J. Membrane Biol. 206, 165–172 (2005)
DOI: 10.1007/s00232-005-0790-3

The Journal of
Membrane
Biology

From Fat to Fat-1: A Tale of Omega-3 Fatty Acids

J.X. Kang
Departments of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA

Received: 15 September 2005

Introduction

Omega-3 (or n-3) fatty acids are fats found within foods and body tissues. They are unique because of the following characteristics: Structurally they appear as a long hydrocarbon chain (18 or more carbons), containing three or more (up to six) double bonds with the first double bond occurring on the 3rd carbon atom (from the methyl end). These fatty acids are termed essential fatty acids because they cannot be produced by the body (whether animal or human) and must be supplied by the diet for good health. The source of n-3 fatty acids is limited. Unlike other fatty acids, which are widely available in foodstuff, these n-3 fatty acids are primarily found in fat fish, certain vegetables and nuts. Functionally, n-3 fatty acids can exert a wide range of effects on cell function. In addition to being a source of energy production, these fatty acids can act as determinants of the physiochemical properties of cell membranes; as substrates for the production of signaling molecules or functioning mediators, and as modulators in the regulation of gene expression. Therefore, n-3 fatty acids can profoundly affect the physiological activity and pathological process through different mechanisms.

The content of n-3 fatty acids in the human diet underwent a dramatic change during evolution and civilization of human beings (Leaf & Weber, 1987). The foods available to our ancestors were quite different from what we eat today. It is believed that the ''ancient'' foods were rich in n-3 fatty acids and had a balance between n-6 and n-3 fatty acids (i.e., n-6/n-3 ratio $\approx$ 1:1). Since such a fatty acid profile existed for so long during evolution, the human (animal) body could establish its genetic pattern based on that condition (loss or gain of fatty acid-related genes might occur to adopt that lipid profile). Under that dietary regime, there was no need of genes that synthesize these fatty acids or convert one to another. Today, the situation is quite different. Deviation began $\sim$10,000 to 15,000 years ago with the adoption of agriculture and animal husbandry, mainly of ruminants. The modern agriculture with dependence on diets of grains led to an increase in total saturated fatty acids and in the n-6 polyunsaturated fatty acids, linoleic and arachidonic acids. In the past century the industrial revolution, the emergence of agribusiness with processed foods, grain-fattened livestock, and hydrogenation of vegetable fats have all further reduced the content of n-3 fatty acids and increased n-6 fatty acids. Consequently, the modern Western diets are deficient in n-3 fatty acids, but have too much of the n-6 fatty acids, with an n-6/n-3 fatty acid ratio of 15–20:1 (Simopoulos, 2000). Obviously, this ratio is contradictory to our genetic profile that was established based on a 1:1 ratio. Unfortunately, our body cannot adjust its gene profile in such a short period of time to adapt to the new ratio.

The shift in n-6/n-3 fatty acid ratio, especially the deficiency of n-3 fatty acids, might have imposed a risk of modern diseases (e.g., cardiovascular disease, cancer, etc.) and thereby a serious threat to public health (Leaf & Weber, 1987; Simopoulos, 2000). This is evidenced by thousands of laboratory and human studies showing that deficiency of n-3 fatty acids is associated with an increased risk of several major diseases and that supplementation with n-3 fatty acids exhibits beneficial effects on numerous clinical problems (Simopoulos, 1999; Connor, 2000). Among many health benefits of n-3 fatty acids, their cardioprotective, anti-inflammatory, anticancer and neuroprotective effects have been most intensively investigated and are now becoming recognized. (I am not going to review these in detail, as other review articles in this issue or elsewhere cover them). Notably, the most important effect of n-3 fatty acids is prevention of sudden cardiac death mainly through their antiarrhythmic action (Leaf & Kang, 1998;

Correspondence to: J.X. Kang; email: jxkang@partners.org

O'Keefe & Harris, 2000). (The details of this effect are described in Dr. Leaf's paper in this issue.) I was very fortunate to have the opportunity to work with Dr. Alexander Leaf on the antiarrhythmic project and have learned so much about the benefits of n-3 fatty acids over the last ten years. Through a series of experiments ranging from molecular level to human trial, our studies have documented the efficacy of the protective effect of n-3 fatty acids against arrhythmias induced by various drugs/agents or by ischemia, and demonstrated that the protective actions result from modulation and stabilization of the electrical activity of the heart cells (Kang & Leaf, 1996, and *see* review by Leaf et al.). In light of the growing evidence for the cardioprotective effects of n-3 fatty acids, the American Heart Association has recommended increased intake of fish for good heath and use of fish oil supplements for patients with documented coronary heart disease (Kris-Etherton, Harris & Appel, 2002).

The available sources of n-3 fatty acids in our diets are from marine vertebrates, but stem from the ability of single-cell phytoplankton and algae to convert the parent n-6 fatty acid, linoleic acid, to the parent n-3 fatty acid, α-linolenic acid, which enters the food chain of marine life and is further elongated and desaturated to produce the fish oil fatty acids EPA and DHA (Leaf & Weber, 1987). As sources of edible fish in the oceans are being depleted by overfishing and the market price of fish keeps rising, where the dietary n-3 fatty acids will come from to meet the growing demand in the future is a question.

In short, the reality we are facing is: on the one hand, the demand for n-3 fatty acids is growing because of their health benefits, but the source is limited; on the other hand, n-6 fatty acids are highly abundant in our food and too much in our body, but we are unable to convert n-6 to n-3 fatty acids because humans as well as most animals (including livestock) do not have a gene for accomplishing that feat. So, what can we do about it? The current practice to enrich tissues with n-3 fatty acids and balance the n-6/n-3 ratio is to feed animals with exogenous n-3 fatty acids (e.g., fish meal or other marine products), which is unsustainable.

These existing problems prompted me to wonder whether we can use biotechnology to enable mammalian cells and animals to produce n-3 fatty acids from the n-6 type. Since some lower life such as plants, microorganisms and *C. elegans* are able to convert n-6 to n-3 fatty acids, and some genes responsible for the conversion have been cloned (Spychalla, Kinney & Browse, 1997), my idea was to transfer a converting enzyme gene from these species to mammals. The gene we used was *fat-1* from the roundworm *C. elegans*. It encodes an n-3 fatty acid desaturase that can introduce a double bond into n-6 fatty acids at the n-3 position of their hydrocarbon chains to form n-3 fatty acids (*see* Fig. 1 for illustration). We tested our idea first in cultured cells followed by generation of transgenic animals.

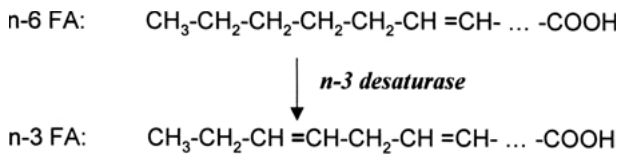

Fig. 1. Conversion of n-6 fatty acids (FA) to n-3 fatty acids by an n-3 desaturase that does not exist in mammalian cells. The n-3 desaturase can catalyze introduction of a double bond into n-6 fatty acids at the n-3 position of their hydrocarbon chains to form n-3 fatty acids.

Transgenic Cells (in Vitro Studies)

In order to introduce the *fat-1* gene into mammalian cells efficiently, we used a virus-mediated gene transfer strategy. We constructed a recombinant adenovirus (Ad.GFP *fat-1*) carrying both the *fat-1* gene and the GFP gene (green fluorescent protein gene) and another adenovirus (Ad. GFP) carrying the GFP gene alone (as control) and used them to infect various mammalian cells, including heart cells, neurons, endothelial cells and human cancer cell lines Kang et al., 2001; Ge et al., 2002a, b; Meiler et al., 2002; Xia, Wang & Kang, 2005).

Following the virus-mediated gene transfer, cellular lipids were extracted and fatty acid composition was analyzed by gas chromatography to determine if the expression of the *fat-1* gene in mammalian cells could change their lipid profile. Our results showed that the fatty acid profiles were remarkably different between cells expressing the *fat-1* gene and control cells (Kang et al., 2001; Ge et al., 2002a, b; Meiler et al., 2002; Xia et al., 2005). In cells expressing the *fat-1* gene (omega-3 fatty acid desaturase), all types of omega-6 fatty acids were largely converted to corresponding omega-3 fatty acids, namely, 18:2n-6 to 18:3n-3, 20:2n-6 to 20:3n-3, 20:3n-6 to 20:4n-3, 20:4n-6 to 20:5n-3, 22:4n-6 to 22:5n-3 and 22:5n-6 to 22:6n-3 (Fig. 2). As a result, the contents of omega-3 fatty acids significantly increased, whereas the levels of omega-6 fatty acids decreased in the *fat-1* transgenic cells, leading to a dramatic reduction of the omega-6/omega-3 ratio from 9–15:1 in the control cells to about 1:1(Table 1) (Kang et al., 2001). Similar effects were observed in all cell types that we have tested (Ge et al., 2002a, b; Meiler et al., 2002; Xia et al., 2005). To examine whether the gene transfer-induced alteration in the ratio of n-6 to n-3 can lead to a change in the profile of eicosanoids generated by the cells, we measured the production of prostaglandin E_2 (PGE_2), one of the major eicosanoids derived from 20:4n6 (AA), in the fat-1 and control cells by using an enzyme immunoassay (Kang et al, 2001; Ge et al.,

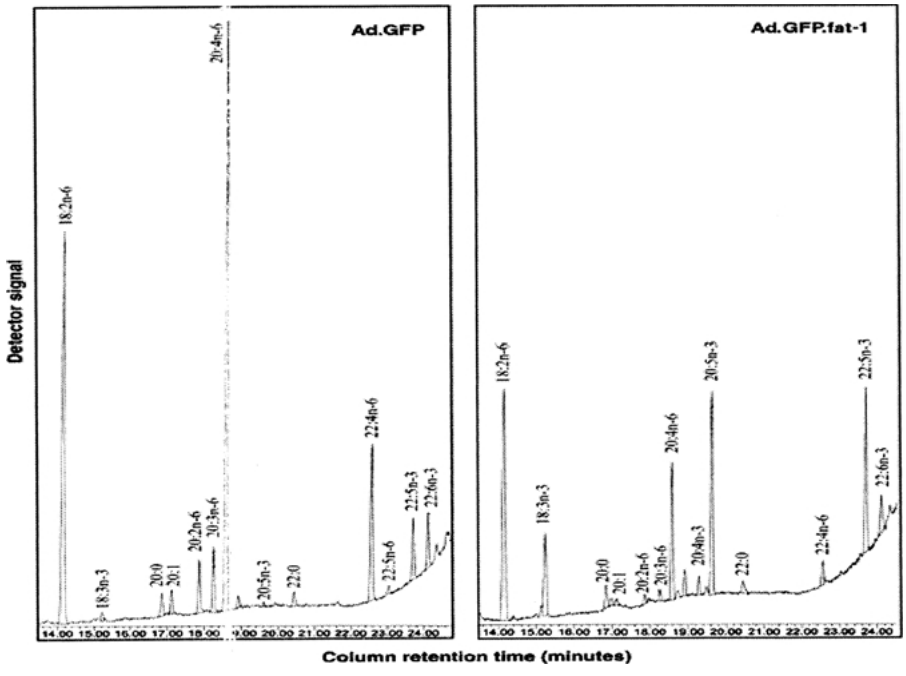

Fig. 2. Partial gas chromatograph traces showing fatty acid profiles of total cellular lipids extracted from cultured neonatal rat cardiac myocytes infected with Ad.GFP (control) and myocytes infected with Ad.GFP.*fat-1*.

Table 1. Polyunsaturated fatty acid composition of total cellular lipids from the control heart cells and the transgenic cells expressing a *C. elegans fat-1* cDNA

Mol % of total Fatty acids	Control	Fat-1
n-6 Polyunsaturates		
18:2n-6	14.2[a]	9.2[b]
20:2n-6	1.2[a]	0.3[b]
20:3n-6	1.6[a]	0.4[b]
20:4n-6	15.2[a]	4.1[b]
22:4n-6	4.4[a]	1.0[b]
22:5n-6	0.2[a]	0.0[b]
Total	36.8[a]	15.0[b]
n-3 Polyunsaturates		
18:3n-3	0.2[b]	3.6[a]
20:4n-3	0.0[b]	0.6[a]
20:5n-3	0.1[b]	6.1[a]
22:5n-3	1.2[b]	5.8[a]
22:6n-3	1.0[a]	1.3[a]
Total	2.5[b]	17.4[a]
n-6/n-3 Ratio	14.7[a]	0.8[b]

Values are means of four measurements. Values for each fatty acid with the same letter do not differ significantly ($P < 0.01$) between control and fat-1.

2002a, b; Meiler et al., 2002). We found that the amount of prostaglandin E_2 produced by the cells expressing the *fat-1* gene was significantly lower than that produced by the control cells (30–50% reduction) (Kang et al, 2001; Ge et al., 2002a, b;. Apparently, this genetic approach is highly effective in balancing the cellular n-6/n-3 fatty acid ratio and in modifying the generation of eicosanoids.

In comparison with supplementation, the gene transfer technology is superior because it not only enhances the absolute quantity of n-3 PUFA but also simultaneously decreases the level of n-6 PUFA. Unlike the supplementation with exogenous fatty acids, this genetic approach needs no incorporation of exogenous fatty acids into cells to alter the n-6/n-3 ratio and therefore does not change the total amount of cellular fatty acids (i.e., no difference in lipid mass between treated cells and control cells). Thus, the transgenic cells created by this technology can serve as a unique model for elucidating the significance of the ratio of n-6 to n-3 PUFA.

Next, we determined whether the gene transfer-induced change in the omega-6/omega-3 ratio would provide the beneficial effects of omega-3 fatty acids as observed with fatty acid supplementation. Our previous studies have demonstrated an antiarrhythmic effect for omega-3 fatty acids when supplemented to cardiac myocytes (Kang & Leaf, 1996). To see whether the gene transfer can provide a similar protective effect, neonatal rat cardiac myocytes expressing the *fat-1* gene were tested for their susceptibility to arrhythmias induced by arrhythmogenic agents, such as high concentrations of extracellular calcium. As shown in Fig. 3, when challenged with a high [Ca^{2+}] (7.5 mM), the control cells promptly exhibited arrhythmia characterized by spasmodic contractures and fibrillation, whereas the cells expressing the *fat-1* gene sustained regular beating (resistant to the arrhythmogenic stimulus), similar to the effect of omega-3 fatty acid supplementation (Kang & Leaf, 1996). This suggests that gene transfer of the omega-3 desaturase into heart cells can provide the antiarrhythmic effect of omega-3 fatty acids.

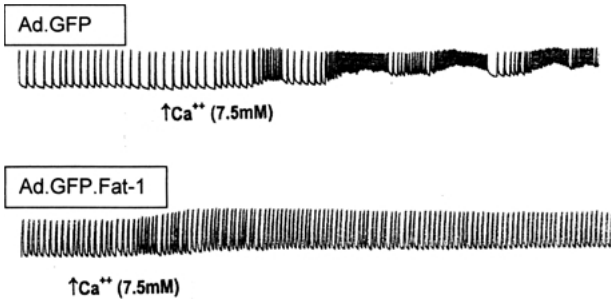

Fig. 3. Effect of expression of the *C. elegans* n-3 fatty acid desaturase in cardiac myocytes on their susceptibility to arrhythmia. Cultured (spontaneously beating) neonatal rat cardiac myocytes infected with Ad.GFP (control) or Ad.GFP.fat-1 were challenged with 7.5 mM extracellular calcium. The control cells promptly exhibited arrhythmia (spasmodic contractures and fibrillation), whereas the *fat-1*cells sustained regular beating.

A number of human cancer cell lines have been tested for their responses to the expression of *fat-1* gene. In human breast cancer cells (MCF-7), gene transfer of the omega-3 desaturase resulted in reduction of both the cellular omega-6/omega-3 fatty acid ratio from 12.0 to 0.8 and the level of PGE_2 by about 40%, leading to an increase in apoptotic cell death and a decrease in cell proliferation (Ge et al., 2002a). As shown in Fig. 4, a large number of the cancer cells expressing *fat-1* gene underwent apoptosis, as indicated by morphological changes (small size with round shape or fragmentation) and nuclear staining (bright spots). Statistical analysis of apoptotic cell counts showed that 30–50% of the cells infected with Ad.GFP.*fat-1* cells were apoptotic, whereas only 10% dead cells were found in the control cells (infected with Ad.GFP). A cell proliferation assay indicated that proliferative activity of cells infected with Ad.GFP.*fat-1* was significantly lower than that of cells infected with Ad.GFP. Accordingly, the total number of viable cells in the cells infected with Ad.GFP.*fat-1* was about 30% less than that in the control cells. In addition, DNA microarray assays showed that the gene transfer-induced change in omega-6/omega-3 fatty acid ratio resulted in a down-regulation of a number of genes involved in cell proliferation, adhesion, angiogenesis and invasion, and in an up-regulation of apoptosis-inducing genes in MDA-MB-231 cells (*unpublished data*). In human lung cancer A549 cells (Xia et al., 2005), reduction of cellular omega-6/omega-3 fatty acid ratio from 5 to 1 as a result of the expression of *fat-1* cDNA led to cell growth arrest and, more importantly, reduced invasive potential as evidenced by a decrease in cell adhesion, migration and expression of invasion-related genes (Fig. 5). These results are consistent with the reported anti-cancer effects of omega-3 fatty acid supplementation (Bougnoux, 1999; Cave, 1997; Rose & Connolly, 1999).

In primary culture of human umbilical vein endothelial cells (HUVEC), expression of *fat-1* significantly reduced the omega-6/omega-3 fatty acid ratio from about 9 to 1 (Meiler et al., 2002). This change in cellular omega-6/omega-3 ratio led to a decrease in the surface expression of adhesion molecules (markers of inflammation). The quantity of the adhesion molecules (as determined by immunoassay), E-Selectin, ICAM-1, and VCAM-1 was reduced by 42%, 43%, and 57%, respectively, in response to cytokine exposure (TNF-α 5 U/ml, 4 h) (Meiler et al., 2002). We then examined whether changes in the adhesion molecule profile were sufficient to alter endothelial interactions with monocytes, the most prevalent white blood cell type found in atherosclerotic lesions. Under laminar flow and a defined shear stress of $\sim$ 2 dynes/cm^2, *fat-1* compared to control vector-infected HUVEC supported $\sim$ 50% less firm adhesion with almost no effect on the rolling interactions of THP-1 cells (Meiler et al., 2002). These results indicate that expression of the *fat-1* gene in HUVEC inhibits cytokine induction of the endothelial inflammatory response and firm adhesion of monocytes, suggesting that a balanced omega-6/omega-3 fatty acid ratio may have an antiatherosclerotic effect.

We have also determined the effect of *fat-1* expression on neuronal apoptosis. We found that the expression of the *fat-1* gene, which could significantly reduce the neuronal omega-6/omega-3 fatty acid ratio from 6 to 1.5 and the production of prostaglandin E_2 by 20%, resulted in protection from growth factor-withdrawal-induced apoptotic cell death of rat cortical neurons (Ge et al., 2002). Following gene transfer, apoptosis was induced by 24 h of growth factor withdrawal and detected by Hoechst staining. As shown in Fig. 6, cortical cultures infected with the Ad.GFP.*fat-1* underwent ($\sim$60%) less apoptosis than those infected with Ad.GFP (Ge et al., 2002b). Accordingly, a cell viability assay indicated that the viability of Ad.GFP.*fat-1* cells was significantly ($\sim$50%) higher than that of cells infected with Ad.GFP. These observations confirm the protective effects of omega-3 fatty acid supplementation on neuron death (Kim et al., 2000; Lauritzen et al., 2000) and highlight the importance of the omega-6/omega-3 ratio in this neuroprotective effect.

These in vitro studies clearly indicate that expression of the *C. elegans fat-1* gene (n-3 fatty acid desaturase) in mammalian cells can quickly and dramatically balance their n-6/n-3 fatty acid ratio, alter the eicosanoid profile, and consequently provide beneficial effects of n-3 fatty acids, without the need of supplementation with exogenous n-3 fatty acids.

Transgenic Animals (in Vivo Studies)

On the basis of the *in vitro* results, we proceeded with generation of fat-1 tansgenic animals capable of producing n-3 from n-6 fatty acids.

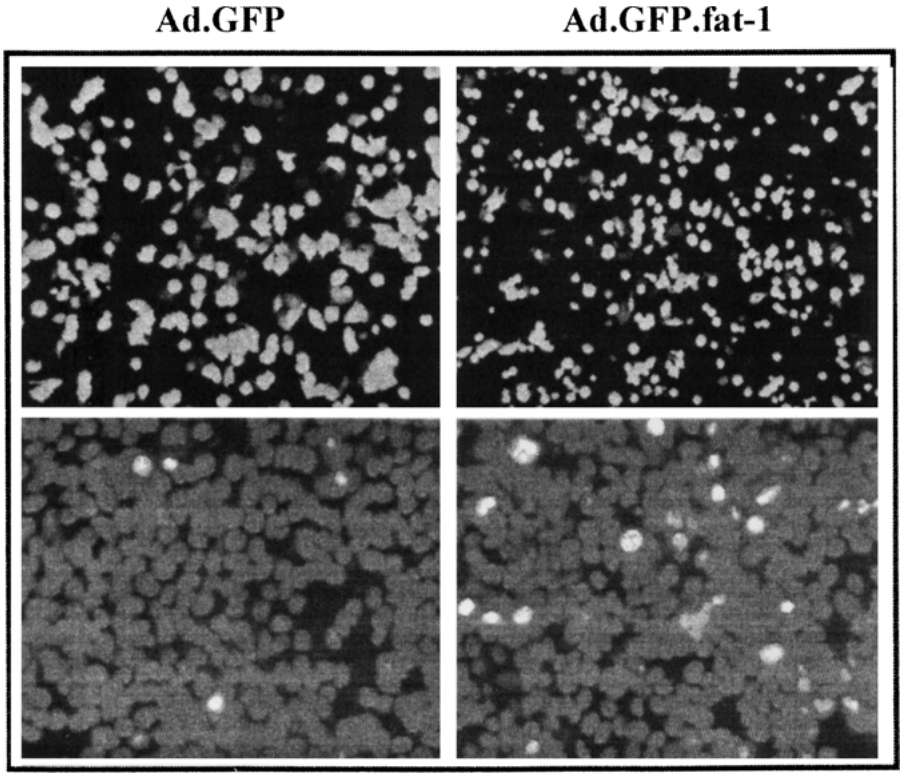

Fig. 4. The gene transfer induces apoptosis of MCF-7 cells. MCF-7 cells were infected with Ad.GFP (*left*; control) or Ad.GFP.*fat-1* (*right*). Three days after infection, cell death was examined using a fluorescence microscope. *Upper panels*: Infected cells were directly visualized at 510 nm of blue light. *Lower panels:* Cells were stained with Hoechst dye for nuclei and observed under 480 nm fluorescent light. The brighter blue spots are the nuclei of apoptotic cells.

To heterologously express the *C. elegans* omega-3 fatty acid desaturase in mice, we modified the *fat-1* gene encoding this protein by optimization of codon usage for mammalian cells and coupled it to a chicken beta-actin promoter. We then microinjected the expression vector into fertilized eggs to produce transgenic mouse lines. We have now successfully generated mice expressing the *fat-1* gene (Kang et al., 2004).

Both transgenic and wild-type mice are maintained at a diet high in omega-6 fatty acids (mainly linoleic acid) with very little omega-3 fatty acids (~0.1% of total fat supplied). Under this dietary regime, wild-type mice have little or no omega-3 fatty acid in their tissues because the animals naturally cannot produce omega-3 from omega-6 fatty acids, whereas the *fat-1* transgenic mice have significant amounts of omega-3 fatty acids (derived from omega-6 fatty acids) in their tissues (Kang et al., 2004). Figure 7 shows the differential fatty acid profiles of total lipids extracted from skeletal muscles of age- and sex-matched wild-type and transgenic mice. In the wild-type animals, the polyunsaturated fatty acids found in the tissues are mainly (98%) the omega-6 linoleic acid (LA, 18:n-6) and arachidonic acid (AA, 20:4n-6) with trace (or undetectable) amount of omega-3 fatty acids. In contrast, there are large amounts of omega-3 polyunsaturated fatty acids, including linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), in the tissues of transgenic mice. Accordingly, the levels of the n-6 fatty acids LA and AA in the transgenic tissues are significantly reduced, indicating a conversion of omega-6 to omega-3 fatty acids. The resulting ratio of omega-6 to omega-3 fatty acids in the tissues of transgenic animals is close to 1. This omega-3-rich profile of lipid with a balanced ratio of omega-6 to omega-3 and an even more balanced AA/(EPA + DPA + DHA) can be observed in all of the organs/tissues, including muscle and milk (Table 2). Our data clearly show that the transgenic mice expressing the *fat-1* gene are capable of producing omega-3 fatty acids from omega-6 fatty acids, resulting in enrichment of omega-3 fatty acids in their organs/tissues without the need of dietary omega-3 supply, which is impossible to achieve in wild-type mammals.

The transgenic mice appear to be normal and healthy. To date, several (more than 5) generations of transgenic mouse lines have been examined and their tissue fatty acid profiles showed consistently high levels of n-3 fatty acids, indicating the transgene is transmittable.

With this model, one can readily address any specific effects of n-3 fatty acids or of the n-6/n-3 ratio in any organs/tissues ranging from gene expression to physiological activity during the whole life cycle. For the studies of comparing the effects of n-3 and n-6 fatty acids or various n-6/n-3 ratios, the use of this model has advantages: 1) It can eliminate the lengthy and costly feeding of different special diets for

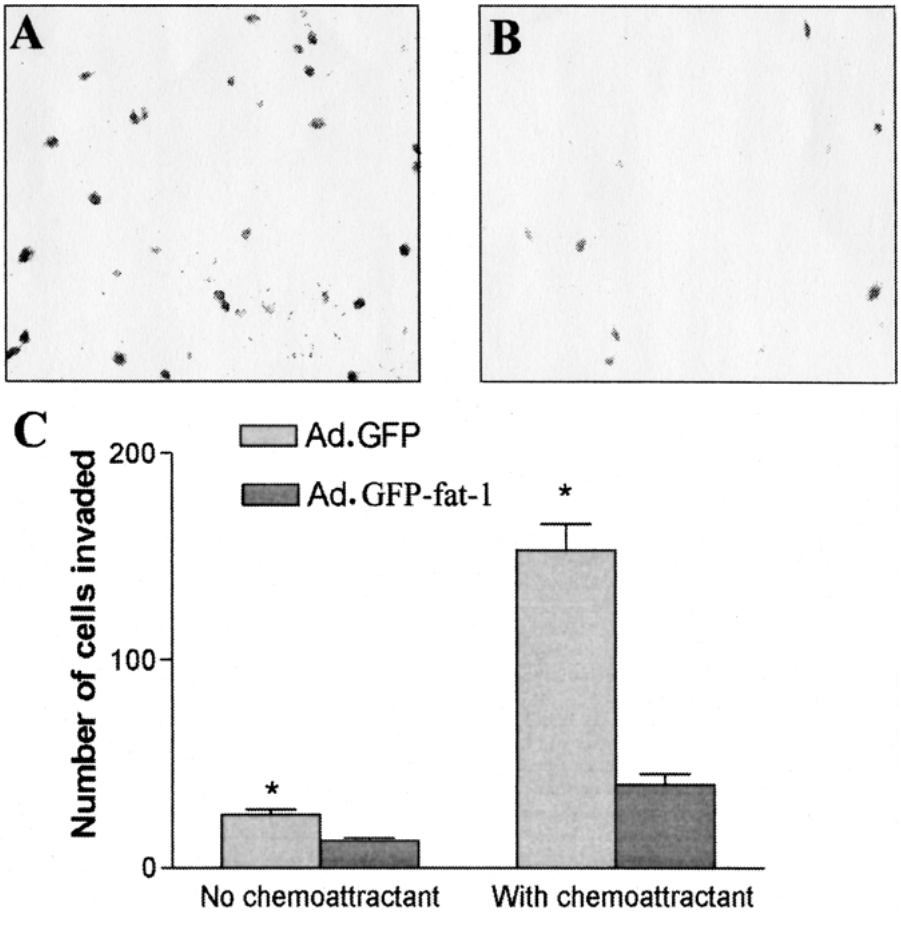

Fig. 5. Fat-1 expression decreased the invasive potential of A549 cells. The cells that invaded through the matrigel-coated *trans*-well inserts toward chemoattractant were stained with 1% crystal purple. Photographs were taken at a magnification of × 100. (*A*) Control cells infected with Ad.GFP; (*B*) transgenic cells infected with Ad.GFP-fat-1; and (*C*) the cells invading through the matrigel were counted under the microscope in five predetermined fields at × 100. Each sample was assayed in triplicate.

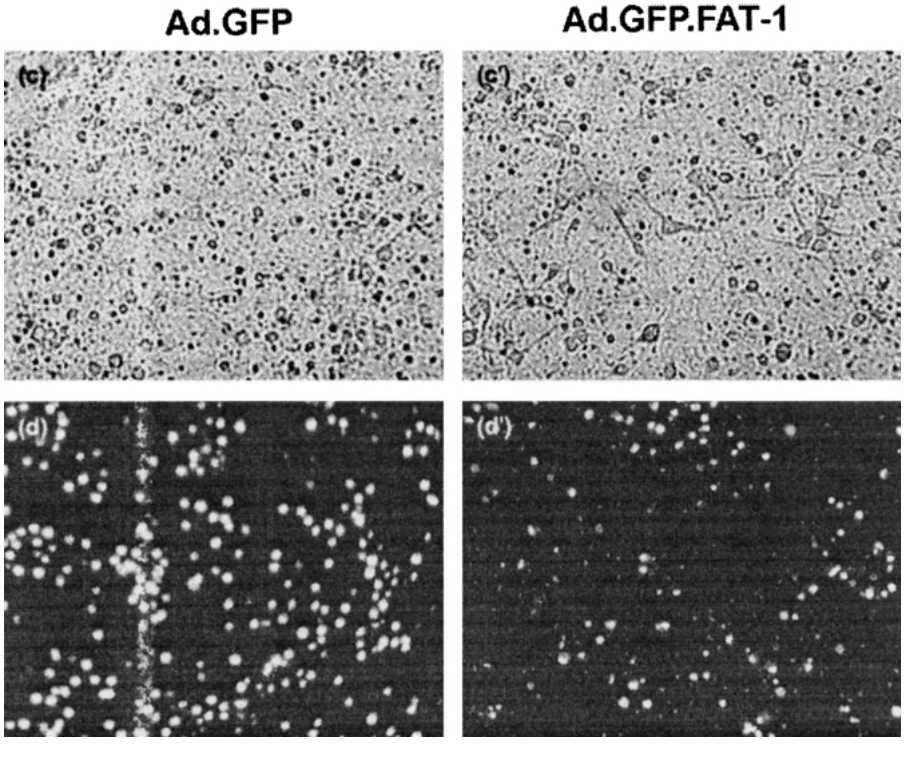

Fig. 6. Photomicrographs showing the protective effect of *fat-1* gene on neuronal apoptosis. Cells were infected with Ad-GFP (*left panels*, control) or Ad-GFP-*fat-1* (*right panels*). Cell death was examined after 24 hours of growth factor withdrawal using a fluorescent microscope. Bright-field (*upper panels*) and Hoechst staining (*lower panels*) images showing apoptotic cells.

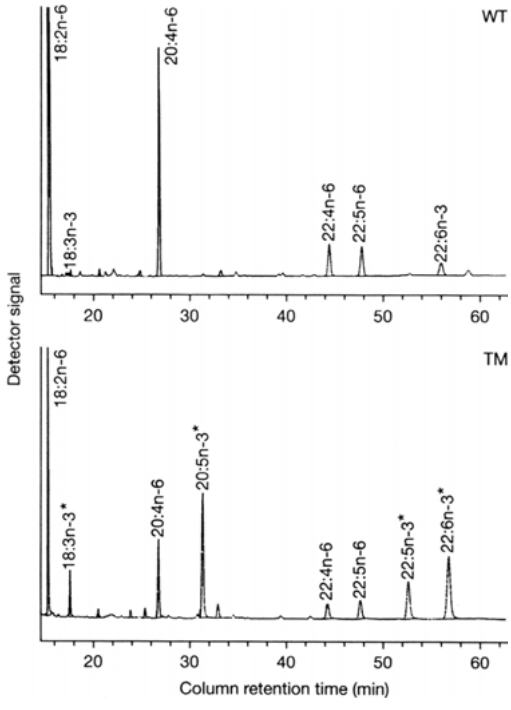

Fig. 7. Partial gas chromatograph traces showing the polyunsaturated fatty acid profiles of total lipids extracted from skeletal muscles of a wild-type mouse (*WT*, *upper panel*) and a *fat-1* transgenic mouse (*TM*, *lower panel*). Both the wild-type and transgenic mice were 8 weeks old, female, and fed with the same diet. Note, the levels of n-6 polyunsaturated acids (18:2n-6, 20:4n-6, 22:4n-6 and 22:5n-6) are remarkably lower, whereas n-3 fatty acids (marked with *) are abundant in the transgenic muscle (*lower panel*) compared with the wild-type muscle, in which there is very little n-3 fatty acid (*upper panel*).

comparative studies (n-6 vs. n-3). 2) It can provide more reliable and definitive results than dietary manipulation. For experiments designed to examine the effects of two different ratios of n-6/n-3 fatty acids, conventionally two different diets must be utilized to feed the animals in order to establish the different fatty acid profiles. Feeding two different diets for months makes it impossible to keep everything identical between two groups of animals. Many variables can arise from the diets and the feeding procedures, including the concentration, impurity or unwanted components of the oils used (e.g., fish oil vs. corn oil), flavor, sensitivity to oxidation, diet storage, duration of diet change, etc., which can impose confounding effects on the absolute content of n-3 PUFA and the n-6/n-3 PUFA ratio. These potential confounding factors in the diet can lead to unreliable (inconsistent or conflicting) results. Availability of fat-1 transgenic mice allows us to produce two different fatty acid profiles in experimental animals by feeding them just a single identical diet, so that carefully controlled studies can be performed without the interference of the potential confounding factors of different diet. And 3), more attractively, *fat-1* transgenic mouse lines can be used to genetically cross with established disease models (transgenic or knockout animals) to generate combined (*fat-1* plus a diseased gene) models, which allow addressing the effects of n-3 fatty acids and or the n-6/n-3 ratio on the pathogenesis and therapy of the disease. Therefore, *fat-1* transgenic mice will serve as a new tool for omega-3 fatty acid research.

Recently, we have begun to explore the potential differences in physiology and pathophysiology between the transgenic and wild-type mice, and have obtained some exciting preliminary data. For example, we implanted mouse melanoma B16 cells into the transgenic and wild-type mice (maintained on a high omega-6/omega-3 diet) and examined the incidence of tumor formation and tumor growth rate. The results showed a dramatic inhibition of melanoma formation and growth in *fat-1* transgenic mice. In a dextran sodium sulfate (DSS)-induced colitis model, fat-1 mice had a much lighter inflammatory response than wild-type animals; when lungs were challenged with lipopolysaccharide (LPS) or bleomycin, pulmonary inflammation and fibrosis in the transgenic mice seemed to be less severe than that in wild-type animals. The *fat-1* transgenic mice have lower levels of blood triglyceride and higher HDL. In addition, the wild-type mice behaviorally exhibit hyperactivity, whereas the *fat-1* mice do not (*unpublished data*). More interestingly, we recently generated *ob*/*ob* plus *fat-1* and $ApoE^{-/-}$ plus *fat-1* mouse lines and examined the development of metabolic syndrome and atherosclerosis in these animals. Our preliminary data showed significantly beneficial effects of omega-3 fatty acids on these conditions (*unpublished data*). Our observations in vivo obtained so far are consistent with the in vitro data and support the notion that a balanced ratio of omega-6/omega-3 fatty acids is more desirable in reducing the risk of many diseases.

Following the successful generation of *fat-1* transgenic mice, we moved to create large transgenic animals, livestock, Very recently, in collaboration with Dr. Yifan Dai (University of Pittsburgh) and Dr. Randall S. Prather (University of Missouri-Columbia), we have successfully created *fat-1* transgenic pigs. The pork from the fat-1 animals is rich in n-3 fatty acids (10 times more than wild-type pigs) and has an n-6/n-3 ratio of close to 1 (Lai et al., 2005). This supports the feasibility of using our genetic approach to provide a land-based source of omega-3 fatty acids. Generation of other fat-1 transgenic livestock (chicken, cow and fish) is under way in our laboratory. Once these food products become available in the market, we could thus readily achieve an n-6/n-3 ratio approximating 1.0 by consuming the foodstuff with such a ratio without the public having to make stringent changes in diet, (that is, to obtain healthy n-3 fatty acids you can eat your favorite

Table 2. Comparison of the n-6/n-3 ratios and AA/(EPA + DPA + DHA) ratio in various organs and tissues between a wild-type mouse (WT) and a *fat-1* transgenic mouse (TG)*

	Omega-6/Omega-3**		AA/(EPA + DPA + DHA)	
	WT	TG	WT	TG
Muscle	49.0	0.7	11.3	0.4
Milk	32.7	5.7	15.7	2.5
RBC	46.6	2.9	27.0	1.6
Heart	22.8	1.8	14.3	0.9
Brain	3.9	0.8	3.6	0.7
Liver	26.0	2.5	12.5	0.9
Kidney	16.5	1.7	11.9	1.2
Lung	32.3	2.2	19.8	1.2
Spleen	23.8	2.4	17.3	1.5

*Both the wild-type and transgenic mice were 8 weeks old, female, and fed with the same diet.
**The n-6/n-3 fatty acid ratio is (18:2n-6 + 20:4n-6 + 22:4n-6 + 22:5n-6)/(18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3).

hamburger, hotdog and eggs if you don't like or can't get fish).

In conclusion, our discovery provides not only a new tool for n-3 fatty acid research, but also a new strategy for producing omega-3 fatty acid-rich foodstuff (e.g., meat, milk and eggs) by generating large *fat-1* transgenic animals/livestock (e.g., cow, pig, sheep and chicken). This genetic approach is a cost-effective and sustainable way of producing omega-3 essential fatty acids for the increasing demand in the future.

The author would like to acknowledge the contributions of Jingdong Wang, Lin Wu and Zhao B. Kang to this work and thank Dr. John Browse for generously providing the *C. elegans fat-1* cDNA and Dr. Jun-ichi Miyazaki for providing the CAG promotor. The author is also grateful to Dr. Alexander Leaf for his long-time support and encouragement. This work was supported by grants from the National Institute of Health, the American Cancer Society and the American Institute for Cancer Research.

References

Bougnoux, P. 1999. N-3 polyunsaturated fatty acids and cancer. *Curr. Opin. Clin. Nutr. Metab. Care* **2:**121–126

Cave, W.T. Jr. 1997. Omega-3 polyunsaturated fatty acids in rodent models of breast cancer. *Breast Cancer Res. Treat*. **46:**239–246

Connor, W.E. 2000. Importance of n-3 fatty acids in health and disease. *Am. J. Clin. Nutr*. **71:**171S–175S

Ge, Y., Chen, Z.H., Brown, J., Laposata, M., Kang, J.X. 2002a. Effect of adenoviral gene transfer of *C. elegans* n-3 fatty acid desaturase on the lipid profile and growth of human breast cancer cells. *Anticancer Res*. **22:**537–544

Ge, Y., Wang, X.Y., Chen, Z.H., Landman, N., Lo, E.H., Kang, J.X. 2002b. Inhibition of neuronal apoptosis by adenoviral gene transfer of *C. elegans* n-3 fatty acid desaturase. *J. Neurochem.* **82:**1360–1366

Kang, Z.B., Ge, Y., Chen, Z.H., Brown, J., Laposata, M., Leaf, A., Kang, J.X. 2001. Adenoviral gene transfer of *C. elegans* n-3 fatty acid desaturase optimizes fatty acid composition in mammalian cells. *Proc. Natl. Acad. Sci. USA* **98:**4050–4054

Kang, J.X., Leaf, A. 1996. Antiarrhythmic effects of polyunsaturated fatty acids. Recent studies. *Circulation* **94:**1774–1780

Kang, J.X., Wang, J., Wu, L., Kang, Z.B. 2004. *Fat-1* transgenic mice convert n-6 to n-3 fatty acids. *Nature* **427:**504

Kim, H.Y., Akbar, M., Lau, A., Edsall, L. 2000. Inhibition of neuronal apoptosis by docosahexaenoic acid (22:6n-3). Role of phosphatidylserine in antiapoptotic effect. *J. Biol. Chem.* **275:**35215–35223

Kris-Etherton, P.M., Harris, W.S., Appel, L.J. for the Nutrition Committee. 2002. AHA scientific statement. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* **106:**2747–2757

Lai, L., Kang, J.X., Witt, W.T., Wang, J., Yong, H.Y., Hao, Y., Wax, D.M., Li, R., Evans, R.W., Starzl, T.E., Prather, R.S., Dai, Y. 2005. Cloned *Fat-1* transgenic pigs rich in omega-3 fatty acids. *Nat. Biotech. in press*

Lauritzen, I., Blondeau, N., Heurteaux, C., Widmann, C., Romey, G., Lazdunski, M. 2000. Polyunsaturated fatty acids are potent neuroprotectors. *EMBO J.* **19:**1784–1793

Leaf, A., Kang, J.X. 1998. Omega-3 fatty acids and cardiovascular disease. *World Rev. Nutr. Diet*. **83:**24–37

Leaf, A., Weber, P.C. 1987. A new era for science in nutrition. *Am. J. Clin. Nutr*. **45:**1048–1053

Meiler, S., Kang, J.X., Rosenzweig, A. 2002. Expression of the *fat-1* gene alters lipid profile and inhibits the inflammation of human endothelium by reducing the transcriptional activity of NFkB. 2002 *Am. Heart Assoc. Annu. Meet*. Abstract (Publication ID:1228)

O'Keefe, J.H., Jr, Harris, W.S. 2000. From Inuit to implementation: omega-3 fatty acids come of age. *Mayo Clin. Proc*. **75:**607–614

Rose, D.P., Connolly, J.M. 1999. Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacol. Ther* **83:**217–244

Simopoulos, A.P. 1999. Essential fatty acids in health and chronic disease. *Am. J. Clin. Nutr*. **70:**560S–569S

Simopoulos, A.P. 2000. Human requirement for n-3 polyunsaturated fatty acids. *Poultry Science* **79:**961–970

Spychalla, J.P., Kinney, A.J., Browse, J. 1997. Identification of an animal omega-3 fatty acid desaturase by heterologous expression in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*. **94:** 1142–1147

Xia, S.H., Wang, J., Kang, J.X. 2005. Decreased n-6/n-3 fatty acid ratio reduces the invasive potential of human lung cancer cells by downregulation of cell adhesion/invasion-related genes. *Carcinogenesis*. **26:**779–784