C concentration, TG, and CHO (25). It is sometimes difficult to clarify these results because most of the data from animal experiments or clinical trials is based on PUFA-rich food and traces (or higher amounts) of other fatty acids in these foods or specific combinations of fatty acids may have affected the results. Confounding factors within a diet often cause bias or inaccurate results. This *sdd17* transgenic model exhibited a decrease in total unsaturated fatty acids in all examined tissues and organs. Thus, it will allow well-controlled studies to investigate the relationship between

health and fatty acid profiles, especially unsaturated fatty acids, without the need for diet-based interventions. In the western diet, meat and milk contribute significantly to the dietary saturated fat and cholesterol contents. However, the proper levels of unsaturated fatty acids are essential for maintaining health. Dietary fat affects the serum concentration of total cholesterol and lipoprotein cholesterol. In the current study, because the *sdd17* mice exhibited low levels of saturated fatty acids and high levels of total unsaturated fatty acids, we further examined the probable changes in total cholesterol, triglyceride, and serum lipoprotein cholesterol levels. As expected, the serum levels of CHO and LDL-C in *sdd17* juvenile and adult females were significantly decreased. This confirms that the low levels of CHO and LDL-C in the serum were attributed to higher endogenous unsaturated fatty acids. Similar results have been reported in humans; i.e., total plasma cholesterol decreased significantly in individuals with MUFA- and PUFA-rich diets. For instance, serum levels of CHO and LDL-C in people from Mediterranean countries were relatively low due to higher monounsaturated fatty acid intakes (26), leading to low rates of coronary heart disease. In addition, increased monounsaturated fatty acid intake lowered LDL-C but did not affect TG and HDL-C in humans (27). In the present study, no changes in triglyceride and HDL-C levels were observed in the *sdd17* female mice. Similar results have also been found in humans. i.e., triglyceride and HDL-C levels did not change significantly in individuals with MUFA- and PUFA-rich diets (27, 28). Interestingly, changes in serum total cholesterol and LDL-C were only observed in *sdd17* females but not in males. It is possible that fatty acid profiles have different effects in males and females. Recently, a human study in Japan demonstrated that healthy female volunteers were a fish-diet intervention (n3 PUFA 3.0 g/day) showed an increase in serum adiponectin, but this result was not observed in men; at the same time, the serum n3 PUFA increased more in female subjects than male subjects (29).

In the current study, we found that the functional expression of *sdd17* induced an increase of total unsaturated fatty acid in various tissues, and a subsequent decrease of total cholesterol and LDL-C in serum, in transgenic mice. These results suggest that endogenous *sdd17* expression is beneficial for mammalian health.

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Abbreviations: FAs: fatty acids; SFAs: saturated fatty acids; MFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; SA: stearic acid; OA: oleic acid; LA: linoleic acid; ALA: alpha linolenic acid; ARA: arachidonic acid; EPA: eicosapentanoic acid; DHA: docosahexaenoic acid; TG: triglyceride; CHO: total cholesterol; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol

Key Words: Desaturase, *sdd17*, Transgenic, Mouse, Fatty Acids, Cholesterol, Lipoprotein

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