Unsaturated Fatty Acids Inhibit Sterol Regulatory Element-Dependent Gene Expression: A Potential Mechanism Contributing to Hypertriglyceridemia in Fat-Restricted Diets (44564C)

RICHARD J. DECKELBAUM,[1] REBECCA A. JOHNSON, AND TILLA S. WORGALL

Institute of Human Nutrition and Department of Pediatrics, Columbia University, New York, New York 10032

Cholesterol and free fatty acids interact at a number of levels in pathways that regulate human lipid metabolism, cellular membrane structure, and function. As examples, both cholesterol and free fatty acids are important substrates for the acyl CoA-cholesterol acyltransferase (ACAT) reaction leading to cell storage of sterol esters. Fatty acids and cholesterol independently regulate expression of the LDL receptor (1). Whereas cholesterol acts to decrease fluidity of cellular membranes, polyunsaturated fatty acids incorporated into membrane phospholipids increase fluidity.

Work from the laboratory of Brown and Goldstein (2) first demonstrated that cholesterol can regulate gene expression *via* sterol regulatory elements (SRE). SREs are found in the promoter regions of genes relating to cholesterol, fatty acid, triglyceride, and carbohydrate metabolism (3). Generally, excess sterol leads to inhibition of SRE-dependent gene expression, and depletion of cellular sterol will lead to activation of SRE-dependent gene expression. The effects of cholesterol on SREs are regulated through distinct proteins: sterol regulatory element binding proteins (SREBP) and the SREBP-cleavage-activating-protein (SCAP). SREBPs contain about 1150 amino acids and are located in the endoplasmic reticulum in an inactive form. Their orientation is similar to a hair pin; both the COOH- and the NH_2-terminal ends protrude from the endoplasmic reticulum into the cytoplasm. Sterol depletion initiates a two-step proteolytic process that results in the release of the transcriptionally active N-terminal end of SREBP. First, SCAP and SREBP translocate to the Golgi where the protein is cleaved at the intraluminal loop. The second proteolysis at an intramembrane site releases the mature SREBP. This transcription factor (MW 68,000 kDa) translocates to the nucleus where it binds to the SRE region and initiates transcription of the respective gene (4). There are two major forms of SREBP: SREBP-1a/1c and SREBP-2. SREBP-1a/1c mainly regulate pathways related to fatty acid and triglyceride metabolism as well as carbohydrate metabolism. In contrast, SREBP-2, which is encoded on a different gene, mainly regulates expression of genes important in sterol or cholesterol metabolism. Nevertheless, there is overlap between the activities of the SREBPs in fatty acid and cholesterol metabolism (5).

Recognizing the frequent and close links between fatty acid and cholesterol metabolism, our recent work explored the possibility that free fatty acids, not only sterols, would regulate SRE expression. In recently published work we showed that mono- and polyunsaturated fatty acids do mediate SRE expression and that this occurs through changes in SREBP maturation (6). Three different cell lines were chosen, each differing in cell cholesterol homeostatic mechanisms so that we might demonstrate the commonality of regulatory pathways. The cells were either transiently or stably transfected with SRE-containing promoters linked to a luciferase reporter gene that originated from the HMG CoA synthase promoter or the fatty acid synthase promoter. In experiments with fatty acids complexed to albumin at physiologic concentrations, monounsaturated and polyunsaturated fatty acids (but not saturated fatty acids) dose-dependently downregulated SRE-mediated gene expression. Effects of fatty acids on SRE promoters originating from different genes in the different cell lines produced very similar results. The effects of the fatty acids were synergistic to those of cholesterol in suppressing SRE-mediated gene expression. Generally, increasing unsaturation led to higher suppression of SRE-mediated gene expression (e.g., linoleic acid had greater effects than did oleic acid, and the effects were most marked with arachidonate and docosa-

[1] To whom requests for reprints should be addressed at PH 15-1512E, Columbia University, 630 West 168th Street, New York, NY 10032. E-mail: rjd20@columbia.edu

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hexanoate). Our experiments showed that free fatty acids alone and synergistic with cholesterol decreased intracellular levels of mature SREBP and decreased mRNA expression for HMG-CoA synthase. In experiments using sterol regulation–defective cell lines and cell lines transfected with mutant SREs, we showed that the effects of fatty acids are dependent upon cellular levels of mature SREBP and are not independent effects of fatty acids acting directly on SREs. As well, our experiments suggested that fatty acids can act upon SREBPs through changes in cell cholesterol partitioning. Recently, other groups also demonstrated that polyunsaturated fatty acids can decrease SREBP-mediated gene expression (7), and this may involve pathways relating to intracellular metabolism of SREBP mRNAs (8).

More recent work in our group has indicated that fatty acids may act to redistribute sterols between the plasma membrane and intracellular compartments by at least two different mechanisms, one physical, and one biochemical. In collaboration with James Hamilton at Boston University, we have shown that free fatty acids decrease the affinity of free cholesterol in model phospholipid membranes and increase its transfer to other membranes (9). We postulate that if such effects occur *in vivo*, then free fatty acids will facilitate the transfer of cholesterol from the plasma membrane to intracellular compartments to decrease SREBP cleavage and decrease cellular levels of mature SREBP. In separate experiments, we have shown that free fatty acids increase cell sphingomyelinase activity and decrease cell sphingomyelin levels (10). Since sphingomyelin has higher affinity and binding capacity to cholesterol than other cell membrane phospholipids, decreasing membrane sphingomyelin would also lead to transfer of free cholesterol toward intracellular membranes and decrease SRE-mediated gene expression through the SREBP cascade.

How might these *in vitro* findings relate to the effects of unsaturated fatty acids on plasma lipoprotein metabolism *in vivo*? Certainly the effect of fatty acids on SREs does not explain upregulation of the LDL receptor activity in diets rich in polyunsaturated fatty acids. In this regard, recent work has demonstrated major control of LDL receptor gene expression in the LDL receptor promoter region distinct from SREs (11, 12). Thus, it is possible that dietary cholesterol or endogenous cholesterol downregulates the LDL receptor through SREs but that fatty acids have another point of action, still to be defined. In contrast, genes regulating fatty acid and triglyceride synthesis undergo major regulation *via* SRE expression (3). Our *in vitro* work suggests that high levels of mono- and polyunsaturated fat would suppress genes important to triglyceride synthesis, and this parallels human *in vivo* studies in which high polyunsaturated fat diets lead to decreased endogenous triglyceride synthesis. In contrast, diets very low in fat are associated with increased plasma triglyceride levels and can also lead to increased endogenous synthesis of triglycerides endogenously, and associated higher plasma levels of triglyceride-rich lipoproteins (13, 14).

We realize that the interactions between cholesterol, fatty acid, and carbohydrate metabolism are complex, and can occur on multiple levels. Work that will emerge within the next few years will help unravel the molecular mechanisms defining the interactions between sterol, fatty acid, and carbohydrate metabolism. Still, linking the results of our group and other laboratories with the impact of low-fat diets in clinical population studies (14, 15), we suggest that a "minimum" level of mono- and polyunsaturated fat intake may be needed to suppress expression of genes directly linked to triglyceride synthesis. We hypothesize that very low levels of exogenous fat intake are associated with higher levels of SRE-mediated gene expression in genes important to *de-novo* triglyceride synthesis, and this will contribute to the hypertriglyceridemia associated with very low dietary intake of fat.

1. Horton JD, Cuthbert JA, Spady DK. Dietary fatty acids regulate hepatic low-density lipoprotein (LDL) transport by altering LDL receptor protein and mRNA levels. J Clin Invest **92**:743–749, 1993.
2. Sudhof TC, Russell DW, Brown MS, Goldstein JL. 42-bp element from LDL receptor gene confers end-product repression by sterols when inserted into viral TK promoter. Cell **48**:1061–1069, 1987.
3. Horton JD, Shimomura I. Sterol regulatory element-binding proteins: Activators of cholesterol and fatty acid biosynthesis. Curr Opin Lipidol **10**:143–150, 1999.
4. DeBose-Boyd RA, Brown MS, Li WP, Nohturfft A, Goldstein JL, Espenshade PJ. Transport-dependent proteolysis of SREBP: Relocation of site-1 protease from Golgi to ER obviates the need for SREBP transport to Golgi. Cell **99**:703–712, 1999.
5. Horton JD, Shimomura I, Brown MS, Hammer RE, Goldstein JL, Shimano H. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. J Clin Invest **101**:2331–2339, 1998.
6. Worgall TS, Sturley SL, Seo T, Osborne TF, Deckelbaum RJ. Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. J Biol Chem **273**:25537–25540, 1998.
7. Yahagi N, Shimano H, Hasty AH, Amemiya-Kudo M, Okazaki H, Tamura Y, Iizuka Y, Shionoiri F, Ohashi K, Osuga J, Harada K, Gotoda T, Nagai R, Ishibashi S, Yamada N. A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J Biol Chem **274**:35840–35844, 1999.
8. Xu J, Nakamura MT, Cho HP, Clarke SD. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids: A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. J Biol Chem **274**:23577–23583, 1999.
9. Johnson RA, Worgall TS, Peng S, Hamilton JA, Deckelbaum RJ. Free fatty acids enhance cholesterol movement between model membranes. FASEB J **14**:A229, 2000.
10. Worgall TS, Johnson RA, Seo T, Peng S, Hamilton J, Deckelbaum RJ. Polyunsaturated fatty acids decrease sterol regulatory element–dependent gene transcription by three interdependent processes. Circulation **100**:I694, 1999.

11. Makar RS, Lipsky PE, Cuthbert JA. Sterol-independent, sterol response element–dependent regulation of low-density lipoprotein receptor gene expression. J Lipid Res **39**:1647–1654, 1998.
12. Makar RS, Lipsky PE, Cuthbert JA. Multiple mechanisms, independent of sterol regulatory element binding proteins, regulate low-density lipoprotein gene transcription. J Lipid Res **41**:762–774, 2000.
13. Hudgins LC, Hellerstein MK, Seidman CE, Neese RA, Tremaroli JD, Hirsch J. Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. J Lipid Res **41**:595–604, 2000.
14. Hudgins LC, Hellerstein M, Seidman C, Neese R, Diakun J, Hirsch J. Human fatty acid synthesis is stimulated by a eucaloric low-fat, high-carbohydrate diet. J Clin Invest **97**:2081–2091, 1996.
15. Stampfer MJ, Hu FB, Manson JE, Rimm EB, Willet WC. Primary prevention of coronary heart disease in women through diet and lifestyle. N Engl J Med **343**:16–22, 2000.