Transgenic Mice Convert Carbohydrates to Essential Fatty Acids

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation
Pai, Victor J., Bin Wang, Xiangyong Li, Lin Wu, and Jing X. Kang. 2014. “Transgenic Mice Convert Carbohydrates to Essential Fatty Acids.” PLoS ONE 9 (5): e97637. doi:10.1371/journal.pone.0097637. http://dx.doi.org/10.1371/journal.pone.0097637.

Published Version
doi:10.1371/journal.pone.0097637

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:12406960

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Transgenic Mice Convert Carbohydrates to Essential Fatty Acids

Victor J. Pai¹*, Bin Wang¹*, Xiangyong Li¹, Lin Wu², Jing X. Kang¹*

¹ Laboratory for Lipid Medicine and Technology (LLMT), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States of America
² Cutaneous Biology Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States of America

Abstract

Transgenic mice (named “Omega mice”) were engineered to carry both optimized fat-1 and fat-2 genes from the roundworm Caenorhabditis elegans and are capable of producing all essential fatty acids from saturated fats or carbohydrates. When maintained on a high-saturated fat diet lacking essential fatty acids or a high-carbohydrate, no-fat diet, the Omega mice exhibit high tissue levels of both omega-6 and omega-3 fatty acids, with a ratio of ~1:1. This study thus presents an innovative technology for the production of both omega-6 and omega-3 essential fatty acids, as well as a new animal model for understanding the true impact of fat on human health.

Introduction

There are several classes of fats that differentially affect human health, such as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), including both essential omega-6 (n-6) and omega-3 (n-3) PUFA. Recent research has shown that our health is impacted not just by the quantity of fat consumed, but more significantly by the types of fat consumed [1],[2]. For example, the worldwide trend of increased SFA and n-6 PUFA intake and decreased n-3 PUFA intake has coincided with the growing prevalence of chronic diseases, such as heart disease, cancer, Alzheimer’s, and diabetes [2].

Normally, mammals readily obtain SFA from either the diet or endogenous synthesis from glucose or amino acids [3], and MUFA can also be obtained from the diet or converted from SFA by the stearoyl-CoA desaturase-1 (SCD-1) gene [4]. On the other hand, n-6 and n-3 PUFA cannot be inter-converted or synthesized de novo in mammals and are mainly acquired through the diet [5]. The primary essential fatty acids in the diet are n-6 linoleic acid (18:2n-6, LA) and n-3 ω-linolenic acid (18:3n-3, ALA), which can be converted into longer-chain n-6 PUFA arachidonic acid (20:4n-6, AA) and n-3 PUFA eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), respectively, through a series of desaturation and chain-elongation enzyme systems [5]. The metabolites derived from n-6 and n-3 PUFA, namely eicosanoids, are functionally distinct and have opposing physiological effects [6]. For example, n-6-derived eicosanoids generally promote inflammation, while n-3-derived eicosanoids have anti-inflammatory properties. Since the synthesis of n-6 and n-3 long-chain PUFA and their metabolites compete for the same enzymes, their ratio in body tissues determines the eicosanoid profile. Recent studies have indicated that the tissue n-6/n-3 PUFA ratio plays an important role in the pathogenesis of many chronic diseases [2],[7]. Many global health organizations now recommend an increased daily intake of n-3 PUFA, particularly EPA and DHA, but these n-3 PUFA are largely limited to marine sources (such as fish and algae) that can be less affordable or accessible [8]. Thus, it is often challenging to balance the different types of dietary fat in a way that optimizes health.

Unlike mammals, the roundworm C. elegans is capable of producing all classes of fatty acids as they possess the required genes. For example, the fat-2 gene encodes a desaturase that catalyzes the conversion of MUFA to n-6 PUFA, and the fat-1 gene enables the conversion of n-6 to n-3 PUFA [9]. Our laboratory previously created the fat-1 transgenic technology to express the fat-1 gene in mammals (both mice and livestock), which successfully demonstrated the conversion of dietary n-6 to n-3 PUFA and exhibited a balanced n-6/n-3 PUFA ratio in body tissues, regardless of diet [10],[11]. Our earlier work generated a unique fat-1 mouse model for n-3 PUFA research and also provided a sustainable, land-based strategy for the production of n-3 PUFA from n-6PUFA.[12],[13],[14].

The objective of this study was to generate a novel transgenic mouse model that can endogenously synthesize all essential fatty acids. Our strategy was to first create a fat-2 transgenic mouse, possessing the C. elegans fat-2 gene encoding an enzyme that converts MUFA into n-6 LA [9], and then cross the fat-2 transgenic mice with fat-1 transgenic mice, which we generated previously to possess the C. elegans fat-1 gene, encoding an enzyme that converts n-6 to n-3 PUFA [10]. Through this procedure, we can generate a compound fat-1/fat-2 transgenic mouse – hereafter
Transgenic Production of Essential Fatty Acids

A

Glucose → SFA → MUFA → **Fat-2** → n-6 PUFA → **Fat-1**

B

WT  Fat-2  Omega

Fat-2

Fat-1

C

WT

Fat-2

Omega

D

Percentage of total fatty acids

WT  Fat-2  Omega

Total PUFA  n-6 PUFA  n-3 PUFA

Omega-6/Omega-3 Ratio

WT  Fat-2  Omega

***
referred to as the “Omega” mouse – that is capable of producing both n-6 and n-3 PUFA from a diet containing only saturated fat or carbohydrates (Figure 1A).

Results and Discussion

To enable the higher expression of the *C. elegans* fat-2 gene in mammals, we first optimized the codon of the fat-2 sequence based on the mammalian desaturase sequence (Figure S1) and utilized a chicken beta-actin promoter to build a transgene construct (Figure S2), and then used microinjection to create the fat-2 transgenic mouse. After the fat-2 phenotype and genotype were confirmed, the heterozygous fat-2 transgenic mice were backcrossed with C57BL6 wild-type (WT) mice for 5 generations, and then crossed with heterozygous fat-1 transgenic mice. The resulting offspring consisted of WT, fat-1, fat-2, and Omega mice.

The fat-1 mouse phenotype has been reported previously [7],[10],[12]. Expression of the fat-1 and fat-2 transgenes was validated by PCR, confirming that WT mice do not express any of the transgenes, the fat-2 mice carry only the fat-2 transgene, and the Omega mice express both the fat-1 and fat-2 transgenes (Figure 1B).

When WT, fat-1, fat-2, and Omega mouse littermates were fed the same diet high in saturated fat and carbohydrates and low in n-6 PUFA (Table S1), phenotype validation by gas chromatography (GC) revealed four distinct tissue fatty acid profiles (Tables 1 and 2). The difference in fatty acid profiles for the new fat-2 and Omega mice are highlighted in Figure 1C and D. As expected, the WT mice exhibited high levels of saturated fat and very low levels of essential fatty acids, primarily n-6 PUFA, with an n-6/n-3 PUFA ratio of roughly 3.5. The fat-2 transgenic mice displayed a significant increase in total tissue PUFA content, with the n-6 PUFA content in the muscle tissue doubling from about 700 mg/g to 1350 mg/g due to the conversion of oleic acid into n-6 LA and AA, without much change in n-3 PUFA levels, resulting in an n-6/n-3 PUFA ratio of about 5. The Omega transgenic mice also

Table 1. Comparison of the fatty acid profiles of various tissues among the four genotypes.

| Muscle | SFA | MUFA | Total PUFA | n-6 PUFA | n-3 PUFA | n-6/n-3 |
|--------|-----|------|------------|----------|---------|---------|
| WT     | 38.96±0.98 | 42.87±1.99 | 18.19±1.29 | 14.23±1.59 | 3.96±0.48 | 3.60±0.95 |
| Fat-1  | 40.51±0.35 | 42.28±1.81 | 16.89±1.55 | 6.23±1.16 | 10.66±0.76 | 0.59±0.10 |
| Fat-2  | 41.15±2.02 | 29.71±2.13 | 29.01±0.99 | 4.29±0.93 | 4.72±0.40 | 5.18±0.52 |
| Omega  | 41.08±0.47 | 33.03±2.12 | 25.90±1.95 | 11.23±2.43 | 14.67±2.09 | 0.78±0.24 |
| Liver  | 36.70±0.83 | 48.78±1.00 | 14.44±1.32 | 11.86±1.33 | 2.57±0.07 | 4.62±0.55 |
| Omega  | 37.49±1.27 | 49.38±0.72 | 13.16±0.77 | 7.28±0.58 | 5.88±0.40 | 1.24±0.11 |
| Fat-2  | 37.33±2.25 | 33.87±3.05 | 28.82±1.55 | 25.03±1.69 | 3.78±0.29 | 6.65±0.82 |
| Omega  | 37.90±2.09 | 34.21±3.75 | 27.90±2.50 | 17.38±1.76 | 10.52±0.74 | 1.65±0.05 |
| Tail   | 39.57±1.55 | 62.39±2.19 | 8.20±0.63 | 7.27±0.41 | 0.93±0.23 | 8.07±1.38 |
| Omega  | 26.99±1.04 | 69.96±1.38 | 8.05±0.38 | 2.49±0.32 | 0.55±0.70 | 0.46±0.11 |
| Fat-2  | 33.16±3.42 | 47.65±3.55 | 19.20±1.57 | 17.85±1.50 | 1.35±0.33 | 13.81±3.87 |
| Omega  | 32.79±2.73 | 49.02±2.15 | 18.20±1.18 | 10.16±1.62 | 8.04±0.44 | 1.27±0.27 |

The four genotypes of mice were fed the same low-PUFA diet for about two months and tissue samples were subject to lipid analysis by gas chromatography. WT: Wild-type; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n-6: omega-6; n-3: omega-3; n = 3 for each group;

*P<0.05, **P<0.01, ***P<0.001.

Tables 1 and 2. Comparison of the n-6/n-3 PUFA ratio among the phenotypes (right). Values expressed as mean ± s.d. (n = 3 per group; *P<0.05, **P<0.01).
doi:10.1371/journal.pone.0097637.g001

![Figure 1A](image_url)

![Figure 1B](image_url)

![Figure 1C](image_url)

![Figure 1D](image_url)
exhibited significantly increased total PUFA content, but the tissue content of n-3 PUFA was increased by roughly five-fold compared to their WT and fat-2 littermates, due to the conversion of almost half the n-6 PUFA content into n-3 PUFA, with a markedly decreased n-6/n-3 PUFA ratio of 0.75 (Figure 1D). MUFA levels were accordingly reduced in the fat-2 and Omega mice, showing that n-6 PUFA had been converted from MUFA. No significant differences in SFA levels were observed among the phenotypes. Detailed fatty acid profiles for the four genotypes (WT, fat-1, fat-2, and Omega) are shown in Tables S2–5. Furthermore, when fed a high-carbohydrate, no-fat diet (Table S1), the fat-2 and Omega mice still exhibited significant tissue levels of n-6 and n-3 PUFA, respectively, confirming their ability to produce essential fatty acids when lacking dietary fat and given only carbohydrates (Table 2). The tissue abundance of n-6 PUFA in the fat-2 mouse and of both n-3 and n-6 PUFA in the Omega mouse, despite the diet containing little of these fatty acids, thus demonstrates the capability of our transgenic mice to produce essential fatty acids from non-essential nutrients – MUFAs, SFA, and even carbohydrates.

The Western human diet today is fundamentally different than it was throughout the majority of human evolution. Among the many shifts in dietary nutrients that have occurred over the last few decades, key changes include increases in saturated fat, carbohydrates, and n-6 PUFA intake with a decrease in n-3 PUFA intake [15,16,17]. As a result, modern humans have an n-6 to n-3 PUFA ratio that favors n-6 PUFA by as much as 20:1; evolutionarily, this ratio would have been closer to 1:1, and the discrepancy is thought to have profound physiological consequences [15,16]. This study presents a new transgenic technology that could produce essential fatty acids, especially the beneficial n-3 PUFA, in animal products (such as meat, milk, eggs, etc.) by feeding animals with just carbohydrates and/or saturated fat. Given that n-3 PUFA are largely limited to marine sources (such as fish and algae), this technology could therefore generate sustainable and accessible n-3 PUFA resources, especially where only carbohydrates are available.

The transgenic technology presented in this study will also be of great utility in elucidating the impact of essential fatty acids on health. Conventionally, dietary modification is used to investigate the effects of different fatty acid profiles, which requires feeding animals with different diets. However, this method is problematic since the diets may not only contain different fatty acid compositions, but also variations in impurities, flavor, calories, or other components used between study groups, ultimately leading to confounding factors that complicate interpretation of results. Our transgenic technology allows us to create four different tissue fatty acid profiles by using a single diet, which makes it possible to evaluate the true health effects of different fats without the confounding factors of diet. This is very important for identification of metabolic biomarkers related to lipid metabolism.

This study clearly demonstrates the feasibility of producing mammals with the capability to convert SFA and MUFA to the essential n-6 and n-3 PUFA. The transgenic mice created by this project will serve as unique animal models, free of dietary confounding factors, for the reliable study of the biological effects of different fatty acid profiles. Ultimately, this transgenic technology serves as a new biotechnology for the production of essential fatty acids, especially n-3 EPA and DHA, to meet increasing demand.

Materials and Methods

Ethics statement

All animal procedures in this study were reviewed and approved by the Massachusetts General Hospital (MGH) Subcommittee on Research Animal Care (SRAC).

Codon optimization

In order to efficiently express a gene from the lower-life C. elegans in mammals in vivo, the codon usage by C. elegans must be adjusted to match those used by mammals. The fat-2 gene sequence was obtained from the gene bank (GenBank accession number NM_070159). We used mammalian desaturases as well as our previously optimized fat-1 gene as references to determine the differences in codon usages of desaturases between C. elegans and mammals. We then manually adjusted the codon to achieve over 90% optimization (Figure S1).

Gene synthesis

After modifying the gene sequence to optimize the codon usage, the fat-2 gene was synthesized by GenScript (Piscataway, NJ). The gene was synthesized to be flanked with EcoR I digestion sites and delivered on pUC57 plasmid. After amplification, the fat-2 sequence was inserted into a pCAGGS expression plasmid containing the chicken beta-actin promoter and cytomegalovirus enhancer (kindly provided by Dr. J Miyazaki, Osaka University Medical School). After ligation, the orientation was confirmed and the plasmid was amplified. Finally, the fragment containing the

### Table 2. Fatty acid profile of tail tissue from mice fed with a high-carbohydrate, no-fat diet.

|        | SFA      | MUFA    | Total PUFA | n-6 PUFA | n-3 PUFA | n-6/n-3 |
|--------|----------|---------|------------|----------|----------|---------|
| WT     | 28.8±5.7 | 64.0±8.1| 7.06±0.89  | 6.4±0.75 | 0.66±0.14 | 9.93±1.2 |
| Fat-1  | 28.2±0.3 | 65.6±1.0 | 6.52±0.74  | 2.32±0.38 | 4.06±0.66 | 0.59±0.16 |
| Fat-2  | 27.78±1.5 | 57.14±2.0 | 15.1±0.65  | 14.44±0.74 | 0.65±0.09 | 22.44±3.82 |
| Omega  | 29.09±3.4 | 56.98±2.65 | 13.97±0.78 | 8.82±0.73 | 5.16±0.28 | 1.71±0.18 |

The four genotypes of mice were fed the same no-fat diet for about two months and tail tissue was subject to lipid analysis by gas chromatography. WT: Wild-type; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n-6: omega-6; n-3: omega-3; n = 3 for each group; *(Fat-1 vs Fat-2), **(WT vs Fat-1), ***(WT vs Fat-2), ****(WT vs Omega), *****(Fat-1 vs Fat-2), ***(Fat-1 vs Omega), One symbol = P<0.05, Two symbols = P<0.01.

doi:10.1371/journal.pone.0097637.t002
promoter, the fat-2 sequence, and the polyA sequence was excised with SpI and Sfi I for microinjection (Figure S2).

**Microinjection**

Transgenic mouse lines were produced at the MGH Transgenic Core Facility by injecting the purified SpI and Sfi I fragment into fertilized eggs from C57BL/6 X C3H mice. The fertilized eggs containing the transgene were transferred to pseudo-pregnant mice (B6CF1) to produce transgenic mice. The founder transgenic mice were then subjected to genotyping and phenotyping.

**Genotyping and phenotyping**

Genotyping was carried out by removing the tip of the tail to acquire a DNA sample for RT-PCR, which was performed with the following primers: fat-2 forward, GCGGCCA GACCCA-GACCATC; and fat-2 reverse, GGGCGAC GTGACCGTTGGTA. PCR products were run through gel electrophoresis on 2% agarose gel. Phenotyping by fatty acid composition analysis using gas chromatography (GC) was performed as previously described [18]. The fat-2 mice were maintained after weaning on a low-PUFA diet (Table S1). GC was carried out after 20 days on the diet to allow for clearer phenotypes. Tissue samples were ground to powder under liquid nitrogen and total lipids were extracted using chloroform/methanol (2:1, v/v). Fatty acids were then methylated by heating them at 100°C for 1 hour under 14% boron trifluoride (BF3)-methanol reagent (Sigma-Aldrich, St. Louis, MO) and hexane (Sigma-Aldrich). Fatty acid methyl esters were analyzed by GC using a fully automated 6890N Network GC System (Agilent Technologies, Santa Clara, CA) equipped with a flame-ionization detector and an Omegawax 250 capillary column (30 m x 0.25 mm ID). Fatty acid standards (Nu-chek-Prep, Elysian, MN) were used to identify peaks of resolved fatty acids, and area percentages for all resolved peaks were analyzed using GC ChemStation Software (Agilent). The fatty acid C23:0 (20 μg/sample) was used as an internal standard to calculate the amount of each fatty acid measured.

After identifying the genotype and phenotype, the fat-2 mice were mated with WT C57BL/6 mice to create the F1 generation. The F1 generation was then backcrossed with WT C57BL/6 mice at least 5 times in order to verify that the gene is transmissible as well as to establish a pure background, so that fat-2 lines could be maintained with a significant phenotype. Each generation was subjected to genotyping by RT-PCR and phenotyping by GC.

**Generation of Omega mice**

The compound fat-1/fat-2 transgenic mice were created by crossbreeding heterozygous fat-2 transgenic mice with heterozygous fat-1 transgenic mice, which were previously generated by our group [10]. After weaning, the offspring were maintained either on low-PUFA diet or a no-fat diet (Table S1), and then genotyped by RT-PCR and phenotyped by GC. Genotyping by RT-PCR of the Omega mice was carried out with the following primers: fat-1 forward, TGTTCAACTGCTTTCCTCTTTTCTCC; fat-1 reverse, GGCACCATTGGATAACATTTGGA; fat-2 forward, GGGCGAC GACCCAGACCATC; fat-2 reverse, GGGCGAC GTGACCGTTGGTA. Phenotyping by fatty acid composition analysis using GC was performed as previously described [18].

**Statistical analysis**

GraphPad Prism 5 (GraphPad Software, San Diego, CA) was used for all statistical analyses. Data sets were analyzed by F-test to verify normal distribution. One-way ANOVA followed by the Tukey test was used to determine statistical significance, set at *P<0.05 and **P<0.01.

**Supporting Information**

Figure S1 Optimized fat-2 sequence. (DOC)

Figure S2 The pCAGGS plasmid and fat-2 construct for microinjection. (DOC)

Table S1 Composition of the low-PUFA diet and the no-fat diet. (DOC)

Table S2 Comparison of the muscle fatty acid profile of mice fed with a low-PUFA diet among the four genotypes. (DOC)

Table S3 Comparison of the liver fatty acid profile of mice fed with a low-PUFA diet among the four genotypes. (DOC)

Table S4 Comparison of the tail fatty acid profile of mice fed with a low-PUFA diet among the four genotypes. (DOC)

Table S5 Comparison of the tail fatty acid profile of mice fed with a non-fat diet among the four genotypes. (DOC)

**Acknowledgments**

The authors are grateful to Marina Kang and Jason Kang for editorial assistance.

**Author Contributions**

Conceived and designed the experiments: JXK. Performed the experiments: VJP BW XYL LW. Analyzed the data: XYL VJP BW JXK. Wrote the paper: JXK.

**References**

1. Joint WHO/FAO Expert Consultation (2003) Diet, Nutrition and the Prevention of Chronic Diseases (WHO technical report series 916). Geneva: World Health Organization. pp. 81–94.

2. Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 233: 674–688.

3. Volpe JJ, Vagelos PR (1976) Mechanisms and regulation of biosynthesis of saturated fatty acids. Physiol Rev 56: 339–417.

4. Paton CM, Ntambi JM (2009) Biochemical and physiological function of stearoyl CoA desaturase. Am J Physiol Endocrinol Metab 297: E29–E37.

5. Leonard AE, Pereira SL, Sprecher H, Huang YS (2004) Elongation of long-chain fatty acids. Prog Lipid Res 43: 36–54.

6. Kang JX, Weylandt KH (2008) Modulation of inflammatory cytokines by omega-3 fatty acids. Subcell Biochem 49: 133–143.

7. Kang JX (2011) The omega-6/omega-3 fatty acid ratio in chronic diseases: animal models and molecular aspects. World Rev Nutr Diet 102: 22–29.

8. Gebauer SK, Piota TL, Harris WS, Kris-Etherton PM (2006) n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr 83: 1526s–1535s.

9. Watts JL, Browse J (2002) Genetic dissection of polyunsaturated fatty acid synthesis in Caenorhabditis elegans. Proc Natl Acad Sci U S A 99: 5854–5859.

10. Kang JX, Wang J, Wu L, Kang ZB (2004) Transgenic mice: fat-1 mice convert n-6 to n-3 fatty acids. Nature 427: 504–506.

11. Lai L, Kang JX, Li R, Wang J, Witt WT, et al. (2006) Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nature Biotechnology 24: 435–436.

12. Kang JX (2007) Fat-1 transgenic mice: A new model for omega-3 research. Prostaglandins Leukot Essent Fatty Acids 77: 263–267.
13. Kang JX, Leaf A (2007) Why the omega-3 piggy should go to market. Nature Biotechnology 25: 505–506.
14. Kang JX (2011) Omega-3: a link between global climate change and human health. Biotechnol Adv 29: 388–390.
15. Leaf A, Weber PC (1987) A new era for science in nutrition. Am J Clin Nutr 45: 1048–1053.
16. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, et al. (2005) Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr 81: 341–354.
17. Institute of Medicine of the National Academies (2002) Dietary fats: total fat and fatty acids. In: Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). Washington, DC: The National Academy Press, pp. 333–432.
18. Kang JX, Wang J (2005) A simplified method for analysis of polyunsaturated fatty acids. BMC Biochem 6: 3.