Role of long-chain fatty acids in the postnatal induction of genes coding for liver mitochondrial \(\beta\)-oxidative enzymes
J.-P. Pegorier*, F. Chatelain, S. Thumelin and J. Girard
Endocrinologie, Métabolisme et Développement, CNRS UPR 1524, 9 rue J. Hetzel, 92190 Meudon-Bellevue, France

Introduction
The regulation of specific gene expression in response to changes in nutrition has become a major aspect of modern nutrition, due to the development of molecular biological techniques that have allowed the cloning of a number of genes involved in the regulation of carbohydrate and fat metabolism. It has been shown that major (glucose, fatty acids, amino acids) or minor (iron, vitamins) dietary constituents participate, in concert with many hormones, in the regulation of gene expression in response to nutritional changes (reviewed in [1]). This report is focused on the fetal–neonatal transition during which dramatic nutritional changes occur spontaneously and during which enzymes catalysing rate-limiting steps of fatty acid oxidation increase in the liver.

The perinatal period is attended by marked changes in the nutritional environment. In utero, the fetus is continuously supplied through the placenta with a diet rich in carbohydrates and amino acids and poor in fat (reviewed in [1]). Immediately after birth, the maternal supply of substrates ceases abruptly, and the newborn has to withstand a brief period of starvation before he is fed at intervals with milk which is a high fat (60% energy) and low carbohydrate (10% energy) diet. To meet the energy needs of the newborn, the capacity for long-chain fatty acid (LCFA) oxidation increases rapidly in many tissues (liver, heart, skeletal muscles, lung, kidney cortex, small intestine, brown adipose tissue). In the liver, the increased oxidation of LCFA results in an enhanced rate of ketone body production.

In the mammalian liver, fatty acid oxidation can occur both in mitochondria and peroxisomes. During the last decade, the role and importance of peroxisomes have received considerable attention after the discovery that hypolipidemic drugs induce the peroxisome proliferation and after the identification of peroxisomal diseases in human (reviewed [2]). During the fetal–neonatal transition, there is an increase in the number of peroxisomes and in the activity of specific enzymes [3]. However, the contribution of peroxisomal \(\beta\)-oxidation represents only 10–15% of overall \(\beta\)-oxidation in the neonatal rat liver [4,5].

The postnatal development of mitochondrial LCFA oxidation and ketogenesis in the liver is closely related to the appearance of the carnitine palmitoyltransferase (CPT) system and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase. The CPT system allows the transfer of LCFA inside the mitochondria and is composed of three distinct entities: CPTI localized in the outer mitochondrial membrane, carnitine-acyl-carnitine translocase and CPTII localized in the inner mitochondrial membrane (reviewed in [6]; Figure 1). In this transfer system, CPTI represents the main site of control for the entry of LCFA into the mitochondria [6] and mitochondrial HMG-CoA synthase is the rate-limiting enzyme of the ketogenic pathway (i.e. the conversion of acetyl-CoA into ketone bodies [7]). However, if one considers ketogenesis as the metabolic pathway converting LCFA into ketone bodies, then the CPT system is the rate-limiting step because it controls the supply of acyl-CoA for \(\beta\)-oxidation and acetyl-CoA for ketogenesis [8]. Indeed, in the adult rat liver the flux control coefficient of CPTI over ketone body formation is very high (close to 1) irrespective of the nutritional state of the animal [9]. During the suckling period, CPTI exerts also a potent control over LCFA oxidation and ketogenesis but the flux control coefficient is slightly lower than in adult rat liver (0.9 versus 1.0) suggesting that other enzymes could participate in the control over pathway flux [10].

Liver CPTI and CPTII gene expression during the fetal–neonatal transition
The activity of CPTI is very low in fetal rat liver and increases markedly during the first 24 h of
extra-uterine life, whereas the activity of CPTII is already high in the fetal liver and does not change after birth [11,12]. This developmental pattern in CPTI and CPTII maximal activities parallels that observed for immunoreactive protein concentrations [12,13]. The postnatal increase in the amount of CPTI results from a stimulation of gene transcription [14], which leads to a marked accumulation of hepatic CPTI mRNA concentration during the first 24 h following birth [12,15] (Figure 2). The liver CPTII mRNA [12,15], immunoreactive protein [12,13] and activity remain remarkably constant during development and are not influenced by nutritional and hormonal changes that occur in the immediate postnatal period (reviewed in [1]).

**Liver mitochondrial HMG-CoA synthase gene expression during the fetal-neonatal transition**

Once inside the mitochondria, LCFA are rapidly oxidized into the β-oxidation pathway, but the metabolic fate of acetyl-CoA produced (Krebs cycle versus ketone body production) is partly dependent upon the activity of mitochondrial HMG-CoA synthase, the rate-limiting enzyme in the HMG-CoA pathway (Figure 1). The activity of mitochondrial HMG-CoA synthase increases during the immediate postnatal period (reviewed in [1,16]). In the fetal rat liver the mRNA encoding mitochondrial HMG-CoA synthase are not detectable before day 18 of pregnancy and then increase slightly until day 21 [17]. This increase

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**Figure 1**

Schematic representation of the mitochondrial metabolism of long-chain fatty acids in the liver

LCFA, long-chain fatty acids; CPT, carnitine palmitoyltransferase; HMG-CoA, hydroxymethylglutaryl-CoA.
Developmental changes in mitochondrial CPTI, CPTII and HMG-CoA synthase gene expression in the rat liver

Results are expressed in arbitrary units, the reference value (1) being the level of mRNA coding for each gene at birth. Results are means ± S.E.M. of four to six different experiments. Data from [12, 17].

Evidence for a role of LCFA in the control of liver mitochondrial CPTI and HMG-CoA synthase gene expression

Modifications of fatty acid metabolism in the postnatal period are attended by changes in plasma free fatty acid (FFA), carnitine and pancreatic hormone concentrations (reviewed in [1, 16]). As the aim of this paper is to review the role of nutrients in the regulation of mitochondrial β-oxidative enzyme gene expression, the role of pancreatic hormones in the control of CPTI, CPTII and HMG-CoA synthase gene expression will be described briefly.

Addition of glucagon or cyclic AMP increases the accumulation of CPTI and HMG-CoA synthase mRNA in cultured hepatocytes in a dose-dependent manner [14, 17]. The accumulation of CPTI mRNA in response to cAMP results from a stimulation of transcription without any effect on the half-life of CPTI mRNA [14]. Insulin antagonizes the effects of cAMP suggesting that the postnatal decrease in plasma insulin concentration potentiates the effect of increased plasma glucagon and liver cAMP concentrations. By contrast, neither cAMP nor insulin affect the CPTII gene expression. This is in agreement with the absence of the C/EBPα or CREB binding site in the promoter region of this gene [20]. Interestingly, the postnatal increase in liver CPTI mRNA concentration is delayed in 6-h-old fasting rats when compared to their suckling counterparts [15], although their hormonal environment is similar (reviewed in [1]). This suggests that some factors contained in the milk affect CPTI gene transcription. Among these factors, carnitine and FFA are good candidates, their concentration being very low in the fasting newborn rats, as during the immediate postnatal period these substrates are only provided by milk (reviewed in [1]). Indeed, addition of carnitine to cultured hepatocytes from fetal rats has no effect on CPTI gene expression [14]. Interestingly, medium-chain fatty acids, whose abundance is particularly important in milk triglycerides from various species (reviewed in [1]), have no effect on CPTI gene expression in fetal rat hepatocytes [14] (Figure 3). Similar observations have been made in hepatocytes from older animals (reviewed in [21]). By contrast, LCFA induce the accumulation of CPTI mRNA in fetal rat hepatocytes as the result of both a stimulation in gene transcription and a stabilization of mRNA [14]. LCFA do not induce the accumulation of CPTII mRNA [14] (Figure 3). Whereas the repressive effects of LCFA on hepatic lipogenic genes appear to be restricted to polyunsaturated LCFA (reviewed in [22, 23]), the stimulatory effect of LCFA on CPTI gene transcription is independent of the degree of saturation of the fatty acids [14] (Figure 3). Similar effects of LCFA have been found for other genes: mitochondrial HMG-CoA synthase [24], long-chain acyl-CoA synthetase (ACS; F. Chatelain, J. Girard and J.-P. Pégorier, unpublished work), fatty-acid binding protein [25] and medium-chain acyl-CoA dehydrogenase [26]. At least two lines of evidence suggest that mitochondrial metabolism of LCFA is not required for the activation of gene expression. Firstly, the addition of 2-bromopalmitate, a non-metabolizable analogue of palmitate, to cultured hepatocytes from fetal rats is more active than
Effects of various fatty acids and clofibrate on CPTI, CPTII and HMG-CoA synthase gene expression in cultured fetal rat hepatocytes

20-day-old fetal hepatocytes were cultured for 48 h in the presence of various fatty acids and clofibrate (a potent peroxisome proliferator) at a final concentration of 0.5 mM. Results are expressed in arbitrary units, the reference value (1) being the concentration of mRNA coding for each gene after 48 h of culture in basal condition. Results are means ± S.E.M. of four to six different cultures. Data from [14]. Legend: open bars, CPTII; filled bars, CPTI; hatched bars, HMG-CoA synthase.

Figure 4
Effects of non-metabolizable fatty acid or inhibitor of fatty acid oxidation on CPTI gene expression in cultured fetal hepatocytes

Results are expressed in arbitrary units, the reference value (1) being the concentration of CPTI mRNA after 48 h of culture in basal condition. Results are means ± S.E.M. of five different cultures. Data from [14]. Palm, palmitate; TDGA, 2-tetradecylglycidic acid; Lino, linoleate.

Respective role of free fatty acids and their CoA esters in the induction of mitochondrial enzyme gene expression

When fatty acid metabolism is blocked by TDGA in cultured fetal hepatocytes, this leads to the accumulation of long-chain acyl-CoA because the fetal hepatocyte has a low capacity for esterification [27]. This suggests that acyl-CoA could be the metabolite responsible for the induction of CPTI, HMG-CoA synthase or ACS gene expression in fetal liver cells. The role of acyl-CoA in the regulation of gene transcription has been clearly established in bacteria. For instance when Escherichia coli is cultured in the presence of LCFA, transport inside the cell by Fad L is coupled to the acyl-CoA synthetase protein (FadD) thus leading to a rapid activation of LCFA into acyl-CoA (reviewed in [28]; Figure 5). Then, an acyl-CoA binding-like protein (FadR) binds acyl-CoA which induces a conformational change of the protein [29]. FadR is a transcriptional factor that controls the fad regulon (genes coding for enzymes involved in transport, activation and β-oxidation of LCFA) and the fabA gene which is required for unsaturated fatty acid biosynthesis (reviewed in [29];
In the absence of LCFA in the culture medium, FadR binds to specific DNA sequences in such a manner that the \( fad \) regulon is repressed and the \( fabA \) gene is induced. By contrast, when LCFA are present in the culture medium, the conformational change of FadR induced by acyl-CoA binding, prevented the association of FadR with the DNA leading to an activation of the \( fad \) regulon and to the repression of the \( fabA \) gene (reviewed in [28]; Figure 5). However, despite similarities with mammalian cells in which LCFA induce \( \beta \)-oxidative enzyme genes (reviewed in [30,31]) and repress lipogenic enzyme genes (reviewed in [32]), the role of long-chain acyl-CoA in the regulation of gene transcription is still controversial. For instance, the gene encoding \( \Delta^9 \) desaturase (\( OLE1 \)) is markedly repressed by unsaturated LCFA [33,34] in lower eukaryotes (yeast \( Saccharomyces cerevisiae \)). When the gene coding for acyl-CoA binding protein (\( ACBI \)) is disrupted in yeast, the transport of acyl-CoA into various cell compartments is blocked and the expression of the \( OLE1 \) gene is up-regulated [35]. Similarly, in yeast the repression of acetyl-CoA carboxylase by LCFA is blocked by mutation of long-chain acyl-CoA synthetase [36]. This suggests that acyl-CoA esters are the components that are active on gene transcription. To what extent does such a regulation of gene expression by acyl-CoA occur in higher eukaryotes (mammals)? To our knowledge, there is no clear-cut demonstration allowing discrimination between FFA or their corresponding CoA esters as modulators of gene expression. However, indirect evidence suggests that FFA rather than acyl-CoA esters could be the components that are active on gene expression. Firstly, in COS-7 cells transfected with the peroxisomal acyl-CoA oxidase promoter linked to chloramphenicol acetyltransferase (CAT) reporter gene, transcription was enhanced more by free arachidonic acid than it was by arachido-

**Figure 5**

Schematic representation of the role of long-chain fatty acids in the control of gene expression in mammalian cells and in bacteria

PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator-responsive element; RXR, retinoic acid receptor. Fad D is an acyl-CoA synthetase-like protein and Fad R is a transcriptional factor that binds acyl-CoA. The \( fabA \) gene is required for the biosynthesis of unsaturated fatty acids; the \( fad \) regulon contains genes encoding enzymes involved in transport, activation and \( \beta \)-oxidation of LCFA. These schemes are adapted from [28,30].

**Mammalian cells**

**E. Coli**

<table>
<thead>
<tr>
<th>Without fatty acid</th>
<th>Long-chain fatty acids</th>
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<tbody>
<tr>
<td>Fad R</td>
<td>Fad R</td>
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<tr>
<td>( \text{Acyl-CoA} )</td>
<td>( \text{CoA-Acyl} )</td>
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**Transcription**

- \( \text{fabA gene} + \)
- \( \text{fad regulon} - \)
- \( \text{fabA gene} - \)
- \( \text{fad regulon} + \)
nel-CoA [37]. Secondly, in the Ob 1771 adipocyte cell line, the LCFA-induced aP2 gene transcription (an adipocyte fatty acid binding protein) precedes the expression of the gene encoding long-chain acyl-CoA synthetase (reviewed in [38]). Finally, in Fao hepatoma cells, when long-chain acyl-CoA synthetase activity was decreased by more than 90% by specific inhibitors, the stimulation of CPTI gene expression by LCFA was similar to that found in the absence of ACS inhibitors (F. Chatelain, J. Girard and J.-P. Pégorier, unpublished work).

It is clear that all these experiments, using chimaeric gene transfection, metabolic inhibitors or in vitro assays, provide only indirect evidence for the role of FFA. However, additional work is necessary to conclude whether FFA or their CoA esters are the active modulators of gene transcription.

**Contribution of nuclear receptors in the LCFA-induced gene expression**

The molecular mechanisms by which FFA or fatty acyl-CoA stimulate gene transcription have not yet been identified in mammalian cells. Based on the comparison between the effects of LCFA and peroxisome proliferators it was suggested that LCFA could regulate gene transcription through the activation of nuclear receptors of the steroid-thyroid superfamily, the peroxisome proliferator-activated receptor (PPAR; reviewed in [30–32]; Figure 5). The modulation of gene transcription is due to the binding of the heterodimer PPAR/RXR (retinoic acid receptor) to specific DNA sequences, the peroxisome proliferator-responsive element (reviewed in [30–32]; Figure 5). In keeping with this, it has been shown that LCFA stimulate the transcription of a DNA construct consisting of HMG-CoA synthase promoter linked to a CAT reporter gene in transfected HepG2 hepatoma cells [39]. However, this stimulation by LCFA required co-transfection with a PPAR-α DNA construct. Indeed, the overexpression of nuclear receptors does not necessarily reflect physiologically relevant processes. As mentioned by the authors of this work [39], if the transcriptional effect of LCFA is due to the synthesis of a putative ligand of PPAR-α, it is difficult to understand why the LCFA-induced HMG-CoA synthase–CAT chimaeric gene does not occur from endogenous PPAR-α. Indeed, the role of PPAR in mediating transcriptional effects of LCFA is still a subject of debate. For instance, it was shown that the repression of hepatic Spot 14 gene transcription (a gene coding for a lipogenic-like enzyme) by polyunsaturated LCFA was not mediated through the activation of PPAR-α [40]. This was recently confirmed using the PPAR-α null mouse in which the induction of peroxisomal β-oxidative genes by polyunsaturated LCFA (which are also potent peroxisome proliferators) was impaired, whereas the inhibition of Spot 14 or fatty acid synthase gene expression occurred normally [41].

Concerning the regulation of CPTI gene expression by LCFA in fetal rat hepatocytes, indirect evidence suggests that their mode of action could be different from that of peroxisome proliferators. Firstly, LCFA induce CPTI but not CPTII gene expression whereas clofibrate (a peroxisome proliferator) enhances both CPTI and CPTII mRNA concentration in the cultured rat hepatocytes [14] (Figure 3). Secondly, the LCFA-induced CPTI mRNA accumulation is impaired by insulin, whereas the clofibrate-induced CPTI and CPTII gene expression is not inhibited by insulin [14]. Thirdly, when Fao hepatoma cells are cultured in the presence of lipoxygenase inhibitors, the clofibrate-induced CPTI gene expression is abolished, whereas LCFA still efficiently induced CPTI mRNA accumulation (F. Chatelain, J. Girard and J.-P. Pégorier unpublished work). Indeed, the lipoxygenase pathway leads to the synthesis of leukotrienes from eicosanoids, the leukotriene B4 being a putative ligand of PPAR-α [42], the predominant subtype expressed in the liver [43]. If this is true in hepatoma cells, it suggests that PPAR-α is not involved in the effects of LCFA on CPTI gene expression.

**Post-transcriptional regulation of mitochondrial CPT and HMG-CoA synthase activities by metabolic effectors**

Although this aspect of regulation is not the purpose of the present review, it must be underlined that following gene expression, protein translation and transport to the outer mitochondrial membrane (CPTI) or mitochondrial matrix (HMG-CoA synthase) and the activities of these enzymes are finely regulated by metabolic effectors. Such mechanisms of regulation are particularly important during the fetal–neonatal transition since they amplify the role of pancreatic hormones in the enhancement of hepatic...
fatty acid oxidation and ketogenesis. This will be briefly reviewed.

**Regulation of CPTI**

LCFA play a pivotal role in the post-transcriptional regulation of CPTI during the immediate postnatal period. In concert with pancreatic hormones, they inhibit the activities and expression of genes encoding key lipogenic enzymes (reviewed in [21]). This allows the maintenance of a low intrahepatic malonyl-CoA concentration, a lipogenic intermediate and a potent inhibitor of CPTI activity [44]. Moreover, the inhibitory effect of malonyl-CoA on CPT (so-called sensitivity of CPTI to malonyl-CoA inhibition) is markedly decreased in the first 24 h after birth (reviewed in [1,16]). Glucagon and cAMP decrease the sensitivity of CPTI to malonyl-CoA inhibition in fetal rabbit hepatocytes, whereas insulin antagonizes their effect [45]. Although the molecular mechanisms responsible for the changes in CPTI sensitivity to malonyl-CoA inhibition are still unknown, recent experiments using heterologous expression of CPTI in yeast [46] and analysis of the topology of the enzyme [47] have provided evidence showing that malonyl-CoA binds to the cytosolic N-terminal region of CPTI.

**Regulation of HMG-CoA synthase**

The activity of mitochondrial HMG-CoA synthase is inhibited by succinyl-CoA through a succinylation/desuccinylation process (reviewed in [1,16]). During postnatal development of ketogenesis, glucagon decreases the mitochondrial succinyl-CoA concentration leading to desuccinylation and subsequent activation of mitochondrial HMG-CoA synthase [48].

**Conclusions and perspectives**

The regulation of specific gene expression in response to changes in nutrition has become a major aspect of modern nutrition studies. The regulation of the genes encoding β-oxidative enzymes during the perinatal period represents a very suitable model since: (i) most of these genes have never been expressed before birth (CPTI, HMG-CoA synthase); (ii) the nutritional changes occur rapidly with a large amplitude. Here we report that long-chain fatty acids in concert with pancreatic hormones (increase in plasma glucagon and decrease in plasma insulin concentrations) enhance the expression of genes coding for key mitochondrial β-oxidative enzymes in neonatal rat liver. The molecular mechanisms by which glucagon (through cAMP), insulin and LCFA regulate transcriptional and post-transcriptional events remain to be fully established. Further investigations will be necessary to characterize the intracellular metabolites (FFA or acyl-CoA esters), the transcription factors and the DNA sequences responsible for the transcriptional activation of mitochondrial β-oxidative enzyme genes by LCFA.

Neonatal brown adipose tissue, UCP1 and the novel uncoupling proteins

D. Ricquier

Centre de Recherche sur l'Endocrinologie Moléculaire et le Développement/Centre National de la Recherche Scientifique, 9 rue Jules Hetzel, 92190 Meudon, France

The existence of fat deposits of a brownish colour, distinct from white adipose tissue deposits, located at various sites in the body of mammals has been recognized for many years. This tissue has been referred to as brown adipose tissue (BAT), and its multilocular lipid storing cells have been referred to as brown adipocytes. The thermogenic function of BAT in newborn rabbits, cold acclimated rats and arousing hibernators was discovered in 1965 (reviewed in [1]). Since this date, intensive research, the main aims of which were the appreciation of the physiological role of BAT and the elucidation of the biochemical mechanisms of heat production, has been carried out.

Research on BAT demonstrated that the brown adipocytes are different from white adipocytes and consist of specialized thermogenic cells. The brown adipocytes have particular locations in the body and they are characterized by

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Abbreviations used: BAT, brown adipose tissue; UCP, uncoupling protein; AR, adrenoreceptor.

References


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