Genetic Manipulation of Adipose Cells to Improve Fatty Acid Composition of Meat

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Summary
The purpose of this study is to modify adipose cells genetically in culture to produce cells that contain less saturated fat. To accomplish this objective, a fatty acid desaturase gene from castor beans will be transferred into adipose cells. Using cultured liver cells as a test system, the gene was successfully transferred into 12 different cultures of liver cells. We are currently studying the production of the corresponding RNA from the incorporated gene and the fatty acid composition of the transformed cells. Our next goal is to conduct similar activities using cultured adipose cells. Saturated fatty acids make up 40 to 55% of the total fat associated with meat. It has been demonstrated in animal and human studies that dietary saturated fatty acids (excluding C18:0) are hypercholesterolemic when compared with polyunsaturated fatty acids. Therefore, nutritional scientists must seek with urgency nutritional, managerial, and genetic protocols for decreasing the fat content and the proportion of saturated fatty acids in animal-derived foods.

Introduction
Animal products clearly are an important part of the American diet. It is estimated that 60% of dietary fat intake in the U.S. is of animal origin. Many people, however, have questioned the wisdom of consumption of animal products relative to risk of heart disease. When asked to identify their major health concern about the healthfulness of the American diet, leading nutritionists most frequently criticize the amount of saturated fatty acids consumed daily. In a recent symposium, the Wisconsin Milk Marketing Board suggested the ideal nutritional fatty acid composition for milk fat would contain 82% monounsaturated fatty acids rather than 25%; the increase would occur at the expense of saturated fatty acids (Grummer, 1991; Figure 1). Nutritionists readily can extend this recommendation to the fatty acid composition of beef and other foods derived from animals. This viewpoint is strengthened by the U.S. Surgeon General’s Report on Nutrition and Health (U.S. Department of Health and Human Services, 1988), in which the number one recommendation is that most people should decrease consumption of fat (especially saturated fat) and cholesterol.

Milk fatty acid composition

Figure 1. Comparison of typical and ideal fatty acid composition of milk. SFA refers to saturated fatty acids, MUFA to monounsaturated fatty acids, and PUFA to polyunsaturated fatty acids.

Our long-term goal is to develop practical procedures for increasing the proportion of monounsaturated fatty acids in meat. During the course of this study, we will accumulate basic knowledge on the cellular, nutritional, and environmental controls of fatty acid composition of meat. This specific project is based on the concept of increasing the total fatty acid desaturase activity of adipose cells in culture by adding a cytosolic plant-derived stearoyl-acyl carrier protein desaturase to cells already containing a membrane-associated stearoyl-coenzyme A desaturase. This specific desaturase catalyzes the conversion of stearic acid, a saturated fatty acid, to oleic acid, a monounsaturated fatty acid. Fatty acid desaturases catalyze the formation of carbon-carbon double bonds (unsaturation) in fatty acids according to the following reaction:
In this reaction, if the precursor or substrate of the reaction is stearic acid, the product is oleic acid.

Adipose tissue of animal species possesses the capacity to convert blood-derived preformed and de novo-synthesized saturated fatty acids to monounsaturated fatty acids (Figure 2). The primary end product of fatty acid synthesis in lipogenic animal cells is palmitic acid, but the principal fatty acid in adipose tissue of meats such as beef is oleic acid. Thus, fatty acid elongase and fatty acid desaturase, which convert palmitic to oleic acid, are physiologically important determinants of the fatty acid composition of animal fats. The fatty acid desaturase is associated predominantly with the endoplasmic reticulum--intracellular membranes--of adipose tissue cells.

Modified adipose cell

We propose to use stearoyl-acyl carrier protein (ACP) desaturase (EC 1.14.99.6), a Δ⁹-desaturase, in this study. Stearoyl-ACP desaturase catalyzes the conversion of stearoyl-ACP to oleoyl-ACP. This enzyme is the only known soluble desaturase in nature and is unique to photosynthetic organisms. Stearoyl-ACP desaturase is located in the chloroplasts of photosynthetic tissues of plants or in plastids of nonphotosynthetic organs.

We have available in our laboratories the complete cDNA clone of the castor bean stearoyl-ACP desaturase. Our hypothesis is that adding the stearoyl-ACP desaturase gene from plants to adipose cells will cause the adipose cell cultures to increase the proportion of monounsaturated fatty acids in the total of fatty acids synthesized by those cells. Completion of this research will determine whether genetic manipulation by gene transfection can modify fatty acids that are synthesized in animal tissue. Concerted action of the endogenous desaturases with the added plant desaturase could enhance the capacity of the lipogenic adipose cells of growing beef animals to synthesize and store greater proportions of monounsaturated fatty acids.

Materials and Methods

1. Cell Cultures
   We have chosen to use Hep G2 cells for our liver cell line because they have retained many characteristics of primary liver cells and because they are relatively easy to work with. These cells retain the ability to synthesize triacylglycerols and secrete lipoproteins in a similar manner to liver cells in vivo (Thrift et al., 1986).

2. Development of DNA Construct
   cDNA clones that encode Δ⁹-stearoyl-ACP desaturase have been isolated from a number of different plant species. We have available the cDNA clone for the Δ⁹-desaturase isolated from castor bean (Shanklin and Somerville, 1991). The cDNA sequence that encodes Δ⁹-stearoyl-ACP desaturase will be ligated into the multiple cloning region of the mammalian expression vector pMAMneo.

3. Transfection of Cultures
   To test for correct expression of the desaturase gene construction, the expressing plasmid will be transfected into cultured Hep G2 liver cells. The recombinant plasmid will be transfected into the cells by using calcium phosphate-mediated DNA uptake. Cells transformed with the vector alone will serve as negative controls.

4. Production of Stably Transformed Cells
   Hep G2 cells transfected as described earlier will be selected for stable transformants by adding the antibiotic GeneticinÒ. Individual clones will be isolated and propagated. Expression of the desaturase gene will be determined by isolation and characterization of cellular RNA from cell lines transformed with recombinant plasmid. Cell lines transformed with the vector alone will serve as negative controls. Northern blotting procedures will be used to confirm the presence and correct size of desaturase messenger RNA (Sambrook et al., 1989).

5. Characterization of Desaturase in Transformed Cells
   Once the transformed cell line has been established and the presence of stearoyl-ACP desaturase has been verified by Southern and Northern analyses, the functionality, specificity, and cellular location of stearoyl-ACP desaturase in our cell lines will be assessed. Fatty acid composition of...
secreted lipids and microsomes plus plasma membranes will be determined. Western analysis to detect expression of the stearoyl-ACP desaturase will be conducted (Sambrook et al., 1989) to verify that the transformed cells express the stearoyl-ACP desaturase.

Results and Discussion

This report is a description of research in progress. The cDNA for the stearoyl-ACP desaturase has been ligated successfully to its expression vector in the proper orientation. The construct then was transferred into numerous different cultures of liver cells. By use of Southern analyses that detect the desaturase gene, we observed that 12 different lines of liver cells have been transformed stably. Figure 3 illustrates a typical Southern analysis. Lane D of the figure indicates that the 1.6 Kb band, which corresponds to the stearoyl-ACP desaturase gene (lane B), was present in the transformed cells. This observation was made on 12 different lines of cells.

Figure 3. Southern analysis of transformed liver cells. Lane A indicates location of specific molecular weight markers, lane B indicates the presence of the desaturase gene in the expression vector at 1.6 Kb, lane C indicates that nontransformed cells contain no desaturase gene (no 1.6 Kb band), and lane D indicates that the transformed liver cells contain the desaturase gene (1.6 Kb band).

Our immediate goals are to document that the transformed cells produce the messenger RNA that corresponds to the desaturase gene via Northern analyses. Then, the presence of the stearoyl-ACP desaturase will be verified by Western analysis. After success with the liver model system, we will complete the same experiments with adipose cells in culture. The ultimate test will be to show that transformed adipose cells produce a higher proportion of monounsaturated fatty acids than do nontransformed adipose cells.

Implications
The technology used in this project represents a potential method for changing the fatty acid composition of animals to meet the demands of health-conscious consumers.

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References