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Can the liver X receptor work its magic in skeletal muscle too?

Published online: 10 March 2006
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Abbreviations apo: apolipoprotein · LXR: liver X receptor · PK: protein kinase · PPAR: peroxisome proliferator-activated receptor · SREBP: sterol regulatory element binding protein · UCP1: uncoupling protein 1

Liver X receptors (LXRs) are members of the nuclear receptor superfamily. As with other nuclear receptors, LXRs alter cellular responses by regulating gene expression. LXRs form obligate heterodimers with the retinoid X receptor, and are activated by physiological concentrations of oxysterols and intermediates in the cholesterol synthetic pathway. LXRs play a key role in regulating whole-body cholesterol and lipid metabolism. Two isoforms have been identified, LXR β , which has a fairly ubiquitous pattern of expression [1], and LXR α , whose expression is largely restricted to tissues known to play important roles in lipid metabolism, the liver in particular (as the name would suggest), but also macrophages, kidney, spleen and adipose tissue [2].

A key effect of LXRs on whole-body cholesterol levels is the initiation of a process called reverse cholesterol transport. This involves cholesterol efflux from peripheral tissues (a process requiring transmembrane proteins of the ABC family), and HDL-mediated transport of cholesterol to the liver, from which it is secreted directly into bile or converted into bile acids. LXRs enhance the expression of the ABCA1 protein, which is critical for cholesterol efflux [3], as well as several lipoproteins (apolipoprotein E [apoE], apoC1, apoCII, apoCIV) that serve as acceptors of ABCA1-mediated cholesterol efflux. In addition to its effects on

cholesterol, activated LXRs antagonise the expression of several inflammatory genes, such as those encoding inducible nitric oxide synthase, cyclooxygenase-2 and interleukin-6, in activated macrophages. This dual anti-inflammatory and cholesterol-lowering effect has focused attention on LXR activators in the treatment and prevention of atherosclerosis [4].

Activated LXR has a few more tricks up its sleeve, and another key target gene is *SREBF1*, which encodes sterol regulatory element binding protein 1c (SREBP1c). SREBPs are synthesised as membrane-bound precursors and cleaved to generate an active nuclear form; this class of transcription factors plays a major role in regulating genes involved in fatty acid and cholesterol synthesis. LXRs stimulate de novo fatty acid synthesis and serum triglycerides via induction of *SREBF1* expression, and also have direct effects on the expression of the genes encoding fatty acid synthase and plasma phospholipid transfer protein.

Given the central role of LXRs in lipid and cholesterol metabolism, possible effects on glucose homeostasis are beginning to be explored. Synthetic LXR ligands have been shown to improve glucose tolerance in mice with diet-induced obesity [5]. In this issue of *Diabetologia*, Cozzone and colleagues [6] demonstrate that the genes for LXR α and LXR β are expressed in human skeletal muscle and adipose tissue, and that levels of LXR α and LXR β mRNA in tissues from type 2 diabetic patients are not different from those in tissues from healthy subjects. Using cultured human muscle myotubes, they show that activation of LXR using a synthetic agonist leads to an increase in the expression of LXR target genes, such as those for SREBP1c, fatty acid synthase and stearoyl-CoA desaturase 1. Furthermore, activation of LXR enhanced triglyceride accumulation in myotubes grown in hyperglycaemic (but not normoglycaemic) conditions. Somewhat surprisingly, neither the accumulation of triglycerides nor the activation of the LXR per se had any effect on insulin action as assessed by insulin-mediated phosphorylation of protein kinase B (PKB)/Akt, thus indicating the absence of the

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usually noted correlation between increased muscle triglycerides and reduced insulin action. Recent studies have suggested that local accumulation of fat metabolites inside skeletal muscle may activate a serine kinase cascade involving PKC- θ , leading to defects in insulin signalling and glucose transport in skeletal muscle [7].

What is the fate of the LXR-mediated accumulation of lipids in the muscle? In a similar series of experiments, Kase and colleagues [8] report that chronic exposure of human muscle cells to an LXR agonist leads to an increase in both fatty acid uptake and subsequent oxidation. Expression of uncoupling protein 2 and 3 was elevated in muscle cells exposed to LXR agonists, suggesting that a greater proportion of energy may be dissipated as heat. Despite the increased uncoupling and fat oxidation, LXR-agonist-treated cells also showed increased net muscle lipid accumulation. LXR activation in skeletal muscle thus appears to increase uptake, synthesis, utilisation and storage of lipid (Fig. 1).

Does activation of LXR also lead to direct effects of glucose metabolism? Cozzone and colleagues report that LXR activation directly increases glucose incorporation into glycogen in human myotubes [6], and work by Kase et al. [8] shows enhanced glucose uptake and glucose oxidation. These effects may be explained in part by increased LXR-mediated expression of GLUT4, the insulin-sensitive glucose transporter [8]. An LXR-responsive element has been identified in the promoter of the gene for GLUT4 (*SLC2A1*) [9], and in vivo treatment of mice with LXR agonists enhances GLUT4 expression in adipose tissue, but not in skeletal muscle [5]. This may have clinical relevance, because people with type 2 diabetes have reduced GLUT4 expression in adipose tissue [10]. In vivo, LXR agonists also lead to reduced expression of gluconeogenic genes in the liver [5], and together these effects should lead to reduced hepatic glucose production and increased glucose uptake in the periphery. The enhanced incorporation of glucose into glycogen noted by Cozzone et al. might also result from a glucose sparing effect through an LXR-mediated shift in substrate utilisation in muscle, thus favouring lipid oxidation in a manner reminiscent of the Randle cycle.

Another class of nuclear hormones that has attracted attention in the metabolic field is the peroxisome proliferator-activated receptors (PPARs). PPAR α is the target of the fibrates, and PPAR γ is the target of insulin-

sensitising thiazolidinediones [11]. There is evidence of important molecular crosstalk between the LXR and PPAR pathways. Thus, for example, the gene for LXR α (*NR1H3*) is itself a direct target of PPAR γ in macrophages, and both nuclear receptors cooperate in modulating ABCA1 expression and regulation of cholesterol efflux [12]. In the liver, the fate of lipid metabolism appears to depend on a fine balance between the PPAR α -induced genes mediating fatty acid degradation, and the LXR-induced genes mediating fatty acid synthesis [13, 14]. It appears that PPAR α activation can suppress the LXR-SREBP1c pathway through reduction of LXR/RXR heterodimer formation [14], whereas LXR activation suppresses PPAR α effects by reducing PPAR/RXR heterodimer formation [13]. A clearer understanding of how this balance is regulated may highlight new means for the therapeutic management of lipid metabolism in the liver.

What is the evidence for crosstalk between LXR and insulin-derived signals? Increased expression of *SREPF1* is also a crucial step in insulin regulation of lipid metabolism, and there is evidence that this 'insulin effect' may be mediated via activation of LXR. Insulin injection does not lead to increased liver expression of *SREBP1c* in mice in which both LXR genes have been deleted (double knock-out), suggesting that insulin may regulate *Srebp1* by inducing expression of the LXR α gene (*Nr1h3*) [15]. Although there is evidence that LXR may in some contexts be upregulated by, and required for, certain effects of insulin, oxysterols and intermediates in the cholesterol synthetic pathway remain the likely endogenous ligands. It is not known how insulin directly regulates expression of the LXR genes, and further insight into the pathways involved will improve understanding of the interplay between insulin and LXR on carbohydrate and lipid metabolism. Evidence from studies of in vitro activation of LXR in human muscle cultures [6] suggests that induction of LXR activity does not impact on the classical insulin signalling pathways, despite the increased accumulation of muscle lipids. Whether this means that LXR-induced muscle lipid accumulation is in some way different and does not affect insulin action, or whether an experimentally longer time frame is needed to observe effects on insulin signalling, is still unclear.

The question remains as to whether activation of LXR may help ameliorate metabolic disease. The potent cholesterol-lowering and anti-inflammatory effects of LXR are

Fig. 1 Effects on glucose and lipid metabolism following activation of liver X receptors (LXRs) in skeletal muscle. LXRs promote increased storage and utilisation of nutrients. RXR Retinoid X receptor; FAT fatty acid transporter; LCFA long-chain fatty acid

certainly attractive. Unfortunately, there appear to be some disadvantages to LXR activation. As mentioned above, the potent induction by LXR of *SREBP1c* leads to increased liver and serum triglycerides [16]. Furthermore, genes important for energy homeostasis such as those for leptin and uncoupling protein 1 (UCP1) are also target genes of LXR activation, such that both these genes are down-regulated in mice treated with a synthetic LXR agonist [17]. A reduction in leptin should increase appetite, and a reduction in UCP1 should (at least in rodents) reduce energy expenditure, both factors increasing the risk of obesity. Of interest is the finding that the increase in skeletal muscle lipid accumulation reported by Cozzzone et al. [6] did not have a negative impact on insulin signalling—although LXR activation also failed to improve insulin sensitivity in skeletal muscle cells from people with type 2 diabetes. A further note of caution comes from the observation that the LXR response is enhanced in cultured muscle from type 2 diabetic patients with respect to lipid accumulation, and, in contrast to the effects of LXR in skeletal muscle from healthy subjects, there is no parallel increase in palmitate oxidation [8]. Individuals with type 2 diabetes may therefore have an altered response to LXR such that they store more, and utilise less, of available lipid. Whether this could be overcome by further pharmacological activation of LXR remains to be studied. The long-term consequences of LXR agonists therefore require further evaluation in order to establish whether such treatment will enhance the synthesis and utilisation of lipids whilst sparing glucose, or whether it will perhaps promote obesity without accompanying metabolic derangements in glucose homeostasis and insulin resistance. If this proves to be the case, you really will be able to have your cake and eat it!

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