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Minireview

Links between fatty acids and expression of UCP2 and UCP3 mRNAs

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Abstract Physiological and pathological states that are associated with elevated plasma fatty acids (FAs) increase uncoupling protein 2 (UCP2) mRNA in white adipose tissue and UCP3 mRNA in skeletal muscle and heart. A direct effect of unsaturated fatty acids from all classes has been shown in various cultured cells. There is evidence that FAs could induce expression of UCPs by acting as ligands for peroxisome proliferator-activated receptors, influencing the function of sterol responsive element binding protein or activating 5′-AMP-activated protein kinase. Oleic acid has been shown to stimulate the activity of the promoter regions of UCP2 and UCP3 genes and the FA responsive regions are beginning to be characterised.

Keywords: Fatty acid; Uncoupling protein; Peroxisome proliferator-activated receptor; Sterol responsive element binding protein; AMP-activated protein kinase; White adipose tissue: skeletal muscle

1. UCP2 and UCP3

UCP2 and UCP3, members of the uncoupling family of proteins, are of interest because of their potential roles in regulating energy expenditure and thus contributing to obesity and type 2 diabetes, and hypermetabolic states such as those associated with sepsis, cancer cachexia and hyperthyroidism.

The uncoupled proteins (UCPs) are found in the inner mitochondrial membrane and are able to uncouple the oxidation of fuels via the electron transport chain from ATP synthesis, thus dissipating energy as heat and potentially affecting metabolic efficiency. The most well-known member of the family is UCP1, which is uniquely expressed in brown adipose tissue and plays an important role in cold- and diet-induced thermogenesis (reviewed in [1]). UCP2 and UCP3 were first cloned and identified in humans in 1997 and subsequently in rodents [1]. They share about 56% amino acid identity with UCP1 and are themselves about 72% identical. UCP2 and UCP3 do not appear to be solely involved in thermogenesis, and have proposed roles in modulating generation of reactive oxygen species and in lipid handling, although their physiological roles have not yet been definitively determined. UCP2 mRNA is found in many tissues and at high levels in white adipose tissue, skeletal muscle, spleen and pancreatic β-cells, whereas UCP3 is predominantly expressed in skeletal muscle, heart, and to a lesser extent adipose tissue. This expression pattern is consistent with their proposed roles since adipose tissue and skeletal muscle are major contributors to overall energy metabolism. Variations in activity or regulation of UCP2 and UCP3 in these tissues could contribute to obesity and associated diseases.

Considerable effort has gone into determining how the expression of UCP2 and UCP3 is regulated. It turns out that the various physiological and pathological states that are associated with raised levels of UCP2 and UCP3 mRNAs in adipose tissue and skeletal muscle are all characterised by elevated plasma fatty acid (FA) levels. This has led to the hypothesis that FA themselves are the molecular signals that bring about the changes in UCP2 and UCP3 expression.

Understanding this link between FA and UCP2 and UCP3 expression could provide new information about how processes related to energy metabolism are controlled in health and disease. Here, we review the evidence for this link and the progress towards determining the molecular mechanisms by which FA bring about transcriptional upregulation of UCP2 and UCP3 mRNA expression (overview in Fig. 1). Functional consequences of such regulation of their mRNA levels are not known at this stage and would depend on protein levels and activities.

2. In vivo regulation of UCP2 and UCP3 is linked with FAs

A number of physiological and pathological states lead to increased expression of UCP2 and UCP3 mRNAs. These include fasting [2–13], high-fat diets [14–19], suckling of newborn pups [20], sepsis [21,22], acute endurance exercise [23–27], and hyperthyroidism (reviewed in [28]), as well as experimental manipulations such as lipid infusion [5,21,29,30] and streptozotocin-induced diabetes [11,32,33]. A common feature of all these conditions is a 2- to 3-fold elevation in plasma FA. The effects of these conditions on the expression of UCP2 and UCP3 mRNAs in various tissues are summarised in Table 1.

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Abbreviations: AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK, 5′-AMP-activated protein kinase; FA, fatty acids; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:4, arachidonic acid; 20:5, eicosapentaenoic acid; PPAR, peroxisome proliferator-activated receptor; SRE, sterol responsive element; SREBP, sterol responsive element binding protein; UCP, uncoupling protein; USF, upstream stimulatory factor; 5′-USR, 5′-upstream region

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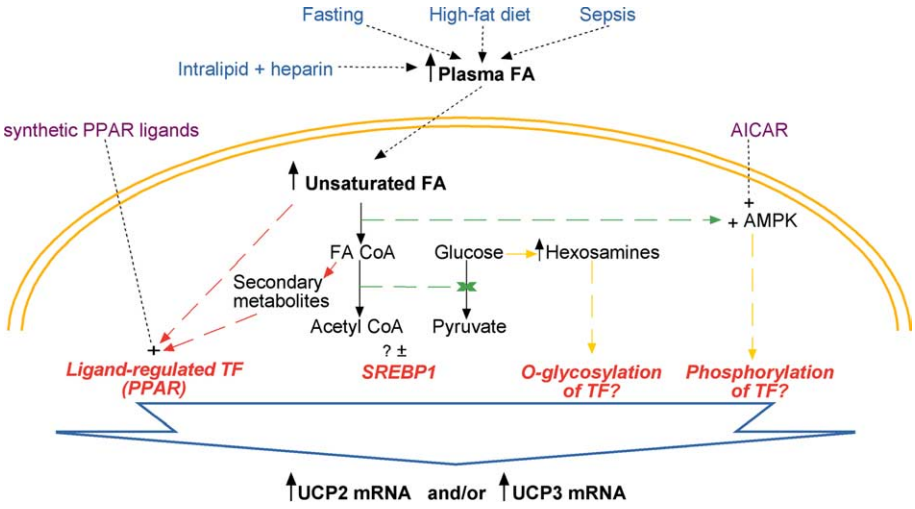

Fig. 1. Molecular routes by which FA could increase the expression of UCP2 and UCP3 mRNAs. Physiological and experimental manipulations (blue) that elevate plasma FA also increase UCP2 and UCP3 mRNAs in a tissue-dependent manner. The changes in mRNA levels need not correlate with changes in protein levels; possible post-transcriptional regulation remains to be studied. Experimental activation of PPARs and AMPK (purple) also increases UCP2 and UCP3 mRNAs. Unsaturated FA could work by activating certain PPARs, influencing SREBP1 function, or via other, as yet unidentified, transcription factors (TF) by stimulating AMPK or the hexosamine pathway.

Table 1
Change in expression of UCP2 and UCP3 mRNAs in response to physiological and experimental states in vivo

Condition†		Skeletal muscle	Heart	White adipose tissue	Brown adipose tissue	Liver
Fasting	UCP2	↔[3,5], ↑ [2,6,8,10]	↔ [2,11,12]	↓ [13]	↔ [3,9], ↑ [8]	↔ [13], ↑ [8]
	UCP3	↑ [2–10]	↑ [11]		↓ [3,9]	
High-fat diet	UCP2	↔ [15–18]		↑ [14–18]	↔ [15]	↑ [17,19]
	UCP3	↑ [15–17], ↔ [18]		↔ [15–17]	↔ [15]	
Streptozotocin	UCP2	↔ [33], ↑ [31,32]	↔ [11,12]	↑ [32], ↔ [31]	↑ [32], ↔ [31]	
induced diabetes	UCP3	↑ [31–33]	↑ [11]	↔ [32]	↔ [32], ↓ [31]	
Sepsis and inflammation	UCP2	↑ [22]		↑ [22]		↑ [22]
	UCP3	↑ [21]				
Acute exercise	UCP2	↔ [24,27]*		↔ [23]		
	UCP3	↑ [23-25,25]				
Intralipid infusion	UCP2	↔ [29]*		↔ [29]*, ↑ [30]*		
	UCP3	↑ [5,21,29]*		↑ [30]*		

Change of expression of mRNA: ↑, increased; ↓, decreased; ↔, no change; *, human subjects; † all these conditions are associated with a 2- to 3-fold elevation of plasma FA. References are given in parentheses.

There are some conflicting results, which probably arise from differences in muscle types sampled, nature of the dietary fats, length of feeding regimes or strains of animals. Despite the incomplete or conflicting reports, taken together there seem to be tissue-dependent trends. In skeletal muscle and heart, the trend is for increased UCP3 mRNA with little change of UCP2 mRNA, whereas in white adipose tissue, the trend is for increased UCP2 mRNA with little change in UCP3 mRNA. For brown adipose tissue, the trend is for decreased UCP3 mRNA. Similar observations have also been made in the limited number of studies on human subjects. For example, infusion with intralipid [29] or acute endurance exercise [27] increased UCP3 mRNA with no change in UCP2 mRNA in skeletal muscle biopsies from human subjects. A positive linear correlation was found between plasma FA and UCP3 mRNA in muscle biopsies from obese human subjects, but not with UCP2 mRNA [34,35].

All these in vivo studies point to a link between FA and expression of UCP2 and UCP3 that occurs in a tissue-dependent manner. This may be related to distinct physiological roles of UCP2 and UCP3 in different tissues.

3. Fatty acids directly affect expression of UCP2 and UCP3 in cultured cells

Studies conducted in vitro using cultured cell models or primary isolated cells provide the most convincing support for a direct effect of FA or their metabolic derivatives on UCP2 and UCP3 expression. These studies are summarised in Table 2.

Myoblast cell lines derived from mouse (C2C12), rat (L6) and human muscle can be induced to differentiate into myotubes and are commonly used as in vitro models of muscle. Many studies using myotubes have shown that the mono-unsaturated 18:1($n-9$) and polyunsaturated FA of both the $n-6$ and $n-3$ class, added directly to the culture medium at physiological concentrations, increase the expression of UCP3 [7,35–40], with no increase in UCP2 mRNA [35,40]. These findings parallel the pattern seen most commonly in skeletal muscle in vivo. However, others found that downstream metabolites of linoleic acid (18:2), linolenic acid (γ18:3), 20:3 and arachidonic acid (20:4) did induce UCP2 in human primary myotubes, whereas 18:2 itself did not [42]. (UCP3 was not measured in these studies.)

Table 2
Observed effects of different classes of FAs on expression of UCP2 and UCP3 mRNAs in cultured cells

		UCP2	UCP3
	16:0	↑INS-1* [46]	
$n-9$	18:1	↔ hSM [42]	↑ hSM [35]
		↔ C2C12 [40]	↑ C2C12 [7,40]
		↑ 3T3-L1 [43]	↑ L6 [36–39]
		↑INS-1 [46,47]	
		↑ hepatocytes [45]	
		↑ cardiomyocytes [12]	
$n-6$	18:2	↔ hSM [35,42]	↑ hSM [35]
		↔ C2C12 [40]	↑ C2C12 [40]
		↑ L6 [41]	↑ L6 [38]
		↑ 3T3-L1 [43]	
		↑ Ob1771 [44]	
	γ18:3	↑ hSM [42]	
	20:3	↑ hSM [42]	
	20:4	↑ hSM [42]	
		↑hepatocytes [45]	
$n-3$	α18:3	↔ C2C12 [40]	↑ C2C12 [40]
		↑ 3T3-L1 [43]	
	20:5	↑ 3T3-L1 [43]	
		↑hepatocytes [45]	

Expression of UCP2 or UCP3: ↑, increased; ↔, no change. Cell lines: hSM, human skeletal muscle myotubes; C2C12, mouse myotubes; L6, rat myotubes; 3T3-L1 and Ob1771, preadipocytes; INS-1, β-cell line; *, no other cell lines have been reported to show upregulation of UCP2 or UCP3 in response to saturated FA. References are given in parentheses.

In pre-adipocyte cell lines, unsaturated FA representative of each of the different classes ($n-9, n-6, n-3$) markedly induced UCP2 mRNA [43,44]. A number of other cultured cell systems representing heart, liver, and pancreatic islets also responded to addition of various FA to the culture medium with increased levels of UCP2 mRNA [12,45–47]. The INS-1 cell line, derived from islet β-cells, is the only one so far to show responsiveness to a saturated FA [46].

The response to FA is most likely due to increased transcription. At least one study has shown that addition of actinomycin D, an inhibitor of transcription, prevented the increase of UCP2 mRNA in 3T3-L1 pre-adipocytes by 18:2, with no effect on turnover [43]. It is possible that actinomycin D prevented transcription of other FA-responsive genes that are required for regulation of expression of UCPs. Additional post-transcriptional regulation by FA cannot be ruled out.

From these studies, it is clear that all classes of unsaturated FA and/or their metabolism can directly bring about the upregulation of UCP2 and UCP3 mRNAs in cultured cells. This apparent lack of specificity for the FA that cause induction seems surprising. It suggests that FA per se are the regulators rather than a specific secondary metabolite derived from a particular pathway. The response is dependent upon the cell type and supports the likelihood of tissue-specific differences in the signalling mechanisms regulating UCP2 and UCP3 expression in response to FA. The tissue-specific complement of transcription factors and/or unique response elements in the regulatory promoter regions of the UCP2 and UCP3 genes could contribute to these distinct patterns of regulation.

4. Do fatty acids work via PPARs?

A family of transcription factors that could be involved in mediating the effect of FA are the peroxisomal proliferator-activated receptors (PPARs). There is already good evidence that FA induce transcription of a cohort of genes involved in lipid oxidation in liver and adipose tissue by acting as ligands of PPARs [48]. It is possible that UCP2 and UCP3 belong to this cohort of genes as their increased expression could potentially increase FA oxidation. Upregulation of UCPs by FA might also be via PPARs. All the classes of unsaturated FA ($n-9, n-6, n-3$) that have been shown to induce either UCP2 or UCP3 in at least one in vitro experimental cell culture system have also been shown to act both as ligands and activators for all three main isoforms of PPARs [49]. Other known synthetic PPAR ligands are able to upregulate UCP2 and UCP3. The pattern of upregulation by selective PPAR ligands in cultured cells matches the predominant tissue-selective expression of the PPAR isoforms: via PPARγ in adipose tissue [44,50–52], via PPARδ in muscle [36,38,53] and via PPARα in liver [19,45]. Ligands for RXR, which forms the active heterodimer with PPARs, can also enhance UCP3 transcription in cultured muscle cells [36,38,54]. All these observations are consistent with, although not definitive proof of, FA acting via PPARs to upregulate UCP2 and UCP3, and also suggest that this may occur in a tissue-specific manner via different PPAR isoforms.

5. Do FA work via SREBP1?

The sterol responsive element (SRE) binding protein (SREBP) family of transcription factors are known for their role in regulating lipid metabolism [55]. SREBP1 induces the expression of genes involved in lipid synthesis in liver and adipose tissue. Interestingly, polyunsaturated FA repress the expression of these same genes [48] and this appears to occur via a mechanism involving SREBP1. Polyunsaturated FA decrease the expression of SREBP1 mRNA and decrease the level of the proteolytic fragment of SREBP1 that enters the nucleus as the active transcription factor, thus decreasing the ability of SREBP1 to activate transcription of the SREBP-responsive lipogenic genes in liver [56,57]. UCP2 and UCP3 are unlikely to belong to this group of lipogenic genes because all conjecture so far give UCPs a role in lipid oxidation. However, SREBP1 has recently been shown to repress the expression of some genes [58]. Is it possible that SREBP1 could also repress UCP2 and/or UCP3 (perhaps in tissue-specific way), and thus when FA decrease SREBP1 activity, repression would be relieved and the expression of UCPs increased? Is there any evidence for this possibility?

As yet there is no direct evidence that SREBP1 represses UCP2 or UCP3 expression, although there are several situations where SREBP1 levels are inversely associated with UCP2 or UCP3 mRNA levels in adipose tissue [59,60] and skeletal muscle [33,61], respectively. These observations support the notion that SREBP1 may repress expression of UCP2 and UCP3. However, data that are contrary to this hypothesis were obtained from INS-1 cells transformed with the active form of SREBP1c; genes of lipogenesis were induced as expected and UCP2 mRNA was also apparently increased, although this was not quantitatively analysed [62]. Involvement of SREBP1 as an activator of UCP2 is also suggested by studies of UCP2 promoter activity, again in INS-1 cells, where cotransfection of the active SREBP1c enhanced expression of a luciferase reporter driven by UCP2 promoter [47]. If all these observations

hold up and are to be reconciled, then the influence of SREBP1 and its involvement in FA regulation of UCP2 and UCP3 expression must depend upon other tissue-specific factors as well.

6. By what other mechanisms could fatty acids induce UCPs?

Another route, which is yet to be fully explored, by which FA could influence gene expression is via activation of the energy-sensing 5′-AMP-activated protein kinase (AMPK) [63]. AMPK could be activated by the AMP generated by conversion of FA to their CoA derivatives during metabolism. Active AMPK in turn may bring about phosphorylation of certain transcription factors and thereby alter UCP2 and UCP3 expression. Experimental activation of AMPK by the AMP analogue 5-aminoimidazole-4-carboxamide-1-β-D-ribofurano- side (AICAR) has been shown to increase UCP3 mRNA in skeletal muscle in vivo [64–66] and in vitro [25,67]. UCP2 expression in INS-1 cells has also been shown to be responsive to AICAR treatment [68]. The missing molecular links are whether FA metabolism does in fact activate AMPK and whether transcription factors that are targets of AMPK are involved in regulating UCP2 and UCP3 expression.

FA metabolism could also influence the metabolism of glucose and change flux through the hexosamine pathway, which has a role as a nutrient sensor [69]. Elevated levels of hexosamines may in turn lead to O-glycosylation of proteins involved in various aspects of gene expression. This potential mechanism for upregulation of UCPs by fatty acids remains to be studied.

7. Can fatty acid responsive regions be identified in the promoters of UCPs?

Some progress is beginning to be made in identifying regions within the 5′-upstream regulatory region (5′-USR) of the UCP2 and UCP3 genes that confer FA responsiveness. Transcription factors that bind to sites within this region might be identified by computer-assisted analysis and ultimately by functional analysis. If FA work via PPARs or SREBP1, then binding sites for these factors might be expected within this region. However, such FA responsive regions are proving elusive.

7.1. UCP2 promoter

The first demonstration that FA can increase transcriptional activity of the UCP2 promoter was made in INS-1 cells transiently transfected with a luciferase reporter driven by the 5′-USR of mouse UCP2 gene [47]. Treatment of these cells with oleic acid (18:1) caused a 2-fold stimulation of luciferase activity. This increase in promoter activity was consistent with a similar increase of endogenous UCP2 expression in the same cell line in response to 18:1 [46,47]. The region responsible for conferring FA responsiveness to the reporter was narrowed down to 42 bp near the transcription start site. Computer-assisted analysis of this region identified a putative Sp1 site, a putative SRE motif and two E-box motifs. Mutations of the SRE site or either of the E-boxes eliminated the response of the UCP2 promoter to 18:1, suggesting that this region corresponded to a FA responsive region. If FA are regulating UCP2 expression via this region, then transcription factors that can bind to them would in turn need to be influenced by FA. Transcription factors to be considered are SREBP1, upstream stimulatory factor 1 (USF1) and USF2, which were shown to bind to this region [47], and also PPARγ, which appears to act indirectly via this same region [70]. A mechanism by which FA regulate these transcription factors needs to be determined. FA could activate PPARγ by acting as ligands. Currently, there is no evidence for FA activating or inducing SREBP1; to the contrary, there is evidence that polyunsaturated FA actually downregulate SREBP1, at least in liver [56]. Nevertheless, there appears to be a connection between SREBP1 and FA responsiveness in INS-1 cells as over-expression of active SREBP1c, together with 18:1 treatment, leads to further augmentation of UCP2 promoter activity beyond the effect of either one alone [47], but the mechanism for this remains to be determined. It will be important to study the promoter activity of 5′-USR of UCP2 in other cells representative of adipose, muscle and liver to establish if SREBP1 effects are tissue-dependent.

7.2. UCP3 promoter

The problem of choosing a suitable cell line in which to functionally analyse promoter regions is again highlighted for UCP3. Substantial basal expression of luciferase reporter driven by the 5′-USR of human UCP3 in an L6 muscle cell line required cotransfection with MyoD [71]. This observation is consistent with UCP3 being expressed highly in differentiated muscle cells, which are characterised by high levels of MyoD, a muscle-specific transcription factor. Incubation of L6 myoblasts with 18:1 increased up to 2-fold the expression of the transfected reporter driven by 5′-USR of human UCP3, but this also required cotransfection with either PPARα or PPARδ as well as with MyoD [71]. The requirement for MyoD along with a requirement for PPARα or δ suggests that these transcription factors functionally interact to bring about FA response. The responsiveness to PPAR agonists via PPARα required an intact direct repeat (DR1) element and MyoD induction depended on E boxes within 165 bp of the transcription start site [71]. It remains to be shown in transient transfection assays whether the responsiveness to 18:1 is also retained within this 165 bp 5′-USR and whether the DR1 repeat element or E-boxes are critical for FA regulation of UCP3. Interestingly, somatic transfer of reporter constructs into the hindlimb of newborn mice showed that an intact DR1 element was required for induction of the promoter by suckling [71]. This provides indirect support for the DR1 element also being involved in FA regulation, as suckling is a situation that is accompanied by elevated plasma FA and induction of endogenous UCP3 [20].

8. Missing links between FA and UCP2 and UCP3 expression

The many studies reviewed here point to unsaturated FAs as key regulators of UCP2 and UCP3 expression in response to changes in nutritional status and whole body energy metabolism. The mechanisms by which FAs upregulate UCP2 and UCP3, in what appears to be a tissue-specific manner, have not yet been fully determined. Further work is required to find:

how the promoters of UCP2 and UCP3 differ to allow tissue-specific responses to FA; the transcription factors involved and how FA influence their function; the molecular route by which FA induce UCP2 in adipose tissue but not in muscle, and UCP3 in muscle but not in adipose tissue; how regulation is associated with the physiological roles of the UCPs in the different tissues; whether regulation is abnormal in various pathological states.

All this work will be worthwhile because there remains the enticing possibility of identifying novel targets in the molecular chain of events that are amenable to therapeutic intervention by new drugs, or even dietary manipulation, to control obesity, type 2 diabetes and other disorders of energy metabolism.

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