Proceedings of the Nutrition Society (2002), **61**, 371–374 DOI:10.1079/PNS2002169

Effects of fatty acids on gene expression: role of peroxisome proliferator-activated receptor α, liver X receptor α and sterol regulatory element-binding protein-1c

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Dietary fatty acids have numerous effects on cellular function, many of which are achieved by altering the expression of genes. The present paper reviews recent data on the mechanisms by which fatty acids influence DNA transcription, and focus specifically on the importance of three transcription factors: peroxisome proliferator-activated receptor α; liver X receptor α; sterol regulatory element-binding protein 1c. These data indicate that fatty acids induce or inhibit the mRNA expression of a variety of different genes by acting both as agonists and as antagonists for nuclear hormone receptors.

Fatty acids: Transcription factors: mRNA expression: Nuclear hormone receptors

Dietary fatty acids have multiple effects on human metabolism. In addition to serving as an important source of dietary energy, they specifically influence numerous metabolic pathways in a variety of organs. Many of these effects are achieved by altering mRNA expression. In the past decade important new information has emerged about the mechanisms by which fatty acids influence DNA transcription and regulate mRNA expression. The present short review focuses on two groups of transcription factors that mediate the effects of fatty acids on gene transcription, the peroxisome proliferator-activated receptors (PPAR) and the sterol regulatory element-binding proteins (SREBP), and specifically addresses their function in hepatic lipid metabolism. The role of PPAR and SREBP will be illustrated by examining the events in liver during fasting or severe energy restriction, when triacylglycerol breakdown in adipose tissue is activated and large amounts of fatty acids are delivered to the liver.

In liver fatty acids can either be esterified to triacylglycerols and exported as VLDL or they can be oxidized, resulting in the formation of CO_2 and ketone bodies. As a result of the huge increase in the amount of fatty acids entering the liver during fasting, in absolute terms both pathways are activated, although relatively fatty acid oxidation becomes more important. At the same time, synthesis of fatty acids (lipogenesis) is strongly suppressed. These alterations in metabolic flux are accompanied by large changes in the expression of many genes involved in fatty acid oxidation and synthesis. It is now clear that one of the signals that govern these changes in gene expression is fatty acids themselves.

Mechanisms of inhibition of lipogenesis by polyunsaturated fatty acids

It has been known for some time that dietary polyunsaturated fatty acids (PUFA) inhibit the expression of several genes involved in lipogenesis, such as fatty acid synthase and stearoyl-CoA desaturase (Clarke & Jump, 1996). However, only recently-detailed insights into the molecular mechanisms behind this regulation have started to emerge. The transcription factor PPARα, which will be discussed later, was ruled out as the factor mediating the effects of PUFA on lipogenic gene expression (Ren *et al*. 1996, 1997). In contrast, it appears that a pivotal role in this regulation is played by SREBP-1c, a helix-turn-helix transcription factor that is present in both liver and adipose tissue (Osborne, 2000). In liver SREBP-1c induces the expression of a whole set of genes involved in fatty acid and triacylglycerol synthesis. Accordingly, mice that overexpress SREBP-1c in the liver display a build-up of hepatic triacylglycerols and have elevated expression levels of lipogenic genes, such as fatty acid synthase and stearoyl-CoA desaturase (Shimano *et al*. 1997). The importance of SREBP-1c in the regulation of hepatic lipogenesis prompted numerous groups to investigate whether PUFA might act via

Abbreviations: LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptors; PUFA, polyunsaturated fatty acids; SREBP, sterol regulatory element-binding protein.

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SREBP-1c. Interestingly, it was found that dietary PUFA potently lower SREBP-1c mRNA levels in mouse liver as well as in hepatoma and other cell lines (Kim *et al*. 1999; Mater *et al*. 1999; Xu J *et al*. 1999; Hannah *et al*. 2001). In addition, it was found that PUFA cause a decrease in the mature nuclear form of SREBP-1 protein but do not affect precursor membrane SREBP-1, suggesting that PUFA may influence proteolytic processing (Thewke *et al*. 1998; Yahagi *et al*. 1999; Hannah *et al*. 2001). Proteolytic maturation is necessary for SREBP-1 to exert its transcriptional regulatory activity. The inhibition of SREBP-1 proteolytic maturation by sterols may also be promoted by fatty acids (Thewke *et al*. 1998; Hannah *et al*. 2001). An important question that remains is how PUFA lower SREBP-1 mRNA levels. One line of evidence suggests that PUFA increase SREBP-1 mRNA decay (Xu *et al*. 2001). Furthermore, elegant work by two groups has provided evidence that the liver X receptor (LXR) α, a nuclear hormone receptor that binds and is activated by oxysterols, is responsible for mediating the effect of PUFA (Ou *et al*. 2001; Yoshikawa *et al*. 2002). LXRα is a potent activator of SREBP-1 gene transcription and stimulates the expression of several lipogenic genes (Schultz *et al*. 2000). Indeed, a response element for LXR, a so-called LXRE, has been identified in the promoter of the SREBP-1 gene (Repa *et al*. 2000). Interestingly, activation of the SREBP-1 promoter activity by LXRα is suppressed by PUFA such as eicosapentaenoic acid by inhibiting binding of the LXR – retinoid X receptor complex, which is the actual binding unit, to the LXRE. It was observed that PUFA compete with oxysterols for binding to LXR, suggesting that PUFA behave as LXR antagonists (Yoshikawa *et al*. 2002). The most potent fatty acid was arachidonic acid, followed by eicosapentaenoic acid and docosahexaenoic acid. Saturated fatty acids showed little or no effect. Thus, it appears that PUFA down regulate lipogenic genes by serving as antagonists of the nuclear receptor LXRα. This finding, however, does not exclude other regulatory mechanisms for which some evidence has been gathered.

Mechanisms of activation of fatty acid oxidation by polyunsaturated fatty acids

In the past few years it has become clear that induction of gene transcription by fatty acids is often mediated by a subclass of nuclear hormone receptors, the PPAR. PPAR are ligand-activated transcription factors that stimulate gene transcription by binding to a small sequence element in the promoter of certain genes. Their activity is induced by binding of small fatty acid-like molecules. Three PPAR subtypes can be distinguished: α, β and γ. While PPARα is mostly expressed in brown adipose tissue and liver, PPARβ is present at high concentrations in numerous tissues but is especially abundant in the intestine (Escher *et al*. 2001). PPARγ, in turn, is most abundant in white adipose tissue and to a lesser extent in colon and macrophages. Each of these receptors binds an overlapping set of fatty acids with low to moderate affinity. Depending on the binding assay used, different results have been obtained with respect to their relative binding affinity for saturated *v.* unsaturated fatty acids (Forman *et al*. 1997; Kliewer *et al*. 1997; Krey *et al*. 1997; Ellinghaus *et al*. 1999; Lin *et al*. 1999; Zomer *et al*. 2000). According to a scintillation proximity assay performed by Xu HE *et al*. (1999), saturated and unsaturated fatty acids bind to the PPARα subtype with approximately equal affinity. The highest binding affinity was found for γ-linoleic acid, an intermediate in the synthesis of arachidonic acid from linoleic acid. In contrast, Lin *et al*. (1999) found, using a fluorescence-based assay, that PPARα has a much higher affinity for arachidonic acid and linoleic acid than for stearic acid. Experiments with mice lacking peroxisomal acyl-CoA oxidase and/or PPARα suggest that substrates for acyl-CoA oxidase, which are very-long-chain fatty acids and their acyl-CoA derivatives, serve as natural ligands for PPARα *in vivo* (Hashimoto *et al*. 1999). Thus, although it is clear that most fatty acids, including saturated and unsaturated fatty acids, bind to and activate PPAR, it is still not entirely clear whether their relative affinities differ greatly.

The physiological significance of PPAR activation by fatty acids is best illustrated by taking a closer look at the metabolic events unfolding in a fasting liver. As discussed earlier, during fasting large amounts of fatty acids are released from adipose tissue and are primarily metabolized in the liver. Experiments with mice that lack PPARα have been invaluable in determining the importance of PPARα in the regulation of liver lipid metabolism (Lee *et al*. 1995; Peters *et al*. 1997; Aoyama *et al*. 1998; Kersten *et al*. 1999; Leone *et al*. 1999; Hashimoto *et al*. 2000). The increased hepatic influx of fatty acid is accompanied by a dramatic increase in the rate of fatty acid β-oxidation, resulting in the elevation of acyl-CoA levels, which serve as substrates for ketone body formation. These processes are severely inhibited in mice that lack PPARα, resulting in a defective influx of fatty acids, inhibition of fatty acid oxidation, and a dramatic reduction in the formation of ketone bodies (Kersten *et al*. 1999; Leone *et al*. 1999). At the same time, glucose output seems to be diminished and major changes occur at the level of amino acid metabolism (Kersten *et al*. 2001). These findings show that PPARα plays a pivotal role in the regulation of intermediary metabolism in the liver, particularly fatty acid oxidation, under conditions where the plasma fatty acid concentration is elevated, such as fasting. Since fatty acids serve as ligands for PPARα, these findings indicate that fatty acids govern their own metabolism, as well as that of other substrates such as glucose and amino acids.

Thus, in conclusion, fatty acids have an important regulatory function in hepatic energy metabolism. Via two different mechanisms, one involving the transcription factors LXRα and SREBP-1 and another involving the transcription factor PPARα, they up or down regulate the expression of a whole set of genes involved in fatty acid synthesis and fatty acid oxidation and/or ketogenesis, thereby controlling their own metabolic fate.

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https://doi.org/10.1079/PNS2002169 Published online by Cambridge University Press

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https://doi.org/10.1079/PNS2002169 Published online by Cambridge University Press

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